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CEREBROVASCULAR DYSFUNCTION FOLLOWING TRAUMATIC BRAIN INJURY

Ph.D. thesis

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ABBREVIATIONS

TBI: traumatic brain injury

CBF: cerebral blood flow

CTE: chronic traumatic encephalopathy

TP receptor: thromboxane/prostaglandin endoperoxide receptor

CVR: cerebrovascular resistance

VCI: vascular cognitive impairment

CBV: cerebral blood volume

Ang II: Angiotensin II

MCA: middle cerebral artery

20-HETE: 20-hydroxy-5,8,11,14-eicosatetraenoic acid

TRPV4: Transient receptor potential vanilloid channel 4

BBB: blood-brain barrier

ROS: reactive oxygen species

BK_{Ca}: large conductance calcium-activated potassium channel

SHR: spontaneously hypertensive rat

OFT: open field test

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I. Introduction

Traumatic brain injury (TBI) is a serious health problem worldwide resulting in significant chronic disabilities. Each year approximately 1.7 million people in the United States [1-3] and another 2.5 million patients in the European Union [3] suffer TBI. In addition to a high mortality rate (35–40%), survivors of severe TBI and patients suffering mild but repetitive trauma are left with significant cognitive, behavioral, and communicative disabilities imparting an even larger burden to the health care systems [2] and the families of these victims. Epidemiological studies show that approximately 5.3 million people live with TBI-related disabilities in the United States [1] and 7.7 million in the European Union.[3] TBI can be mild, moderate, or severe defined by the Glasgow Coma Scale (14-15, 9-12 and < 9, respectively), imaging findings, and various biomarkers.[4] Mild TBI (mTBI) is the most frequent form of head trauma, typically affecting young athletes and the elderly population, who are prone to fall.[5]

After the mechanical force-induced primary brain injury, TBI initiates a variety of pathophysiological processes leading to secondary brain damage[6-8], which contributes to the development of long-term psychiatric problems and cognitive decline[7]. While little can be done to reverse the initial primary brain damage caused by trauma, secondary brain injury, which is (in part) due to vascular/microvascular alterations, and dysregulation of cerebral blood flow (CBF) might be potentially preventable or treatable.

1. Impaired autoregulation after traumatic brain injury

Despite of wide changes in systemic blood pressure due to daily activities CBF has to be relatively constant to avoid great changes in intracranial pressure, which would negatively impact the function of brain tissues. The physiological mechanism fulfilling these requirements is called autoregulation of CBF. Although the overall CBF is relatively constant there are regional heterogeneity in CBF distribution and local functional hyperemia according to the increased neural/glial function. Thus regulation of CBF is achieved by the

integration of mechanosensitive (such as pressure-induced myogenic), metabolic, neural and other mechanisms.[9,10]

The myogenic mechanism adjust the diameter of cerebral resistance vessels and thus cerebrovascular resistance (CVR) to the changes of perfusion pressure: they increase CVR in case of increasing perfusion pressure and decrease it when blood pressure drops. In other words, cerebral autoregulation is a negative feedback process maintaining stable and constant blood flow when perfusion pressure changes: 1) in hypotension an intact autoregulation prevents hypoperfusion and ischemia of cerebral tissue; and 2) in hypertension it protects the cerebral microvascular bed against hyperemia and hypervolemia (Fig. 1). Mechanisms of CBF autoregulation include static and dynamic components. Static autoregulation of CBF adjusts vascular resistance (thus blood flow) to a steady-state perfusion pressure value, and it dictates how large changes in perfusion pressure can be compensated. Dynamic cerebral autoregulation restores CBF after rapid transient changes in perfusion pressure and thus determines how fast the autoregulatory compensation can be implemented. Dynamic and static cerebral autoregulation act on a continuum to maintain CBF when blood pressure changes.[11-14]

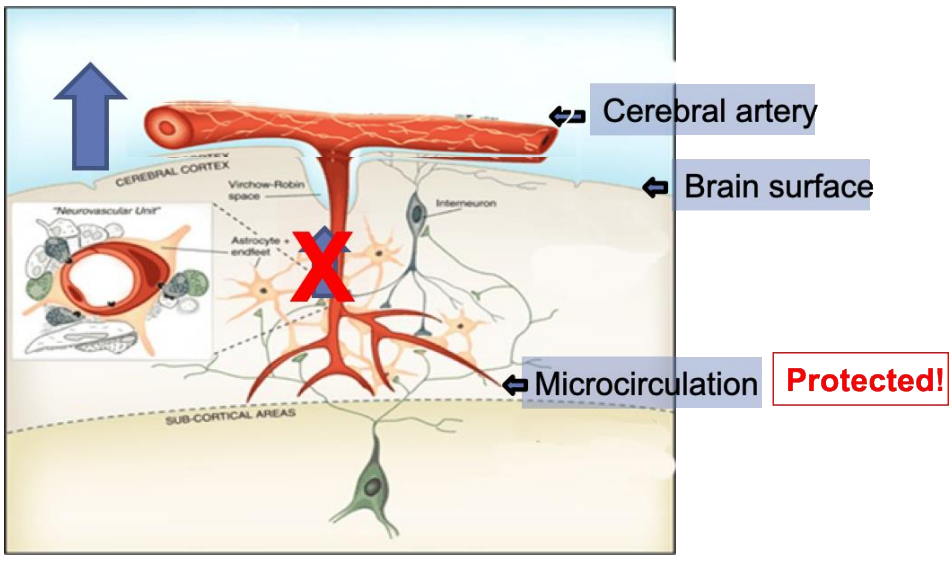

Figure 1. Pressure-induced myogenic constriction of cerebral arteries and arterioles protects the microcirculation. *When perfusion pressure increases myogenic constriction of cerebral vessels*

adjust cerebrovascular resistance to the change in perfusion pressure, therefore hydrodynamic pressure is relatively unchanged in the cerebral microcirculation and the capillary bed is protected from increases in intraluminal pressure and volume.

As mentioned above, when autoregulatory mechanisms, due to their impaired capacity to decrease cerebrovascular resistance, cannot maintain approximately unchanged cerebral perfusion when blood pressure decreases, then autoregulatory dysfunction leads to ischemia of cerebral tissue. On the contrary, at high pressure values, the significance of autoregulatory mechanisms responsible for maintaining constant blood flow can be appreciated when taking into account the anatomical fact that the brain is situated in the closed cranium consisting of three main volume compartments: cerebral tissue, cerebrospinal fluid, and intravascular blood. Volume expansion of one of the compartments can only be compensated by a decrease in the others, as stated in the Kellie-Monroe doctrine and its modified versions[15]. Thus, in case of low intracranial compliance (when compensatory capacity of CSF dynamics is attenuated), an uncontrolled increase in cerebral blood volume (which is the only compartment with higher pressure than normal or even pathological ICP) would lead to sudden increases in ICP and may damage the cerebral microcirculation (Figure 2.).[16,17]

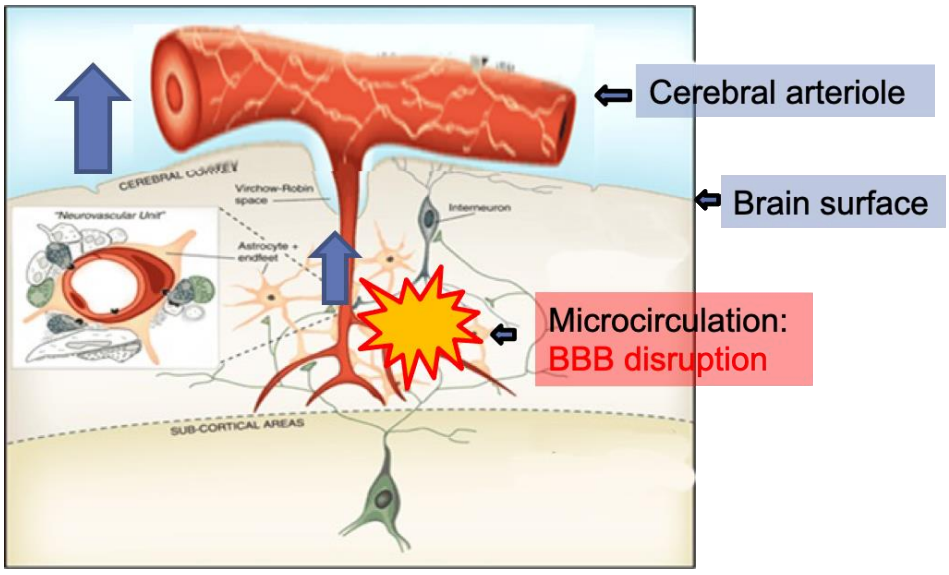

Figure 2. Myogenic autoregulatory dysfunction of cerebral arteries leads to injury of the cerebral microcirculation. *In autoregulatory dysfunction cerebral arteries and arterioles cannot*

constrict in response to increases in intraluminal pressure, therefore high pressure (and blood volume) can penetrate the distal microcirculation, damaging the cerebrovascular capillary bed (for example disruption of the blood-brain barrier BBB).

There is growing experimental and clinical evidence that TBI impairs autoregulation of cerebral blood flow (CBF)[16-28], in which impairment of pressure-induced myogenic constriction of cerebral resistance vessels may play a significant role[29-32]. As mentioned above, on the one hand, TBI-induced myogenic autoregulatory dysfunction of cerebral vessels results in ischemia with relatively small reductions in systemic blood pressure. On the other hand, with modest increases in blood pressure it permits marked increases in CBF and penetration of high pressure to the vulnerable distal portion of the cerebral microcirculation. Thus, TBI-induced myogenic autoregulatory dysfunction exacerbates ischemic brain damage, contributes to vascular congestion and intracranial hypertension and promotes blood brain barrier disruption, vasogenic edema and cerebromicrovascular injury[33]. Despite its pathophysiological importance[18-21,32,34] the cellular and molecular mechanisms underlying myogenic autoregulatory dysfunction of cerebral arteries following TBI are not well understood. Early studies suggested that TBI-related oxidative stress exerts vasodilator effects in pial vessels following trauma[35], however, the source of increased reactive oxygen species (ROS) and the mechanisms by which ROS contribute to TBI-induced myogenic autoregulatory dysfunction of cerebral arteries remained obscure.

2. The effect of traumatic brain injury on blood-brain barrier integrity in in hypertension: downstream consequences of autoregulatory dysfunction

As it is mentioned above autoregulation of cerebral blood flow is an important homeostatic mechanism, which maintains stable cerebral perfusion and thus cerebral volume when systemic blood pressure changes, and autoregulatory dysfunction following traumatic brain injury plays a central role in the development of secondary brain damage.[9,12] Accordingly, when blood pressure drops the injured brain is exposed to hypoperfusion and ischemia due to inefficient vasodilation; on the contrary, when pressure increases inefficient vasoconstriction leads to increase in intracranial volume and pressure thus exacerbating

disruption of the blood-brain barrier (BBB) [16,17]. BBB disruption plays a central role in the secondary pathological processes[36], leading to accumulation of blood-borne substances in the cerebral parenchyma, neuroinflammation and consequent decline in higher brain function. Mild TBI is the most frequent form of head trauma, typically affecting young athletes and the elderly population, who are prone to fall[5]. Interestingly, mild TBI is likely to induce BBB disruption, but it has only a transient effect[37-39]. A growing body of research has established that preexisting comorbid medical conditions exacerbate the deleterious effects of TBI (resulting in longer ICU stay and increased risk for complications)[40]. The most frequently identified comorbid condition in TBI is hypertension (in older individuals its prevalence: ~38.8%)[41,42], which has been demonstrated to exacerbate disruption of the BBB in various pathological conditions. For example, hypertensive aged mice were found to exhibit increased permeability of the BBB, which is associated with neuroinflammation and cognitive decline of the animals[33].

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II. Objectives

Based on the aforementioned we sought two test two hypothesis regarding the effect of traumatic brain injury on the proximal and distal part of the cerebral circulation:

1) The studies presented here were designed to test the hypothesis that following brain trauma myogenic response of cerebral arteries is compromised due to the excessive mitochondrial production of vasodilator H_2O_2 in the vascular smooth muscle cells. To test this we induced diffuse brain trauma in rats by the impact acceleration technique and compared vascular H_2O_2 production and pressure-induced myogenic responses of isolated middle cerebral arteries in the presence and absence of scavengers of mitochondria-derived H_2O_2. We also aimed to elucidate the downstream targets of increased H_2O_2. Specifically, we tested the hypothesis, developed based on previous findings[43-4647], that after TBI excessive mitochondria-derived H_2O_2 activates large conductance calcium-activated potassium (BK_{Ca}) channels[48,49] via a TRPV4-dependent pathway in the vascular smooth muscle cells, which impairs pressure-induced constriction of cerebral arteries. To achieve this goal we assessed the effects of specific blockers of TRPV4 and BK_{Ca} channels on TBI-induced myogenic autoregulatory dysfunction of cerebral arteries, determined the role of these channels in vasomotor responses elicited by exogenous H_2O_2 and used patch clamp to characterize the effects of H_2O_2 and TRPV4 inhibitors on BK_{Ca} ion currents.

2) We tested whether pre-existing chronic hypertension exacerbates the damaging effect of mild TBI on the BBB. To achieve this goal we induced mild traumatic brain injury in normotensive and hypertensive rats, and assessed blood-brain barrier integrity, accumulation of blood borne substances in the brain parenchyma, production of neuroinflammatory cytokines in the cerebral cortices and hippocampi and cognitive function of the animals two weeks after TBI.

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III. Results

1. Role of mitochondria-derived H_2O_2 in impaired myogenic constriction of cerebral arteries after severe TBI

We found that myogenic constrictions of MCAs isolated from rats 2 hours after TBI were intact, whereas myogenic responses of MCAs 24 hours after traumatic brain injury were significantly decreased compared to control MCAs in the autoregulated pressure range (between 60-140 mmHg) (Figure 3.A-B). These results confirm the findings of Golding et al[29] (obtained in controlled cortical impact model) for the first time after impact acceleration diffuse brain injury. We continued our studies with MCAs isolated from rat brains 24 hours after TBI. The thromboxaneA2 analog U46619 agonist-induced constrictions of basilar arteries from the same animals were intact after TBI and did not differ from control responses (Figure 3. B inlet). We demonstrate here that administration of the mitochondrial antioxidant mitoTEMPO restored myogenic constriction of MCAs of TBI rats to the level of control MCAs ($p<0.05$ vs. TBI) suggesting a key role of mitochondria-derived ROS in the TBI-induced impairment of cerebral myogenic responses (Figure 3.B). Our results that administration of catalase (CAT) restores TBI-induced impaired myogenic responses of MCAs, as well ($p<0.05$ vs. TBI), and co-administration of mitoTEMPO did not have any additional effects suggest that mitochondria-derived H_2O_2 is the primary factor that attenuates myogenic constriction of MCAs after TBI (Figure 4.A). This is supported by our further findings that TBI enhances cerebrovascular H_2O_2 production significantly ($p<0.05$), as shown by the catalase-sensitive increased CM-H2DCFDA fluorescence in isolated MCAs (Figure 4.B) in TBI vessels compared to controls.

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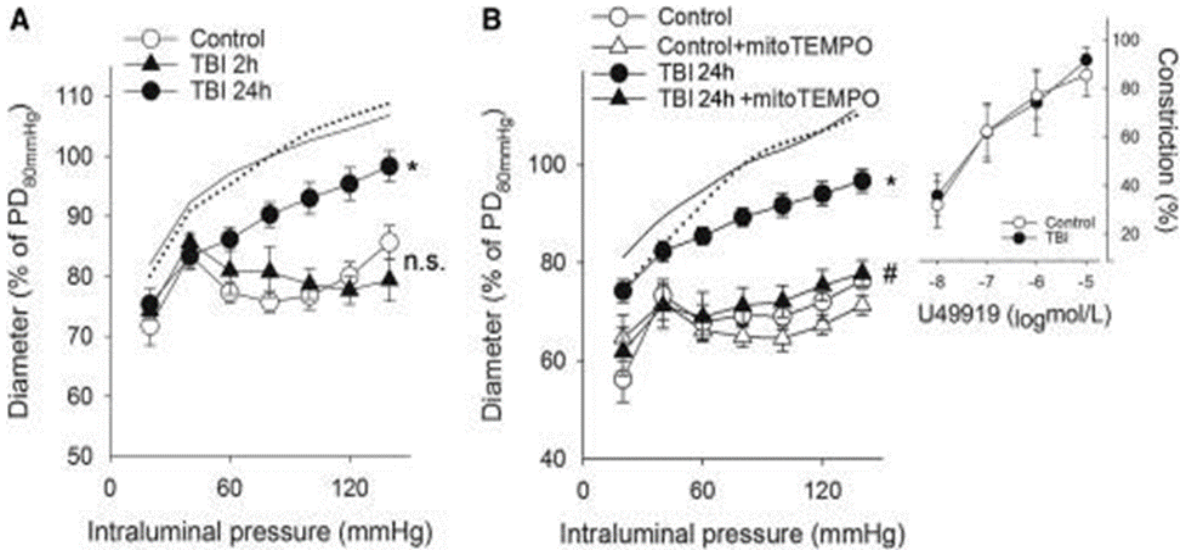

Figure 3. *TBI impairs myogenic constriction of cerebral arteries: role of mitochondrial ROS production. A: Diameter responses (as % of passive diameter (PD) at 80 mmHg intraluminal pressure) of isolated middle cerebral arteries (MCA) are shown as a function of intraluminal pressure (myogenic response) in control rats and in rats 2 (TBI 2h) and 24 (TBI 24h) hours after severe traumatic brain injury. Note that the pressure induced constrictor response is intact 2 hours after the impact, and it is significantly attenuated 24 hours post-injury. Data are mean ± S.E.M. (n=5 for each group) *P<0.05 vs. Control (lines without symbols show passive pressure-diameter curves of MCAs). B: Myogenic responses of MCAs are depicted in control and TBI 24h-rats in the absence and presence of the mitochondrial antioxidant mitoTEMPO. Inlet depicts the constriction of basilar arteries of control and TBI 24h rats in response to the thromboxane analogue U46619. Data are mean ± S.E.M. (n=5 for each group) *P<0.05 vs. Control; #P<0.05 vs. TBI 24h.*

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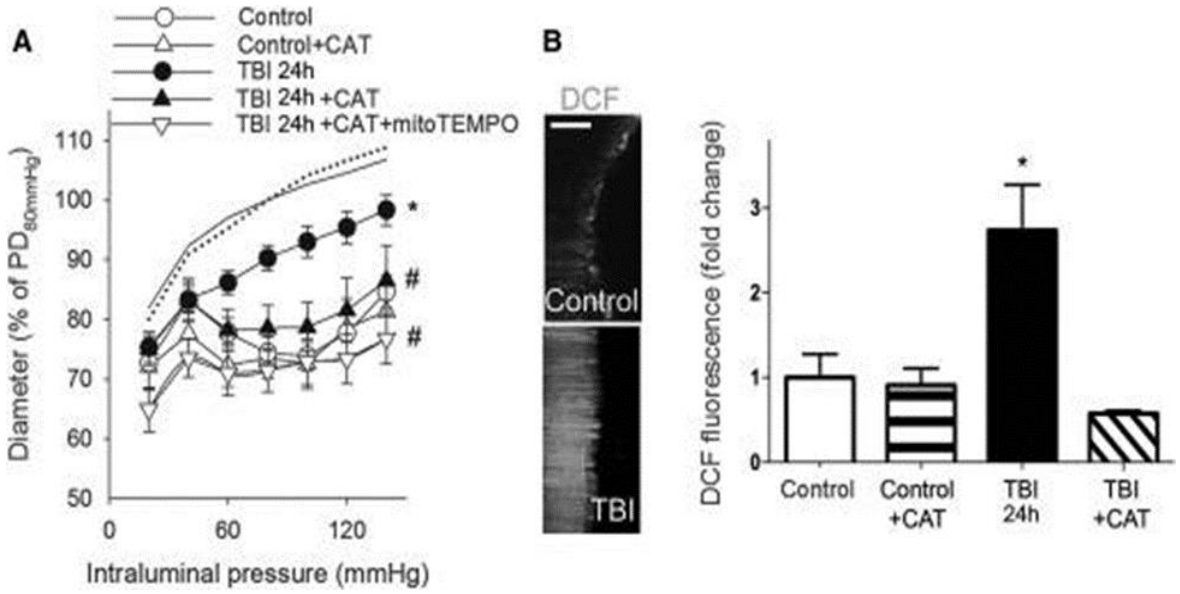

Figure 4. *TBI impairs myogenic constriction of cerebral arteries: role of mitochondrial H2O2. A: Diameter responses (as % of passive diameter (PD) at 80 mmHg intraluminal pressure) of isolated middle cerebral arteries (MCA) are shown as a function of intraluminal pressure (myogenic response) in control and TBI 24h (24 hours after the impact) rats after the administration of catalase (CAT). Please note that additional administration of mitoTEMPO does not augment the effect of CAT on the diameter responses. Data are mean ± S.E.M. (n=5 for each group) *P<0.05 vs. Control; #P<0.05 vs. TBI 24h. B: Summary data and representative images of cerebrovascular H2O2 production in endothelium-denuded MCAs of control rats, TBI 24h rats and control and TBI 24h rats after incubation of the vessels in catalase (CAT) shown by the fluorescence of the cell-permeant oxidative fluorescent indicator dye DCF (5 (and 6)- chloromethyl-2',7'dichlorodihydrofluorescein diacetate-acetyl ester). Scale bar is 50 µm. Data are mean±S.E.M. (n=5 for each group). *P<0.05 vs. Control.*

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2. Role of BK_{Ca} channels in impaired myogenic constriction of cerebral arteries after severe TBI

Activation of BK_{Ca} channels with the consequent hyperpolarization of vascular smooth muscle cell membrane is a negative feed-back regulator of myogenic constriction[45,50,51], and inhibition of BK_{Ca} channel was shown to constrict cerebral arteries after TBI[31]. These previous findings are supported by our present results (Figure 5.A) that specific inhibition of BK_{Ca} channels on isolated MCAs by paxilline restores myogenic constriction of MCAs of TBI rats to the control level. We found that H_2O_2-induced dilations of MCAs of TBI rats are 1) significantly larger than dilator responses of MCAs from control animals and 2) inhibited by blocking BK_{Ca} channels (Figure 5.B). These results (Figure 5.A and B) suggest that TBI-related increased production of cerebrovascular H_2O_2 (Figure 4.B) impairs myogenic constriction of MCAs via activation of BK_{Ca} channels. TBI up-regulates the cerebrovascular mRNA expression of BK_{Ca} channels, which is likely to explain the augmented dilator effect of the channels after brain trauma, as well.

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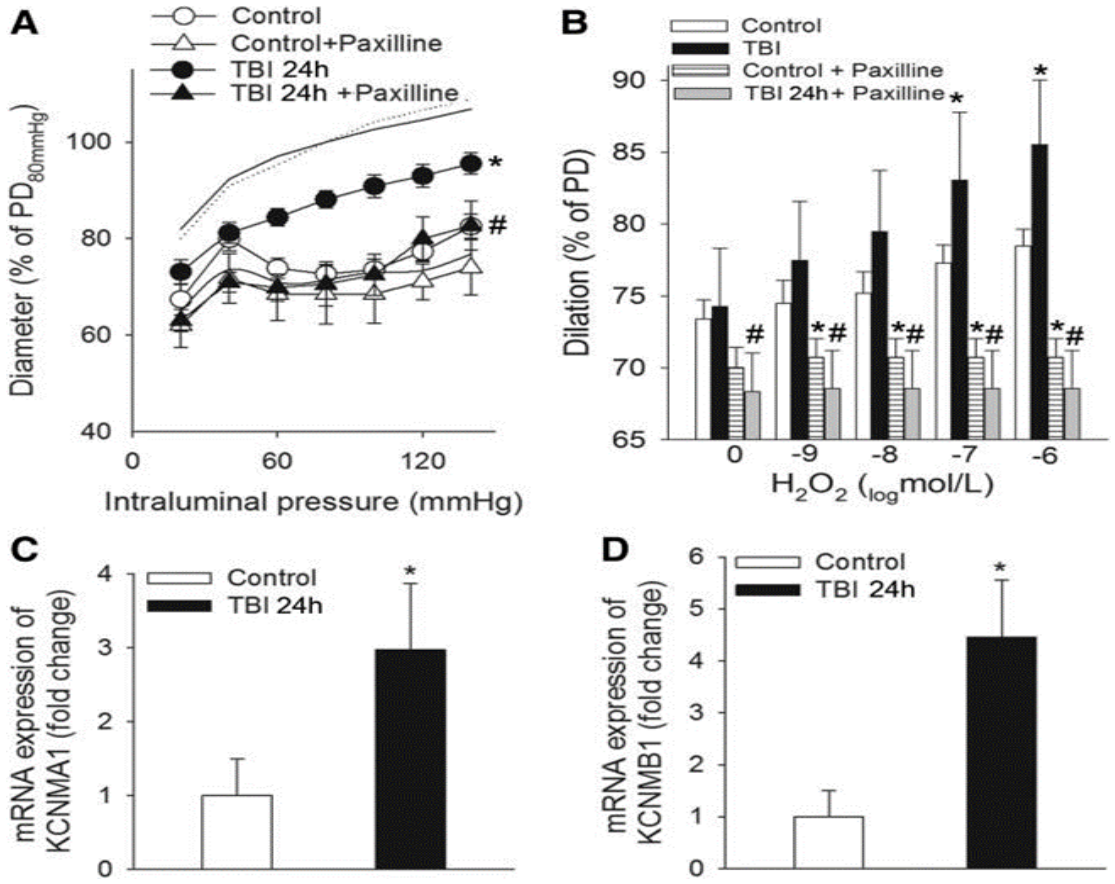

Figure 5. *TBI impairs myogenic constriction of cerebral arteries: role of Ca2+-activated K+ (BK) channels. A: The effect of paxilline, a specific blocker of calcium-activated potassium (BK_{Ca}) channels on pressure induced myogenic constriction of middle cerebral arteries (MCAs) of control rats and rats 24 hours after severe TBI (TBI 24h). Data are mean ± S.E.M. (n=5 for each group) *P<0.05 vs. Control; #P<0.05 vs. TBI 24h. B: Blocking BK_{Ca} channels by paxilline inhibits H2O2-induced dose-dependent dilations of MCAs of control and TBI 24h rats. Note that H2O2-induced dilations are significantly augmented in MCAs isolated from TBI 24h rats. Data are mean ± S.E.M. (n=5 for each group); *P<0.05 vs. Control, #P<0.05 vs. TBI 24h. QRT-PCR data of mRNA expression of α (panel C) and β (panel D) subunits of BK_{Ca} channels (KCNMA1 and KCNMB1, respectively) in MCAs of control and TBI-rats. Data are mean ± S.E.M. (n=5 for each group) *P<0.05 vs. Control.*

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3. Role of TRPV4 channel activation in impaired myogenic constriction of cerebral arteries after severe TBI

TRPV4 channels have been suggested to be redox sensitive[52] and capable to activate BK_{Ca} channels[53-55]. Therefore, we tested the hypothesis that H_2O_2 activates BK_{Ca} channels via TRPV4 channels. Here we show for the first time that TBI-induced impaired myogenic response of MCAs is improved and restored to the control level in the presence of HC067047, a specific blocker of TRPV4 channels (Figure 6.A). Establishing the link between H_2O_2, TRPV4 and BK_{Ca} channels, we demonstrate that H_2O_2-evoked dilations of MCAs are diminished in the presence of HC067047 (10^{-6} mol/L) in both control and TBI MCAs, and that the TRPV4 agonist GSK1016790A-induced dose-dependent dilations of MCAs are 1) are significantly greater in MCAs after TBI than in control vessels and 2) blocked by the specific BK_{Ca} channel blocker paxilline (Figure 6.B-C). TBI significantly enhances the cerebrovascular mRNA expression of the TRPV4 gene, which is likely to contribute to the demonstrated effect of TRPV4 channels in the impaired myogenic constriction of MCAs after TBI (Figure 6.D) and explains the attenuated dilator responses to the TRPV4 agonist GSK1016790A.

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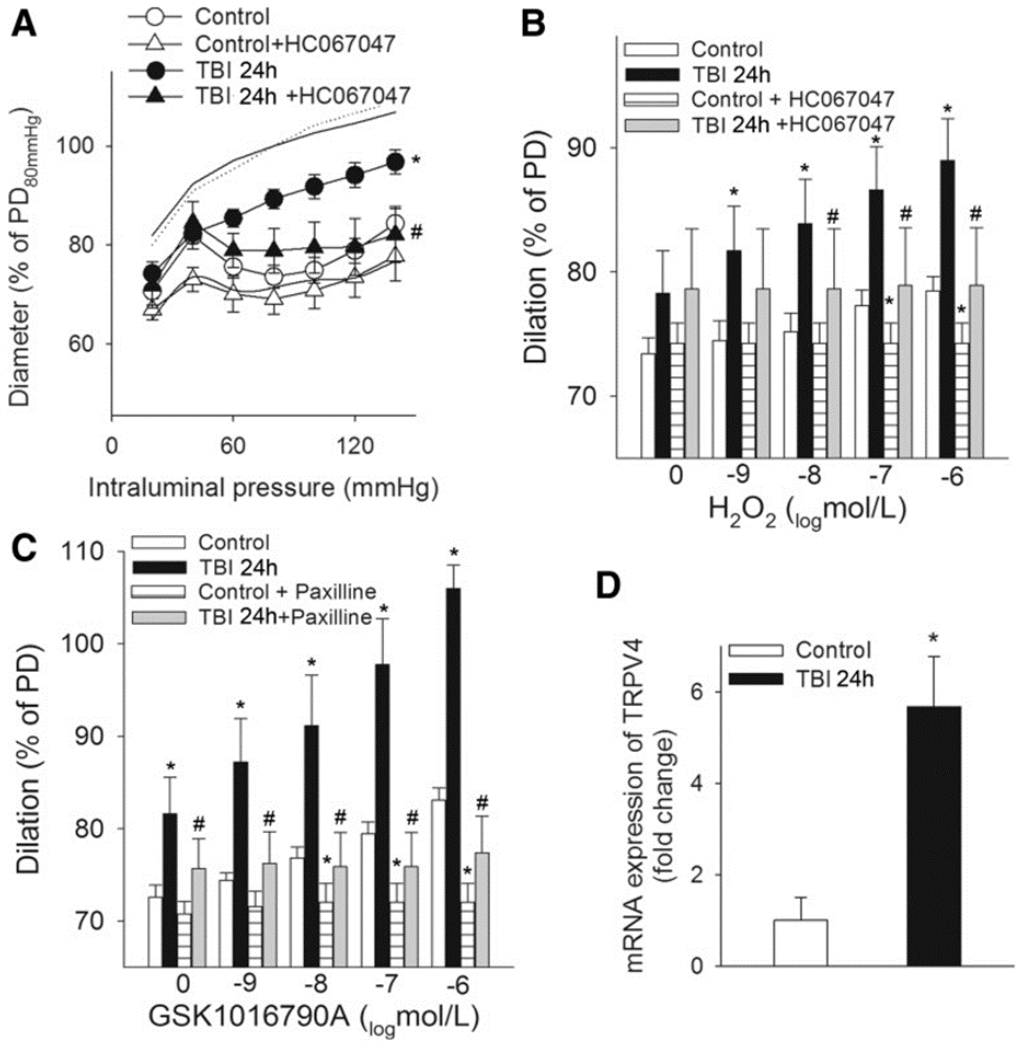

Figure 6. *TBI impairs myogenic constriction of cerebral arteries: role of TRPV4 channels. A: Myogenic constriction of middle cerebral arteries (MCAs) of control rats and rats 24 hours after traumatic brain injury (TBI 24h) in the absence and presence of the specific TRPV4 channel blocker HC067047. Data are mean ± S.E.M. (n=5 for each group) *P<0.05 vs. Control; #P<0.05 vs. TBI 24h. Panel B depicts the effect of the TRPV4 channel blocker HC067047 on H_2O_2-induced dilations of MCAs of control and TBI rats, and C shows the effect of blocking BK_{Ca} channels on dilations of MCAs evoked by the TRPV4 agonist GSK1016790A in the same groups of animals. Please note that both H_2O_2-induced and GSK1016790A-induced dilations of MCAs are significantly higher after TBI. Data are mean ± S.E.M. (n=5 for each group) *P<0.05 vs. Control; #P<0.05 vs.TBI 24h. D: QRT-PCR data of mRNA expression of TRPV4 channels in MCAs of control and TBI 24h rats. Data are mean ± S.E.M. (n=5 for each group) *P<0.05 vs. Control.*

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4. H2O2-induced activation of calcium-activated potassium (BK) channel currents in vascular smooth muscle cells (VSMCs) is mediated by TRPV4 channels

We measured BK_{Ca} channel currents from vascular smooth muscle cells (VSMC) that are isolated from middle cerebral arteries of SD rats using patch clamp method. Supporting our findings in isolated MCAs we found that H_2O_2 significantly increased BK_{Ca} currents on VSCMs, and inhibition of TRPV4 channels (10-6 mol/L MHC067047 for 5 minutes) returned BK_{Ca} channel activity to the control level. H_2O_2 has no effect in the presence of BK_{Ca} channel inhibitor paxilline (100nM) (Figure 7).

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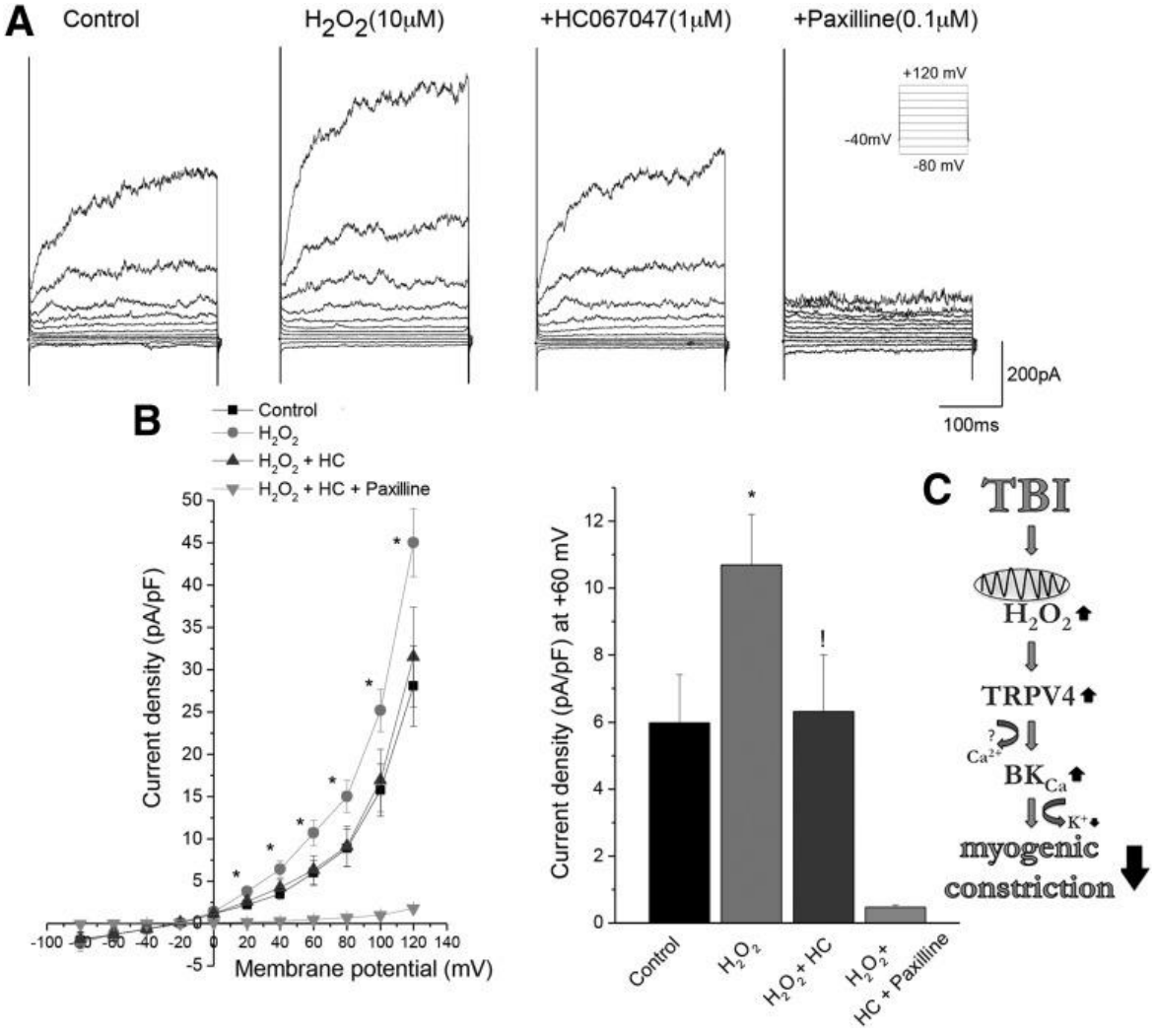

Figure 7. *H_2O_2-mediated increase in BK_{Ca} channel activity requires TRPV4 channel. Whole cell BK_{Ca} currents were recorded with 100nM free cytosolic calcium in the presence and absence of H_2O_2, TRPV4 channel inhibitor (1μM HC067047) and BK_{Ca} channel inhibitor (Paxilline 100nM). BK_{Ca} currents were elicited by 20ms pulses from -60 to +120 mV from a Vh of -40mV (Inset). 2-3 smooth muscle cells from 4 Wistar Kyoto rats were studied in each group (8-12 cells/group). Panel A represents whole cell BK_{Ca} currents before and after 10μM H_2O_2, in the presence of 1μMHC067047 and/or 100nM Paxilline. Panel B represents current voltage curves and the current density at +60mV membrane potential. *p<0.05 before and after application of H2O2. !p<0.05 before and after application of 1μM HC067047 in the presence of H_2O_2. Data are mean ± SEM. Number in the parenthesis is the number of cells studied. C: Scheme depicting the mechanisms of impaired myogenic constriction of cerebral arteries after TBI.*

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5. Mild TBI induces persistent disruption of the blood-brain barrier which is associated with extravasation of blood borne fibrin in cerebral tissue of spontaneously hypertensive rats

Spontaneously hypertensive rats (SHR) had significantly higher blood pressure than normotensive Wistar rats (Figure 8.A). Blood pressure was not affected by mTBI in either normotensive or hypertensive rats (Figure 8.A). We found that mTBI led to a significant disruption of the blood-brain barrier detected two weeks after mTBI in hypertensive rats (n = 7), as shown by increased Evans blue content of cerebral tissue of these animals. No leakage of Evans blue dye could be observed in normotensive rats (n = 7), indicating intact

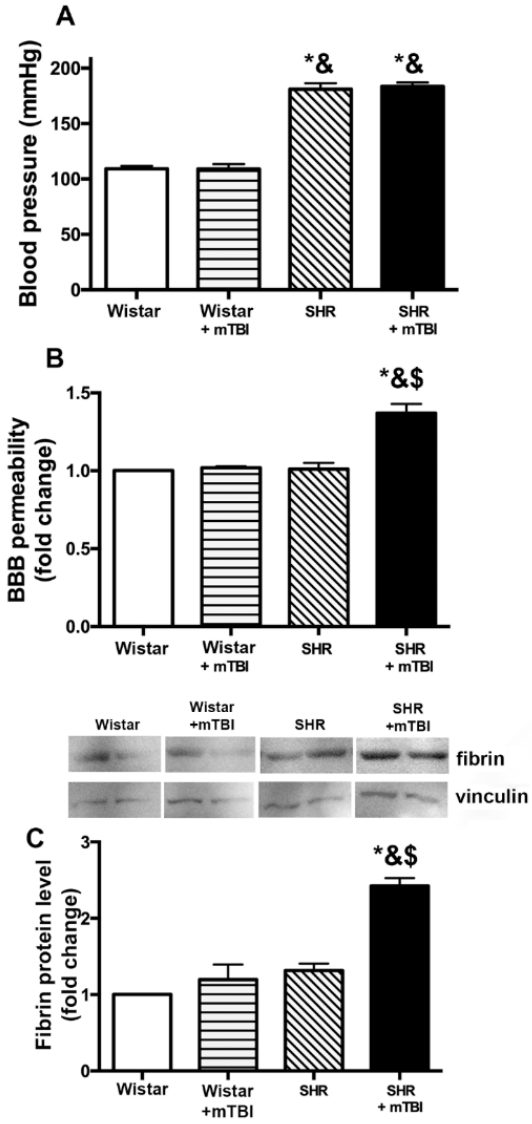

Figure 8. *Mild traumatic brain injury (TBI) induces persistent disruption of the blood brain barrier and extravasation of blood borne substances in hypertensive rats. (A) shows blood pressure of Wistar rats and spontaneously hypertensive rats (SHR) with and without mild traumatic brain injury (mTBI) measured by the tail-cuff method. Data are means ± S.E.M. (n = 6 in each group) * p < 0.05 vs. Wistar, p < 0.05 vs. Wistar + mTBI. (B) Summary data show blood brain barrier permeability indicated by extravasated Evans blue content of cerebral tissue (depicted as fold change compared to control) in sham operated Wistar rats and SHRs, and in rats two weeks after mTBI. Data are means ± S.E.M. (n = 6 in each group) * p < 0.05 vs. Wistar, p < 0.05 vs. Wistar + mTBI, $ p < 0.05 vs. SHR. (C) One representative Western blot presents fibrin level in perfused cerebral tissue from Wistar and spontaneously hypertensive rats (SHR) with and without mild traumatic brain injury (mTBI) (showing two in each group) two weeks after trauma. Summary data depicts cerebral fibrin protein level in cortical tissue of the above groups of animals. Data are means ± S.E.M. (n = 6 in each group) * p < 0.05 vs. Wistar, p < 0.05 vs. Wistar + mTBI.*

BBB after mTBI (Figure 8. B). We found that two weeks after mild TBI fibrin accumulated in cortical tissue of hypertensive rats (n = 6), which could not be observed in SHRs without mTBI (n = 6) or normotensive rats with and without mTBI (n = 6, in both groups) (Figure 10.C).

6. Mild TBI Induces Persistent Neuroinflammation and Cognitive Decline in Spontaneously Hypertensive Rats

We found that expression of inflammatory cytokines IL-1, IL-6 and TNF was significantly ($p < 0.05$) increased in both cortical and hippocampal tissue of spontaneously hypertensive rats (n = 8) two weeks after mTBI compared to sham-operated SHRs (n = 8) and to normotensive Wistar rats with and without mTBI (n = 8 in both groups) (Figure 8. A,B). We show that two weeks after trauma normotensive Wistar rats (n = 11) exhibited a significant ($p < 0.05$) decrease in the number of crossings (locomotor activity and exploration) in the open field arena, indicating habituation to the environment thus functioning learning and memory (Figure 9.C). In contrast, SHRs (n = 11) did not show any changes in the number of crossings during the repeated session two weeks after mTBI, indicating a decline in their learning and memory functions (Figure 9.C). We studied intermediate-term declarative memory by the novel object recognition test. We found that mTBI resulted in a significant ($p < 0.05$) decrease in the Discrimination Index (DI) in SHRs (n = 11) assessed two weeks after trauma indicating impaired memory function. Whereas DI was not changed in normotensive Wistar rats (n = 5), indicating preserved memory function (Figure 9.D).

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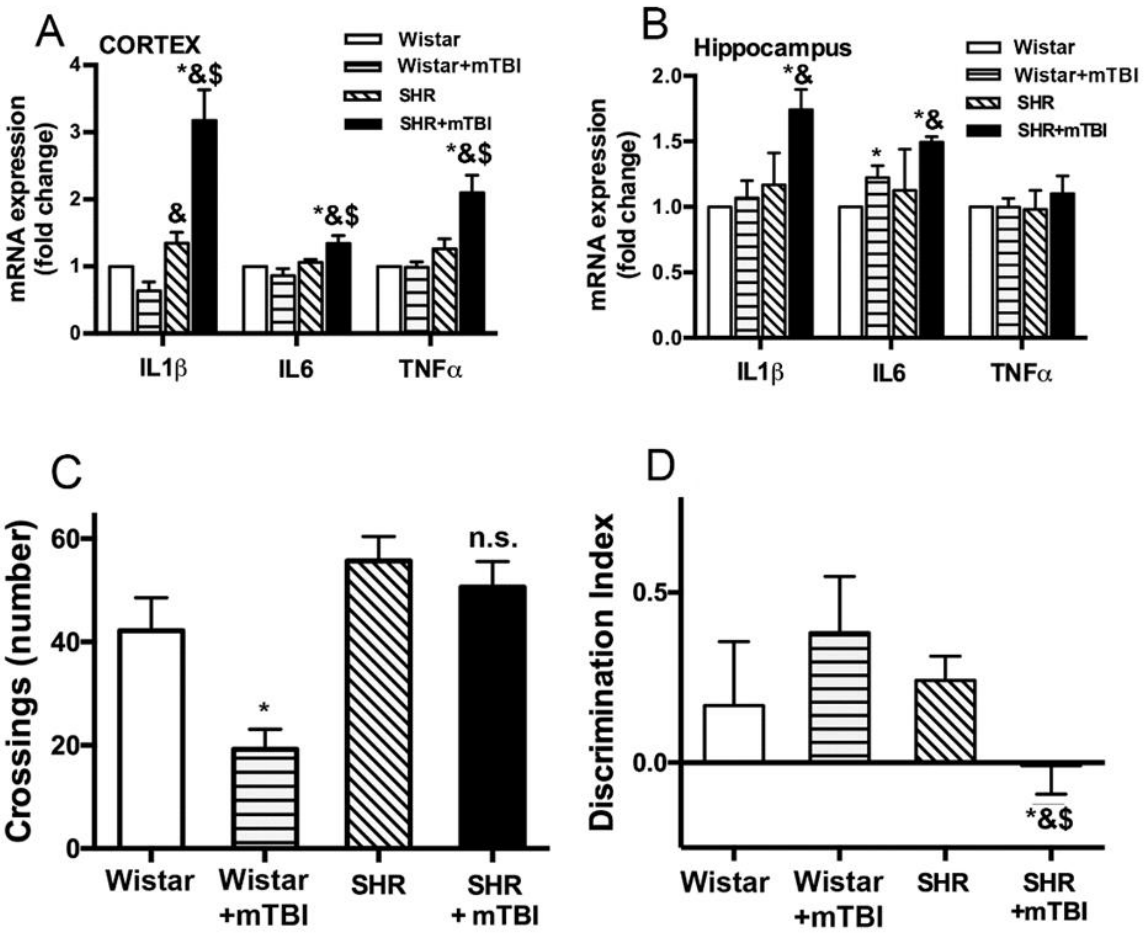

Figure 9. *Mild TBI induces persistent neuroinflammation and cognitive decline in hypertensive rats. (A,B) mRNA expression of inflammatory cytokines IL1, IL6 and TNF in cortical (A) and hippocampal (B) tissue of sham operated normotensive Wistar rats and SHRs, and of animals two weeks after mild TBI, expressed as fold change compared to control. Data are means ± S.E.M. (n = 8 in each group) * p < 0.05 vs. Wistar, p < 0.05 vs. Wistar + mTBI, $ p < 0.05 vs. SHR. (C) In a standard open field test normotensive Wistar animals showed attenuated exploratory activity (number of crossings) two weeks after mTBI (Wistar + mTBI) indicating habituation to the environment and intact locational memory function. In contrast, SHRs did not show habituation to the environment in the repeated open field test (OFT) session two weeks after mTBI (SHRmTBI), indicating impaired locational memory. Data are means ± S.E.M. (n = 15 in each group) * p < 0.05 vs. Wistar. (D) Intermediate-term declarative memory was tested two weeks after mTBI by the novel object recognition test. Discrimination index was not changed in normotensive Wistar rats two weeks after mTBI, but it was significantly decreased in SHRs, indicating impaired declarative memory of the animals. Data are means ± S.E.M. (n = 5 Wistar and n = 11 SHR) * p < 0.05 vs. Wistar, p < 0.05 vs. Wistar + mTBI, $ p < 0.05 vs. SHR.*

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IV. Discussion of findings

TBI and the myogenic response

Traumatic brain injury is a serious health problem worldwide. Beside a high mortality rate (35–40%), survivors are left with significant cognitive, behavioral, and communicative disabilities.[2] In addition to the primary impact, secondary brain injury determines the outcome of TBI patients significantly. It has been demonstrated that TBI-related impairment of autoregulation of cerebral blood flow plays a central role in the processes of secondary brain injury, and there is growing experimental and clinical evidence that impairment of pressure-induced myogenic constriction of cerebral resistance vessels play a significant role in TBI-induced autoregulatory dysfunction.[29-32] Here we demonstrate that myogenic constriction of cerebral arteries is intact 2 hours after the impact, but compromised 24 hours after trauma in the constrained impact acceleration model of TBI (Figure 3.), extending earlier findings in different models of TBI (Golding et al. used the controlled cortical impact model, and Villalba et al. studied the fluid percussion injury model)[29,31]. Our results and the findings of the mentioned studies suggest that impairment of myogenic mechanisms is a consequent result of TBI regardless of animal models used, and that it develops sub-acutely after the impact, most likely being involved in the development of secondary injury of cerebral tissue. The consequences of TBI-induced impairment of myogenic constriction are likely multifaceted. First, it is likely to contribute to increased blood volume in the closed cranium. Second, when blood pressure increases lack of myogenic protection likely allows high pressure to penetrate the cerebral microcirculation promoting blood brain barrier disruption and microvascular injury, which exacerbate vasogenic edema. Both increased CBV and vasogenic edema contributes to rise in intracranial pressure, especially when intracranial compliance (to compensate increases in ICP) is attenuated by cytotoxic edema[16,17,33,56,57].

Involvement of ROS

This is the first study to demonstrate that mitochondrial ROS production plays a central role in impaired myogenic constriction of cerebral arteries after diffuse TBI (Figures

3-4). Our studies provide direct evidence that following TBI the production of ROS is increased in the vascular smooth muscle cells, extending previous findings[35,58]. The mechanisms by which TBI promotes mitochondrial oxidative stress in smooth muscle cells may involve changes in the hemodynamic environment/mechanosensitive mtROS production[59], factors released from the damaged brain parenchyma (including glutamate neurotoxicity)[60,61], and/or humoral factors[62,63]. These possibilities should be tested in future studies. Complex I and III of the electron transport chain are possible major sites of premature electron leakage to oxygen, generating superoxide in the mitochondria[64,65]. Future studies using specific inhibitors should elucidate how ROS generation is affected by TBI at these sites in the smooth muscle mitochondria. Mitochondrial superoxide is readily dismutated to H_2O_2 by MnSOD, which is abundantly expressed in VSMCs.[66-69] While superoxide is not membrane permeable, H_2O_2 can readily penetrate the mitochondrial membranes, increasing cytosolic H2O2 levels. Importantly, H_2O_2 is a potent vasodilator in the cerebral circulation[43]. Thus, it is significant that H_2O_2 levels are substantially increased in VSMCs of cerebral arteries after TBI (Figure 4.). The findings that administration of catalase restores myogenic responses of MCAs derived from rats with TBI provide experimental evidence that increased mitochondria-derived H_2O_2 production plays the key role in dysregulation of arterial myogenic constriction after diffuse brain trauma. Recent studies raise the possibility that activation of nitric oxide synthesis may also contribute to the decreased myogenic constriction after TBI[31]. As there are data showing that a cross talk exists between NO and mitochondria-derived H_2O_2 production[70,71], the possibility that such interaction is also present after TBI and the role of TBI-related endothelial impairment in the decreased myogenic tone should be also tested in future studies.

Role of BK_{Ca} channels

The mechanisms by which H_2O_2 induced vasodilation in the cerebral circulation likely involve activation of large conductance Ca^{2+}-activated potassium (BK_{Ca}) channels[48,49]. In support of this concept we demonstrate that selective blockade of BK_{Ca} channels restored myogenic constriction of MCAs derived from rats with TBI, and that H_2O_2-induced dilations of MCAs were inhibited in the presence of BK_{Ca} channel blocker paxilline (Figure 5.).

Furthermore, H_2O_2 induced a significant increase of BK channel currents on vascular smooth muscle cells (Figure 7.). There is strong evidence that activation of BK_{Ca} channels readily hyperpolarizes smooth muscle cells, which inhibits pressure-induced activation of voltage-sensitive calcium channels and thereby myogenic constriction of cerebral arteries[45,50,51]. It has to be noted here, that Armstead et al. demonstrated that TBI impairs the function/activation of Ca^{2+} activated potassium channels, which mechanism might be involved in the processes that lead to decreased dilation (thus cerebral hypoperfusion) in response to hypotension after brain trauma[72,73]. Although these results cannot be directly compared to our present studies because the authors used an in vivo approach to measure dilation of pial arterioles to hypotension in newborn piglets, location- and vessel-dependent changes in function/activation/expression of BK_{Ca} channels after TBI should be established by future studies. Previous studies reported that the mechanisms by which H_2O_2 activates BK_{Ca} channels in different cell types are multifaceted and may involve the synthesis of eicosanoid mediators[74], protein kinase G pathway[75] and/or protein kinase C[76]. Importantly, the activity of BK_{Ca} is regulated by Ca^{2+} sparks, the frequency/amplitude of which can also be modulated by H_2O_2[77].

Transient receptor potential cation channels

Transient receptor channels type 4 from the vanilloid family (TRPV4) are mechanosensitive, non-selective cation channels, which regulate Ca^{2+}-sparks in vascular smooth muscle cells[53] and there are data extant linking activation of TRPV4 channels to regulation of vasomotor tone[47]. Our findings demonstrate that selective blocking of TRPV4 channels inhibits H_2O_2-induced vasodilation (Figure 6.) and restores myogenic responses of cerebral arteries isolated from rats with TBI (Figure 6.). Further, dilations of cerebral arteries evoked by a TRPV4 agonist are abolished by a BK_{Ca} channel blocker (Figure 6.). These results support the concept that in TBI increased H_2O_2 levels activate BKCa channels via a pathway that involves activation of TRPV4 channels in the smooth muscle cells. Direct experimental support for this concept is offered by our findings that H_2O_2-induced increases in BK_{Ca} currents in VSMCs are diminished by the TRPV4 blocker HC067047 (Figure 7.).

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Disruption of the blood-brain barrier

As a distal injury in the cerebral circulation after traumatic brain injury we demonstrated that even mild TBI is sufficient to induce persistent disruption of the blood-brain barrier when pre-existing arterial hypertension is present, which is associated with the development of cognitive dysfunction (Figure 8-9). These findings extend previous results that mild TBI- induced BBB disruption reaches a peak within hours and after 2 to 3 days post-injury[37-39], and have an important translational aspect: hypertensive patients should probably be assessed and treated after mild brain trauma differently than normotensive subjects. Our results also raise the possibility, that other co-morbidities known to increase the vulnerability of the cerebral vessels to various insults, such as diabetes or obesity [78] would also exacerbate BBB disruption following mTBI and lead to cognitive decline. These possibilities should be verified by future studies.

The mechanisms by which hypertension and mild TBI interact to promote persistent disruption of the BBB are likely multifaceted. Both TBI and hypertension have been shown to induce overproduction of reactive oxygen species[79-81]. Although mild TBI results in a transient increase in ROS generation[82], co-existing hypertension may exaggerate and extend production of ROS in the cerebrovasculature. ROS can directly damage tight junction proteins of the BBB[83], and activate redox sensitive matrix metalloproteinases [84], resulting in increased permeability of the barrier. Other proteases, like caspase, known to be activated in hypertension[85,86], may contribute to the process. These possibilities should be tested by future studies.

TBI and neuroinflammation

We found that mild TBI leads to accumulation of fibrin in cerebral tissue of hypertensive rats (Figure 8). Previous studies suggested that the link between BBB disruption and neurological dysfunction is the accumulation of toxic blood borne substances in the brain parenchyma. For instance, in mice deficient of pericytes the injured BBB allows the deposition of fibrinogen in brain parenchyma [87], which is associated with activation of the monocyte/macrophage system [88], the increasing number of microglia and the increased

production of ROS [89], further activation of proteases and production of inflammatory cytokines. According to this pathological process, we also found that two weeks after mild TBI production of inflammatory cytokines IL-1β, IL-6 and TNFα is significantly enhanced in the cortices and hippocampi of hypertensive rats compared to normotensive animals (Figure 9.), indicating mTBI-induced persistent neuroinflammation in SHRs. Inflammatory cytokines further promote BBB damage [90], probably producing a positive feedback loop and perpetuating the pathological process. There is most likely a causal link between mild TBI-induced persistent neuroinflammation and cognitive deficit (Figure 9). This supported by studies showing that in the mouse hippocampus chronic neuroinflammation structurally modifies axons and dendrites of neurons and dysregulates genes involved in regulation of neuronal function (such as Bdnf, Homer1, and Dlg4) [91,92], which may have a role in impaired synaptic plasticity and impaired cognitive function [91].

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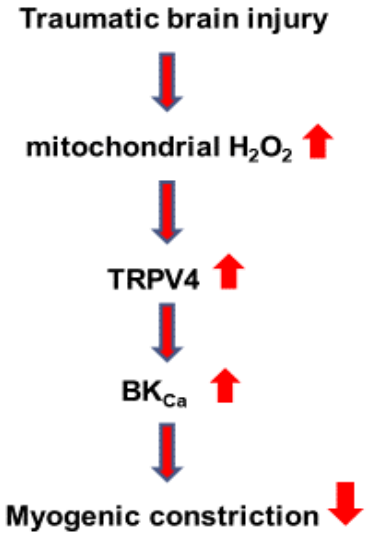
V. Summary

Diffuse brain trauma leads to excessive production of mitochondria-derived H2O2, which dampens myogenic constriction of cerebral arteries by a mechanism that involves TRPV4-dependent activation of BKCa channels.



In hypertensive rats mild brain trauma is sufficient to cause a persistent disruption of the blood-brain barrier, which is associated with accumulation of toxic blood borne substances in the brain parenchyma, neuroinflammation and cognitive decline of the animals. We propose that hypertensive patients with mild TBI should be assessed differently than normotensive patients (by quantifying BBB function, cognitive function etc.), and the mechanisms by which hypertension and mild TBI interact should be established in order to selectively target BBB function and achieve neuroprotection in this patient population.

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VI. Novel findings: conclusions of the thesis and clinical implications

- **Diffuse brain trauma leads to excessive production of mitochondria-derived cerebrovascular H_2O_2, which reduces myogenic constriction of cerebral arteries.**

- **H_2O_2 – induced attenuation of myogenic constriction of cerebral arteries involves TRPV4-dependent activation of BK_{Ca} channels.**

We propose that this pathway may contribute to autoregulatory dysfunction in TBI patients and could be targeted pharmacologically in order to restore autoregulation of cerebral blood flow and prevent the development of secondary brain injuries.

- **In hypertensive rats mild brain trauma is sufficient to cause a persistent disruption of the blood-brain barrier.**

- **Persistent BBB disruption in hypertensive rats following mild brain trauma leads to accumulation of toxic blood borne substances in the brain parenchyma, which is associated with neuroinflammation and cognitive decline of the animals.**

We propose that hypertensive patients with mild TBI should be assessed differently than normotensive patients (by quantifying BBB function, cognitive function etc.), and the mechanisms by which hypertension and mild TBI interact should be established in order to selectively target BBB function and achieve neuroprotection in this patient population.

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X. Peer-reviewed publications of the author (IF: 22,999)

- The thesis is based on the following publications:

1. Szarka N, Pabbidi MR, Amrein K, Czeiter E, Buki A, Koller A, Toth P. Traumatic brain injury impairs myogenic constriction of cerebral arteries: role of mitochondria-derived H2O2 and TRPV4-dependent activation of BKCa channels. J Neurotrauma. 2018. 1;35(7):930-939.

2. Szarka N, Toth L, Czigler A, Kellermayer Z, Ungvari Z, Amrein K, Czeiter E, Bali Zs, Tadepalli A, Wahr M, Hernadi I, Koller A, Buki A, Toth P. Single mild traumatic brain injury induces persistent disruption of the blood-brain barrier, neuroinflammation and cognitive decline in hypertensive rats. Int J Mol Sci. 2019 30;20(13):3223.

- Other publications:

3. Toth P, **Szarka N**, Farkas E, Ezer E, Czeiter E, Amrein K, Ungvari Z, Hartings JA, Buki A, Koller A Traumatic brain injury-induced autoregulatory dysfunction and spreading depression-related neurovascular uncoupling: Pathomechanisms, perspectives, and therapeutic implications. Am J Physiol Heart Circ Physiol. 2016 1;311(5):H1118-H1131.

4. **Szarka N,** Amrein K, Horvath P, Ivic I, Czeiter E, Buki A, Koller A, Toth P. Hypertension-induced enhanced myogenic constriction of cerebral arteries is preserved after traumatic brain injury. J Neurotrauma. 2017 15;34(14):2315-2319.

5. Czigler A, Toth L, **Szarka N**, Szilágyi K, Kellermayer Z, Harcid A, Vecsernyes M, Ungvari Z, Szolics A, Koller A, Buki A, Toth P. Prostaglandin E2, a postulated mediator of neurovascular coupling, at low concentrations dilates whereas at higher concentrations constricts human cerebral parenchymal arterioles. Prostaglandins and Other Lipid Mediators 2020 146:106389.

6. Czigler A, Toth L, **Szarka N**, Berta G, Amrein K, Czeiter E, Lendvai-Emmert D, Bodo K, Tarantini S, Koller A, Ungvari Z, Buki A, Toth P. Hypertension exacerbates cerebrovascular oxidative stress induced by mild traumatic brain injury: protective effects of the

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8. **Szarka N**, Oberling J, Vegh M, Nagy L. Patient satisfaction with care in gastrooesophageal reflux disease. Orv Hetil. 2013 27;154(43):1713-8.

- Abstract of oral and poster presentations

1. Deak F.*, Logan S.*, **Szarka N**.*, Orock A., Giles C., Mitschelin CM., Wren J., Koller A., Sonntag EW. Novel model of age-related cognitive impairment and molecular mechanism of synaptic failure. Poster presentation (Joint meeting of FEPS and the Hungarian Physiological Society, Budapest, 2014)

2. Orock A., Logan S., **Szarka N**., Deák F. Munc18-1 mediates age dependent synaptic dysfunction. Poster presentation (Meeting of the American Aging Association, San Antonio, TX 2014)

3. Logan S., Landoll J., Orock A., **Szarka N**., Sonntag EW., Deák F. Age-related decline in synaptic function is rescued by IGF-1 treatment. Poster presentation (Meeting of the American Aging Association, San Antonio, TX 014)

4. **Szarka N,** Toth P, Koller A. Hypertension enhances flow-induced constriction of rat cerebral arteries, which is lost prior to stroke. Oral presentation (Meeting of the European Research Council for Cardiovascular Research, Garda, Italy 2014)

5. **Szarka N,** Toth P, Koller A. Flow-induced constriction of cerebral arteries in hypertension: a protective mechanism against stroke? (FASEB J. 2014)

6. **Szarka N,** Pabbidi MR, Koller A, Toth P. Traumatic brain injury impairs myogenic constriction of rat cerebral arteries by mitochondria-derived H2O2-induced, TRPV4

dependent activation of BKCa channels Poster presentation (Vascular remodelling in biology and medicine, Fribourg, Switzerland 2016.)

7. **Szarka N,** Amrein K, Czeiter E, Buki A Koller A, Toth P. Enhanced myogenic response of cerebrak arteries induced by pre-exesting hypertension is intact after traumatic brain injury Poster presentation (Experimental biology, Chicago, USA, 2017)

8. **Szarka N.** Mechanism of Cerebral Myogenic Dysfunction Following Traumatic Brain Injury. Oral presentation (7th Pannonian Symposium on Central Nervous System Injury 2017)

9. Szénási A, **Szarka N**, Amrein K, Tóth P, Koller Á. A traumás agysérülés a konstriktor 20-HETE termelés csökkentése révén, gyengíti a nyomás és áramlás-indukált konstriktor mechanizmusokat. (FAMÉ 2019.Budapest Semmelweis University)