Neurobiology of Disease 91 (2016) 155–165

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Synaptic plasticity in area CA1 of rat hippocampal slices following intraventricular application of albumin

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ARTICLE INFO

Article history:
Received 16 December 2015
Revised 16 February 2016
Accepted 9 March 2016
Available online 10 March 2016

Keywords:
Blood-brain barrier
Albumin
Synaptic plasticity
Hippocampus
Hyperexcitability
Epilepsy

ABSTRACT

Epileptogenesis following insults to the brain may be triggered by a dysfunctional blood-brain barrier (BBB) associated with albumin extravasation and activation of astrocytes. Using *ex vivo* recordings from the BBB-disrupted hippocampus after neocortical photothrombotic stroke, we previously demonstrated abnormal activity-dependent accumulation of extracellular potassium with facilitated generation of seizure like events and spreading depolarizations. Similar changes could be observed after intracerebroventricular (icv) application of albumin. We hypothesized that alterations in extracellular potassium and glutamate homeostasis might lead to alterations in synaptic interactions. We therefore assessed the effects of icv albumin on homo- and heterosynaptic plasticity in hippocampal CA1, 24 h after a single injection or 7 days after continuous infusion of icv albumin. We demonstrate alterations in both homo- and heterosynaptic plasticity compared to control conditions in *ex vivo* slice studies. Albumin-treated tissue reveals (1) reduced long-term depression following low-frequency stimulation; (2) increased long-term potentiation of population spikes in response to 20 Hz stimulation; (3) potentiated responses to Schaffer collateral stimulation following high-frequency stimulation of the direct cortical input and low-frequency stimulation of alveus and finally, (4) TGFβ receptor II (TGFβR-II) involvement in albumin-induced homosynaptic plasticity changes. We conclude that albumin-induced network hyperexcitability is associated with abnormal homo- and heterosynaptic plasticity that could partly be reversed by interference with TGFβR-II-mediated signaling and therefore it might be an important factor in the process of epileptogenesis.

1. Introduction

Dysfunction of the blood-brain barrier (BBB) is a shared finding of common neurological disorders such as tumors, stroke, neurodegenerative, infectious and inflammatory diseases (Zlokovic BV, 2008; Shlosberg et al., 2010; Schoknecht and Shalev 2012; Abbott and Friedman 2012). In recent years, a growing number of studies have focused on the direct role of the dysfunctional BBB in disease pathophysiology and particularly in epilepsy (Seiffert et al., 2004; van Vliet et al., 2007; for review, Friedman 2009). Blood-brain barrier dysfunction after hyperosmotic mannitol application was long before demonstrated to induce paroxysmal changes in the EEG of rats and later in the pig (Fieschi et al., 1980; Marchi et al., 2007). More recent experiments revealed that disruption of BBB, for example by bile salts, led to delayed epileptogenesis (Seiffert et al., 2004; Tomkins et al., 2007). Damage to BBB was shown to result in extravasation of serum albumin (as well as other proteins) into the brain and to be associated with vasogenic edema (Klatzo et al. 1980; Kuroiwa et al., 1985; Wahl et al., 1988). Interestingly, the uptake of albumin by astrocytes was thought to be a

Abbreviations: ACSF, artificial cerebrospinal fluid; Ab, antibody; BBB, blood-brain barrier; BCM, Bienenstock, Cooper and Munro; dCI, direct cortical input; ES coupling, field excitatory postsynaptic potential-spike coupling; fEPSP, field excitatory postsynaptic potential; HFS, high frequency stimulation; icv, intracerebroventricular; IO, input-output; LFS, low frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; ISI, inter-stimulus interval; SC, Schaffer collaterals; SD, spreading depolarization; si, single injection; SLE, seizure-like event; SO/A, stratum oriens/alveus; SP, stratum pyramidale; SR, stratum radiatum; PPI, pair pulse index; PS, population spike; TGFβR, transforming growth factor beta receptor.

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Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2016.03.008
0969-9961/

compensation mechanism for osmotic disturbances (Ivens et al., 2007; Raabe et al., 2012). In addition, the uptake of albumin into astrocytes was found to be mediated by transforming growth factor beta receptors (TGFβRs) (Ivens et al., 2007), and that the binding of albumin to TGFβ receptors was shown to activate TGFβsignaling. This, in turn, was associated with robust changes in gene expression (Cacheaux et al., 2009; David et al., 2009), including downregulation of astrocytic inward rectifier potassium channels (K_{ir} 4.1), glutamate transporters and gap junction connexins 30 and 43 pointing to alterations in the functions of astrocytes (for review see Kovács et al., 2012; Heinemann et al., 2012). Activation of TGFβsignaling and consecutive hyperexcitability could partially be antagonized by application of TGFβR-II antibodies (Ab) (Ivens et al., 2007) and be prevented in rats by the application of Losartan, a blocker of the ALK5 pathway involved in TGFβ dependent signaling (Bar-Klein et al., 2014) as well as in mice by the specific TGFβsignaling blocker, SJN2511 (Weissberg et al., 2015). Direct activation of the TGFβ signaling pathway has comparable effects to BBB opening by bile salts and to albumin-induced activation of astrocytes (Cacheaux et al., 2009). Additional recent experiments have shown that, a single injection of albumin into the ventricles led to increased susceptibility to spreading depolarizations (SDs) in rats (Lapilover et al., 2012) and prolonged injection of albumin for 7 days was associated with excitatory synaptogenesis and spontaneous seizures in mice (Weissberg et al., 2011 and 2015).

Blood vessels also display increased permeability in acute ischemic stroke, whose ischemic core is surrounded by a peri-ischemic region (often referred to as penumbra). This peri-ischemic region shows a complex and dynamic progression of injury, as recently shown in the photothrombotic stroke model in rodents (Schoknecht et al., 2014). While it is increasingly recognized that the dysfunctional BBB is associated with complex changes within the neurovascular unit, the critical mechanisms that determine the destiny of a particular affected brain region are still not known. We recently confirmed (Lapilover et al., 2012) a report by Stoll et al., 2009 that there is opening of the BBB in the hippocampus following a photothrombotic stroke with intact calvarium. This was surprising since hippocampal blood supply in rats is independent of the neocortex (Coyle 1976). *Ex vivo* recordings from such slices revealed abnormal potassium accumulation during repetitive neuronal activation and facilitated generation of seizure like events (SLE) and SDs. This effect could be partly mimicked by intracerebroventricular (icv) application of albumin (Lapilover et al., 2012). Such alterations would not only lower the SLE threshold acutely but they may also have effects on synaptic coupling and plasticity (David et al., 2009; Weissberg et al., 2015). In fact, following activation of astrocytes via albumin, tripartite glia-neuronal synapses may no longer function properly (for review of astrocytic regulation of synaptic transmission see Araque et al., 1999). Loss of this shielding function is expected to affect synaptic plasticity in different ways and ultimately facilitate lasting

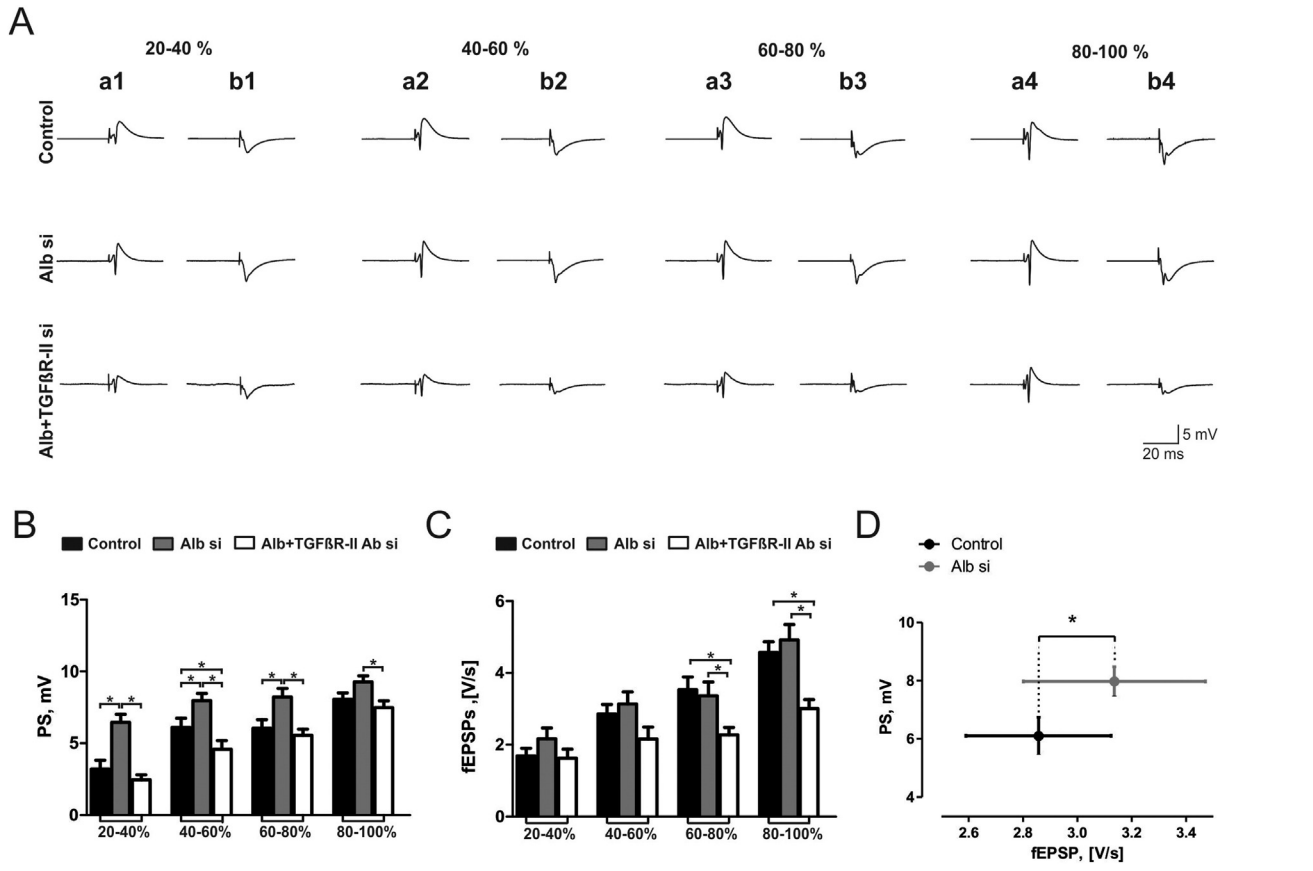

Fig. 1. Changes in input-output interactions and ES coupling after single icv albumin and combined albumin and TGFβR-II Ab application. (A) Sample PS (a1–4) and fEPSP (b1–4) traces corresponding to different stimulus intensities (% of the maximum) in slices from control, albumin and albumin + TGFβR-II Ab-treated animals are shown. (B) Albumin application results in increase in the PS amplitudes when 20 to 80% of maximum stimulus intensities are used to elicit PSs ($control_{20–40\%}$ vs $albumin_{20–40}$, $^*p = 0.001$; $control_{40–60\%}$ vs $albumin_{40–60\%}$, $^*p = 0.026$; $control_{60–80\%}$ vs $albumin_{60–80\%}$, $^*p = 0.03$). Combined albumin and TGFβR-II treatment reverses the changes induced by albumin application (albumin + TGFβR-II $Ab_{20–40\%}$, $^*p = 0.03$; albumin + TGFβR-II Ab $_{40–60\%}$, $^*p < 0.001$ and vs controls $^*p = 0.035$); albumin + TGFβR-II Ab $_{60–80\%}$, $^*p = 0.001$; albumin + TGFβR-II Ab $_{80–100\%}$, $^*p = 0.007$). (C) Albumin application has no effect on fEPSP slopes in tested stimulus intensities. However, combined albumin and TGFβR-II Ab application decreases fEPSP slopes at 60–100% of the maximum stimulus intensities compared to both controls (albumin + TGFβR-II Ab $_{60–80\%}$, $^*p = 0.02$ and albumin + TGFβR-II Ab $_{80–100\%}$, $^*p < 0.001$) and albumin-treated (albumin + TGFβR-II Ab $_{60–80\%}$, $^*p = 0.024$ and albumin + TGFβR-II Ab $_{80–100\%}$, $^*p < 0.001$) animals. (D) ES coupling increases after albumin application compared to controls. PS amplitudes ($^*p = 0.026$) but not fEPSP slopes increase after albumin application. Only responses obtained by 40–60% of the maximum stimulus intensity are shown here. All comparisons are carried out by Kruskal-Wallis test.