Sodium selenate retards epileptogenesis in acquired epilepsy models reversing changes in protein phosphatase 2A and hyperphosphorylated tau

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There are no treatments in clinical practice known to mitigate the neurobiological processes that convert a healthy brain into an epileptic one, a phenomenon known as epileptogenesis. Downregulation of protein phosphatase 2A, a protein that causes the hyperphosphorylation of tau, is implicated in neurodegenerative diseases commonly associated with epilepsy, such as Alzheimer’s disease and traumatic brain injury. Here we used the protein phosphatase 2A activator sodium selenate to investigate the role of protein phosphatase 2A in three different rat models of epileptogenesis: amygdala kindling, post-kainic acid status epilepticus, and post-traumatic epilepsy. Protein phosphatase 2A activity was decreased, and tau phosphorylation increased, in epileptogenic brain regions in all three models. Continuous sodium selenate treatment mitigated epileptogenesis and prevented the biochemical abnormalities, effects which persisted after drug withdrawal. Our studies indicate that limbic epileptogenesis is associated with downregulation of protein phosphatase 2A and the hyperphosphorylation of tau, and that targeting this mechanism with sodium selenate is a potential anti-epileptogenic therapy.

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Introduction

Epilepsy is a common and disabling group of neurological conditions characterized by an enduring tendency of the brain to generate spontaneous seizures (Fisher et al., 2005). Current interventions for epilepsy primarily consist of symptomatic treatment with anti-epileptic drugs that merely suppress seizures. However, anti-epileptic drugs are ineffective in many epilepsy patients and have not been demonstrated to mitigate epileptogenesis—the neurological processes that convert a healthy brain into an epileptic brain. Therefore, new therapeutic strategies that target the underlying mechanisms of epileptogenesis are a major goal of translational research in this field (Fisher et al., 2005; Galanopoulou et al., 2012; Simonato et al., 2014).

Tau proteins play an important role in stabilizing microtubules in neurons in the CNS. Hyperphosphorylated tau (p-tau) dissociates from microtubules and can result in the destabilization of microtubules and formation of neurofibrillary tangles, which may induce dysfunction and death of neurons (Ittner and Gotz, 2010). Abnormal expression of p-tau has been observed in a number of neurodegenerative diseases that are commonly associated with epilepsy, in particular Alzheimer’s disease, traumatic brain injury and focal cortical dysplasia (Ittner and Gotz, 2010). A recent post-mortem analysis of the brains of patients with long-term, mainly drug-resistant, epilepsy found an increase in p-tau and neurofibrillary tangles (Thom et al., 2011). Neurofibrillary tangles have also been identified in the brains of patients with drug resistant epilepsy and focal cortical dysplasia (Sen et al., 2007) or traumatic brain injury (Thom et al., 2011). Furthermore, our previous research found that p-tau expression is increased in the kindling model of epilepsy (Jones et al., 2012), and in a rat model of traumatic brain injury where a proportion of rats develop post-traumatic epilepsy (Shultz et al., 2015). Together, these results suggest that p-tau could be involved in epileptogenesis, and therefore represent a potential target for disease-modifying therapies by removal of p-tau (Iqbal et al., 2009). In particular, the PP2A 55 kDa regulatory B subunit (PR55) is associated with a catalytic subunit (PP2Ac) and is essential for PP2A to dephosphorylate p-tau (Xu et al., 2008). Furthermore, the downregulation of PP2A activity promotes an increase in p-tau, and PP2A activity and PR55 levels are decreased in tauopathies such as Alzheimer’s disease and traumatic brain injury (Sontag et al., 2008; Bolognin et al., 2011). These data infer that downregulation of PP2A activity and PR55 expression could promote the accumulation of p-tau as has been observed in epileptogenesis.

Sodium selenate, an oxidized, less toxic form of selenium, has been identified to specifically activate PP2A containing the PR55 regulatory subunit, and to decrease the level of p-tau (Corcoran et al., 2010; van Eersel et al., 2010). This effect is not observed with other types of selenium salts. In support of a role for p-tau in epilepsy, sodium selenate attenuates seizures in rodent models (Jones et al., 2012). Conversely, okadaic acid, a PP2A inhibitor, can induce seizures in rodents (Arias et al., 2002; Ramirez-Munguia et al., 2003). However a critical unanswered question is whether downregulation of PP2A activity could promote the process of epileptogenesis, and whether stimulation of PP2A activity has anti-epileptogenic effects. To investigate this we used three complementary well-validated rat models of limbic (temporal lobe) epileptogenesis: amygdala kindling, post-kainic acid status epilepticus and the post-fluid percussion injury (FPI) model of post-traumatic epilepsy. In each of these models we found that PP2A activity and PR55 levels were significantly reduced in limbic brain regions, and that this loss of PP2A activity was associated with increases in p-tau. Sodium selenate treatment during the epileptogenic period (4 weeks for amygdala kindling, 8 weeks for post-kainic acid status epilepticus and 12 weeks for FPI) attenuated the decrease in PP2A activity and PR55 levels, reduced p-tau, and mitigated brain damage in the models. Importantly, rats treated with sodium selenate had fewer and shorter epileptic seizures during treatment, and this effect was sustained after washout of the sodium selenate treatment. Taken together, these findings indicate that PP2A activity is downregulated in epileptogenesis and that modulation of PR55 forms of PP2A activity, via...

Abbreviations: FPI = fluid percussion injury; PP2A = protein phosphatase 2A; PR55 = PP2A 55 kDa regulatory B subunit

Keywords: epilepsy; animal model; MRI; traumatic brain injury; PR55
treatment with sodium selenate, may be a novel and effective anti-epileptogenic intervention.

Materials and methods

Reagents and antibodies

The rabbit polyclonal antibodies pS198 and pS262, which recognized phospho-tau at Ser198 and Ser262, respectively were purchased from Epitomics. The mouse monoclonal antibody Tau-5, which recognized total tau, was purchased from BD Biosciences. The mouse monoclonal anti-PP2Ac and PR55 were purchased from Millipore. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control and recognized by rabbit monoclonal anti-GAPDH antibody, which was purchased from Cell Signaling Technology. Bicinoninic acid protein assay kit (BCA kit) was purchase from Pierce Biotechnology. PP2A immunoprecipitation phos- phatase assay kit was purchased from Millipore. Enhanced chemiluminescence detection kit was purchased from GE healthcare. Sodium selenate was purchased from Sigma-Aldrich. General chemicals, such as sodium deoxycholate (DOC), sodium dodecyl sulphate (SDS) and IGEPAL CA-630 (NP-40), were purchased from Sigma-Aldrich. Osmotic mini-pumps were purchased from DURECT (ALZET® models 2004 and 2006).

Experimental animals

Adult male Wistar rats were used in kindling and post-kainic acid status epilepticus experiments. Adult male Long-Evans rats were used in the FPI experiment. These rats were obtained from our breeding colony in the Department of Medicine (RMH), University of Melbourne, individually housed and maintained on 12-h light/dark cycles with food and water available ad libitum. All animal experiments were approved by the Animal Ethics Committees of The University of Melbourne, and were performed in accordance with the guidelines set by the Australian NHMRC Code of Practice for the Care and Use of Animals for Scientific Purpose.

Amygdala kindling surgery and implantation of osmotic mini-pumps

All rats in the amygdala kindling experiments received surgical implantation of stimulating and recording electrodes at 10 weeks of age, as described previously (Jupp et al., 2005; Powell et al., 2008). Under isoflurane general anaesthesia, a stainless steel bipolar electrode (Plastics One) for stimulation was stereotactically implanted into the left basolateral amygdala nucleus (3.0 mm posterior, 5.0 mm lateral from bregma, and 6.5 mm ventral from dura). In addition, three extradural electrodes were implanted bilaterally for recording EEG (two electrodes at 2.0 mm anterior and 2.0 mm lateral, and one electrode 2.0 mm postural and right lateral, to bregma). All electrodes were fixed to the skull by dental acrylic cement. In the same surgery session, ALZET® osmotic mini-pumps were implanted subcutaneously at the shoulder. These pumps were filled with the appropriate sodium selenate (Na2SeO4) or sodium chloride (NaCl) solution to continuously release drugs, beginning immediately, at a dose of 1 mg/kg/day for 4 weeks (Supplementary Fig. 1). The pumps were filled by a different operator and the animals coded so that the researcher was blinded to the treatment group until the completion of all experiments and analyses. The treatments (i.e. selenate versus saline) were randomly allocated to the animals in a 1:1 ratio. It was confirmed that the osmotic pumps were working and had delivered the appropriate amount of drug by measuring the residual fluid in the pump after explanation. Rats were allowed 7 days to recover from surgery before amygdala kindling.

Amygdala kindling

Amygdala kindling commenced on Day 8 post-surgery. The bipolar electrode was electrically stimulated with a 1-s train of 1 ms biphasic square wave pulses at a frequency of 60 Hz. The after discharge threshold (ADT) was determined at the first stimulation. Then, rats were stimulated at ADT current intensity twice daily, 5 days per week for 3 weeks (30 stimulations in total). The EEG was recorded by Labchart 7.0 software (ADInstruments Pty Ltd). The total and primary after discharge duration were measured from the EEG trace (by a reviewer blinded to treatment). The behavioural progression of kindling-induced seizures was scored according to the Racine classification: class I, facial clonus; class II, chewing and head nodding; class III, contralateral forelimb; class IV, rearing and bilateral; class V, rearing and loss of balance. The total and primary after discharge duration and Racine class of each seizure were expressed as a mean per three stimulations. Sham rats underwent identical surgery and handling, but did not receive any stimulations.

Induction of status epilepticus, implantation of osmotic mini-pumps and video-EEG recordings and analysis

Rats of 12 weeks of age were injected with repeated low doses of kainic acid (5 mg/kg, i.p., followed by 2.5 mg/kg, i.p., injections once per hour) until status epilepticus behaviour was observed, and other rats underwent saline treatment as ‘sham’ controls (Jupp et al., 2012). After 4 h of status epilepticus, all rats were given diazepam injection (5 mg/kg i.p.) to terminate the status epilepticus. The osmotic mini-pump filled with sodium selenate or sodium chloride was implanted subcutaneously at the shoulder after the diazepam injection in all rats, and released drug at a dose of 1 mg/kg/day. After 6 weeks post status epilepticus, all rats were placed under general anaesthetic and underwent surgical implantation of four electrodes on the skull to record EEG (2 × 2.0 mm anterior and lateral, and 2 × 2.0 mm postural and lateral, to bregma). At this time, the osmotic pumps were replaced with fresh pumps filled with the same drugs. Rats were then left to recover for 1 week before 2 weeks of continuous video-EEG monitoring (Compumedics) as previously described (Powell et al., 2008; Jupp et al., 2012; Shultz et al., 2013). After this period, the pumps were removed and a 2-week washout period occurred, which was followed by another 2-week video-EEG recording.
(see Supplementary Fig. 1 for experimental timeline). A reviewer blinded to treatment group used Compumedics software to review the video-EEG recordings to determine the number of seizures recorded in each rat and the duration of each seizure (Powell et al., 2008; Jupp et al., 2012; Shultz et al., 2013). The criteria for determining that a recorded event was a seizure was: high-amplitude, rhythmic discharges that represented a clearly new pattern of tracing, including repetitive spikes, spike-and-wave discharges, and slow waves, that had a duration of at least 5 s and showed an evolution in the dominant frequency (Kharatishvili et al., 2006, 2007; Shultz et al., 2013).

It is recognized that following the initial diazepam injections to terminate the status epilepticus, rats can experience more seizures and even relapse back into status epilepticus over the next 24–48 h. This could potentially result in a more severe epileptogenic insult and therefore a more severe long term epileptic condition. Therefore, there is the potential that if selenate modified these early post status epilepticus seizures that this could confound the assessment of the effect of the selenate treatment on the long term epileptic state. To assess this, we studied a cohort of male Wistar rats at 12 weeks of age that had EEG electrodes implanted. After recovery, video-EEG was commenced and 3 days later, these animals underwent kainic acid-induced status epilepticus. After 4 h status epilepticus, animals were injected with diazepam, and then subcutaneously implanted with an osmotic minipump filled with either sodium selenate of saline control (n = 7/group). The video-EEG was continued for the next 3 days, and analysed. We then compared the number of rats that had recurrent seizures and recurrence of status epileptics following the initial diazepam injection, the average number of seizures, and the total time in seizure activity between treatment groups.

**Induction of post-traumatic epilepsy, implantation of osmotic mini-pumps and video-EEG recordings and analysis**

To investigate the anti-epileptogenic potential of sodium selenate in a model of post-traumatic epilepsy, 12-week-old male Long-Evans rats were administered a lateral FPI as previously described (Shultz et al., 2013). Briefly, under anaesthesia a 5-mm craniotomy, positioned 4-mm right lateral and 4-mm posterior to bregma, was performed to create a circular window exposing the intact dura mater of the brain. A modified female Luer-Lock cap was secured over the craniotomy window by dental acrylic. The rat was then removed from anaesthesia and attached to the fluid percussion device via the Luer-Lock. Once the rat responded to a toe pinch, a severe-intensity (320–350 kPa) fluid pulse of silicone oil generated by the fluid percussion device was delivered to the brain. Rats were resuscitated with pure oxygen post-injury if required. On resumption of spontaneous breathing, and return to pre-FPI levels of heart rate and oxygenation status, the dental acrylic caps were removed and the wound sutured closed.

This injury results in post-traumatic epilepsy in 30–50% of rats (Kharatishvili et al., 2006, 2007; Shultz et al., 2013). Following injury, rats were re-anaesthetized and implanted with osmotic pumps to deliver sodium selenate or vehicle as described above for 12 weeks (pumps were replaced after 6 weeks). Nine weeks after FPI, all the rats were placed under general anaesthetic and underwent surgical implantation of four electrodes on the skull to record EEG as described above for the post-kainic acid status epilepticus rats (2 × 2.0 mm anterior and lateral, 1 × 2.0 mm postural and right lateral, and 1 × 6.0 mm postural and right lateral, bregma). Rats were then left to recover for 1 week before 2 weeks of continuous video-EEG monitoring, as described above for the post status epilepticus rats. After this period, the pumps were removed and a 2-week washout period occurred, which was followed by another 2-week video-EEG recording (see Supplementary Fig. 1 for experimental timeline). A reviewer blinded to treatment used Compumedics software to review the video-EEG recordings to determine the number of seizures recorded in each rat and the duration of each seizure (Powell et al., 2008; Jupp et al., 2012; Shultz et al., 2013).

To investigate whether the FPI induced acute seizures post-injury, and if so whether this was modified by the selenate infusions, a separate cohort of 12-week-old male rats were implanted with EEG electrodes 7 days prior to the FPI. Treatment was initiated immediately after injury, and continuous EEG recordings acquired for 3 days (n = 6 sodium selenate and n = 6 saline). The video-EEG files were then assessed in a blinded fashion for the occurrence of acute post-traumatic seizures, the frequency and duration of which were compared between treatment groups.

**Assessment of sodium selenate treatment on animal health and behaviour**

The effect of the sodium selenate infusions on the health and behaviour of the animals was assessed using a standardized neurotoxicity scale (0–4) that we have used in our previous preclinical studies of drug effects (Tringham et al., 2012; Casillas-Espinosa et al., 2015), where a score of 0 indicates no sedation, normal movement; a score of 1 is for slight sedation, slow movement but alert when startled; a score of 2 is for mildly sedated, struggles when restrained; a score of 3 shows a sedated animal that is not moving in cage, but does respond to provocation; and, the highest score of 4 indicates an animal that is very sedate, catatonic and unable to stand when provoked. This was performed at least weekly for the duration of the drug infusion periods, along with weighing the animal, by an observer blinded to the nature of the treatment the animal was receiving.

**MRI acquisition**

Five weeks post status epilepticus, in vivo MRI scanning was performed using a 4.7 T Bruker Avance III scanner with 30 cm horizontal bore and fitted with a BGA12S2 gradient set and actively decoupled volume transmit and 4-channel surface receive coils. Anaesthetized rats were positioned supinely on a cradle with stereotactic fixation and a nose cone positioned over the rat’s snout to maintain anaesthesia. Body temperature was maintained throughout the experiment with a hot water circulation system built into the cradle.

The scanning protocol consisted of a 3-plane localizer sequence followed by multi-slice axial, coronal and sagittal...
scout images to accurately determine the position of the rat brain. A T2-weighted image was acquired using a 2D rapid acquisition with relaxation enhancement (RARE) sequence (Higuchi et al., 1992) with the following imaging parameters: recovery time = 3000 ms, RARE factor = 8, effective echo time = 50 ms, field of view = 25.6 × 25.6 mm², matrix size = 320 × 320, number of slices = 24, slice thickness = 600 μm and number of excitations = 12.

Diffusion-weighted imaging was performed using a 2D echo planar sequence (Stejskal and Tanner, 1965) with the following imaging parameters: repetition time = 3000 ms, echo time = 48 ms, field of view = 25.6 × 25.6 mm², matrix size = 160 × 160, number of slices = 12 and slice thickness = 600 μm. Diffusion-weighted imaging was performed with diffusion duration (Δ) = 4 ms, diffusion gradient separation (δ) = 11 ms and b-value = 1200 s/mm² in 30 non-collinear directions with five non-diffusion images.

Point resolved spectroscopy was acquired with VAPOR water suppression and outer volume saturation. Other parameters were: repetition time = 2500 ms, echo time = 20 ms, number of excitations = 256, spectral width = 6 ppm, number of points = 2048 and voxel size = 2 × 9 × 4 mm³.

**MRI analysis**

Volumetric analysis of brain structures followed procedures previously described (Shultz et al., 2013, 2014). Briefly, T2-weighted MRI volumes of the cortex, hippocampus, corpus callosum, and lateral ventricles from each hemisphere were quantified with manually drawn regions of interest using FSL (Analysis Group, Oxford, UK). Regions of interest were drawn on consecutive axial MRI slices by an investigator blinded to experimental conditions. Fractional anisotropy measures within each region of interest were calculated using FSL’s FDT software. Magnetic resonance spectroscopy (MRS) data were processed using LCModel (Provencher, 2001), and regions of interest in MRS analysis contained only bilateral hippocampus. The MRS region of interest was 2 × 9 × 4 mm³ (height × width × length) and encompassed the dorsal hippocampi. The region of interest was centred along the midline and the most dorsal aspect of the region of interest was aligned with the most dorsal aspect of the hippocampi. The length of the region of interest spanned from −2.0 to −6.0 posterior relative to bregma. N-acetyl aspartate and myo-inositol metabolite concentrations were expressed as a ratio to creatine.

**Tissue collection and processing**

Twenty-four hours after the last kindling stimulation, or after the final video-EEG recording in the post status epilepticus model, rats were sacrificed with a lethal dose of pentobarbital. The brains were rapidly removed and split into two hemispheres on ice-cold artificial CSF: 125 mM NaCl, 3 mM KCl, 6 mM MgCl₂, 1 mM CaCl₂, 1.25 mM NaH₂PO₄, 25 mM NaHCO₃, 10.6 mM glucose. The left hemisphere was fixed with 4% paraformaldehyde for 48 h at 4°C and then sectioned for histological verification of the electrode position in the left basolateral amygdala for the amygdala kindling rats. The right hemisphere was micro-dissected to extract three regions: amygdala, hippocampus and cortex, which are highly relevant to kindled seizures. The blocks of tissue were rapidly frozen in liquid nitrogen and reserved at −80°C.

**Measurement of PP2A activity**

The PP2A activities of samples were measured with PP2A immunoprecipitation phosphatase assay. The frozen tissues were grind on dry ice, and dissolved in 20 mM imidazole-HCl, pH 7.0 with protease inhibitors cocktail (Begum and Ragolia, 1996). These brain lysates were centrifuged at 12 000g for 15 min at 4°C and the supernatants were used to assay phosphatase activity. After protein concentration of the supernatants was determined with BCA kits, 100 μg total protein of tissue was used to assay phosphatase activity. PP2A was immunoprecipitated by anti-PP2Ac, and the background was pulled down by mouse IgG in parallel samples. These immune complexes were pulled down by protein A agarose beads. The PP2A of these immune complexes were incubated with threonine phosphopeptide for 15 min at 30°C to release free phosphate, which was assayed with malachite green phosphate detection solution. The PP2A activities were calculated as pmol released free phosphate/min/mg protein, which were subtracted background, and expressed as relative of the enzymatic activities in sham-operation or sodium chloride treatment rats.

**Western blotting**

The frozen tissues were ground on dry ice and dissolved in RIPA buffer [50 mM Tris (pH 7.4), 150 mM NaCl, 0.1% sodium dodecyl sulphate (SDS), 0.5% sodium deoxycholate and 1% NP-40] with protease inhibitors cocktail and phosphatase inhibitors cocktail. The brain extracts were centrifuged at 12 000g for 15 min at 4°C and the supernatant was used for western blotting. After the protein concentration of supernatant was determined with a BCA kit, the supernatant was mixed with a sample buffer containing 300 mM Tris-HCl (pH 6.8), 300 mM dithiotreitol, 12% SDS, 0.6% bromophenol blue, and 60% glycerol [5:1 (v/v) ratio], boiled for 10 min at 95°C, then centrifuged at 12 000g for 10 min, and the supernatant was stored at −80°C for western blotting analysis. The proteins in samples were separated with SDS-PAGE, and the bands of proteins were electro-blotted onto polyvinyl difluoride (PVDF) membranes. The blots on PVDF membranes were respectively developed with pS198 (1:1000) and pS262 (1:1000). Then, these membranes were stripped and reprobed for Tau-5 (1:1000) and then GAPDH (1:10 000). To measure the expression of PP2A, we ran other western blots, in addition to the tau western blotting. We first blotted the PVDF membranes with anti-PR55 (1:1000) and then anti-PP2Ac (1:1000) after stripping. These membranes were stripped again and rebotted by anti-GAPDH (1:10000). All protein blots were visualized by enhanced chemiluminescent substrate kit and exposure to X-ray film. These blots were scanned and the mean intensity of the blots was quantified using NIH ImageJ software (Abramoff et al., 2004). The ratio of immunoreactivity associated with phospho-tau (pS198 and pS262) to total tau (Tau-5) and other proteins to loading control (GAPDH) was calculated and the results were expressed as relative of the average control value (sham operation, sodium chloride treatment as control).
**Statistical analyses and sample size determinations**

Statistical comparisons were performed using SPSS 20.0. Western blotting, PP2A activity, and the number and duration of seizures/day were analysed with independent-samples t-tests. The proportion of rats experiencing seizures were analysed using Fisher’s exact test. Repeated measures ANOVA were used to assess seizure duration and severity in the kindling experiments. MRI data were analysed using Kruskal-Wallis ANOVA and Dunn’s multiple comparison post hoc. For the rats in the post-kainic acid status epilepticus treatment study a Pearson’s correlation analysis was performed investigating for a relationship in individual animals between the molecular brain measures that were significantly affected by the selenate treatment and the primary epilepsy measures from the pre- and post-drug washout video-EEG recording. A P-value of 0.05 was used to determine statistical significance.

An a priori power calculation was not performed to select the sample size, but based on our previous experience and the capacity of the laboratory it was aimed to study 15 rats each in the selenate and vehicle treatment arms for the amygdala kindling and post-kainic acid status epilepticus experiments, and 30 rats per treatment arm in the post-FPI experiment (assuming that 50% of these animals would become epileptic after 12 weeks based on our previous experience) (Shultz et al., 2013). Using data from our previously published studies in the post-kainic acid status epilepticus rat model (Machnes et al., 2013) and the post-FPI rat model (Shultz et al., 2013), it was calculated using the Student t-test this group size would give a 60% power to detect a 50% decrease in the primary analysis endpoint of the number of seizures per day in the epileptic rats during a 2 week video-EEG recording with a type I error rate of 5%.

**Results**

**PP2A activity is downregulated in chronic acquired epilepsy rat models**

To investigate whether PP2A is affected in epileptogenesis, we measured the activity of PP2A using an immunoprecipitation phosphatase assay kit. We found that PP2A activity in the amygdala, hippocampus and cortex was significantly decreased in amygdala kindled (Fig. 2A) and post status epilepticus rats (Fig. 2C) relative to their sham controls. In previously published work we have demonstrated similar changes in PP2A activity in the injured cortex of post-FPI rats, with a 20–30% decrease compared to sham injured rats (Shultz et al., 2015).

**PP2A 55 kDa regulatory subunit B expression is decreased in chronic acquired epilepsy models**

To investigate the mechanisms underlying the inhibition of PP2A activity in epilepsy, we examined the expression levels of the PP2A catalytic subunit (PP2Ac) and the PP2A 55 kDa regulatory B subunit PR55 with western blotting. We found that amygdala kindled (Fig. 2B) and post status epilepticus (Fig. 2D) rats had significantly decreased ratios of PR55 immunoreactivity to GAPDH immunoreactivity in the amygdala, hippocampus and cortex. However, the ratio of PP2Ac to GAPDH in the amygdala was not significantly different from sham operated control rats (Shultz et al., 2015). These results imply that downregulation of PP2A activity in limbic epilepsy could occur due to the diminishing regulatory expression of the subunit PR55, with no direct effect on the levels of the conserved catalytic domain.

**Acquired limbic epileptogenesis is associated with increased phosphorylation of tau**

PP2A is a major brain protein phosphatase implicated in the dephosphorylation of p-tau, and downregulation of PP2A activity promotes the accumulation of p-tau (Benecib et al., 2000; Qian et al., 2009). To explore further the role of PP2A in epileptogenesis, we examined the levels of phosphorylation on two pathological p-tau epitopes, Ser198 and Ser262, which are regulated by PP2A and affected in neurodegenerative disease (Benecib et al., 2000; Qian et al., 2009). Using western blotting, we found that the ratio of pS198 and pS262 immunoreactivity to Tau-5 (total tau) immunoreactivity in amygdala, hippocampus and cortex were significantly increased in both amygdala kindled and post status epilepticus rats, compared with the same brain regions from sham controls (Fig. 3A and B). Levels of total tau were not significantly influenced by either model (Fig. 3A and B). In previously published work we have demonstrated similar changes in the brains of post-FPI rats, with an increase of ~200% in pS198 and pS262 immunoreactivity to Tau-5 immunoreactivity (Shultz et al., 2015).

**Sodium selenate suppresses epileptogenesis in rat models**

We next investigated whether the upregulation of PP2A activity could suppress epileptogenesis by testing the effects of continuous sodium selenate treatment, which boosts PP2A activity (Corcoran et al., 2010a), in the three rat models of epileptogenesis (see Supplementary Fig. 1 for timelines). We demonstrated that sodium selenate significantly delayed the progression of amygdala kindling epileptogenesis compared to sodium chloride treatment, as evidenced by slower progression of seizure class (Fig. 4A), and a greater number of stimulations to reach the different stages of kindling (Fig. 4B). Importantly, all rats eventually reached the same convulsive stage of kindling, suggesting a
true modulatory effect (Fig. 4A and B). Furthermore, the progressive increase in total (Fig. 4C) and primary (Fig. 4D) seizure duration (after discharge) was slower in sodium selenate-treated rats throughout the course of kindling. The cumulative total after-discharge duration through the kindling period was significantly less in the selenite-treated rats (2371 ± 78 s versus mean 5377 ± 181, P < 0.0001). These results indicate that sodium selenate attenuates epileptogenesis in the amygdala kindling rat model.

We then investigated the effect of sodium selenate treatment on epileptogenesis in the post-kainic acid status epilepticus rat model of acquired epilepsy (Supplementary Fig. 1B). All rats had at least one seizure recorded during the video-EEG monitoring period. During the recording in the last 2 weeks of the treatment period, the number of seizures per day and the average seizure duration in rats treated with sodium selenate were significantly decreased compared with sodium chloride treatment (Fig. 4E and F). To assess the anti-epileptogenic, or disease-modifying, effects of sodium selenate, independent from any acute seizure suppressing effect (Jones et al., 2012), we also assessed seizure frequency 2 weeks after cessation of sodium selenate treatment. Sodium selenate has a short half-life (~2 h in humans, and significantly less in rats; Corcoran et al., 2010b), and therefore 2 weeks should provide more than sufficient time for any anti-seizure effect of the selenate treatment to have ‘washed out’. We found that the average number of seizures per day, and the average duration of these seizures, remained significantly lower in the rats previously treated with sodium selenate, compared to the rats previously treated with sodium chloride-vehicle (Fig. 4E and F).

We also investigated the anti-epileptogenic effects of sodium selenate treatment in the rat FPI model of post-traumatic epilepsy (Supplementary Fig. 1C). During the 2 weeks of video-EEG monitoring at the end of the 12-week post-injury treatment period, 14/26 rats in the sodium selenate treatment group and 16/27 rats in the sodium chloride treatment group were recorded to have at least one seizure. Of the
Figure 2 PP2A activity and PR55 expression are decreased in epileptogenesis. (A) Amygdala kindling (n = 4) significantly decreased PP2A activity in amygdala, hippocampus and cortex, compared with sham operation (n = 4). (B) Amygdala kindling (n = 8) also significantly decreased the ratio of PR55 immunoreactivity to GAPDH immunoreactivity in amygdala, hippocampus and cortex, compared with sham operation (n = 8), but had no effect on the ratio of PP2A immunoreactivity to GAPDH immunoreactivity. (C) Post-status epilepticus (n = 4) significantly decreased PP2A activity in amygdala, hippocampus, and cortex, compared with sham group (n = 4). (D) Post-status epilepticus (n = 8) also significantly decreased the ratio of PR55 immunoreactivity to GAPDH immunoreactivity in amygdala, hippocampus and cortex, compared with sham (n = 6), but had no significant effect on the ratio of PP2A immunoreactivity to GAPDH immunoreactivity. The data were expressed as mean ± SD (**P < 0.01). IR = immunoreactivity.
Figure 3  Phosphorylation of tau is increased in epileptogenesis. (A) Amygdala kindling (n = 8) significantly increased the ratio of pS198 and pS262 immunoreactivity to Tau-5 immunoreactivity in amygdala, hippocampus and cortex, compared with sham (n = 8). Total tau (Tau-5) was not affected by kindling. (B) Post-status epilepticus (SE, n = 8) also significantly increased the ratio of pS198 and pS262 immunoreactivity to Tau-5 immunoreactivity in amygdala, hippocampus and cortex compared with sham (n = 6). Total tau was not significantly affected by post status epilepticus. The data were expressed as mean ± SD (**P < 0.01). IR = immunoreactivity.
Figure 4 Sodium selenate attenuated epileptogenesis in chronic acquired epilepsy models. (A) Compared with sodium chloride (NaCl) treatment ($n=11$), treatment with sodium selenate ($n=12$) reduced the average Racine seizure class experienced by rats as the number of stimulations increased during kindling. (B) Sodium selenate treatment ($n=12$) significantly increased the number of stimulations required to attain at the same Racine class of seizure compared with sodium chloride treatment ($n=11$). (C and D) Sodium selenate treatment ($n=12$) significantly decreased the primary and total after-discharge duration compared with sodium chloride treatment ($n=11$) during kindling. (E and F) In the post status epilepticus model, during treatment (pre-washout), selenate ($n=9$) significantly decreased the number of seizures/day and...
Sodium selenate retards epileptogenesis

Chronic treatment with sodium selenate was well tolerated by the rats

No adverse health effects of the sodium selenate treatment were observed in all three chronic epilepsy models, and there were no significant differences in rat weights between the sodium selenate and sodium chloride treated groups. After the first week, all animals in both the selenate and the saline control treated groups scored 0 on the neurotoxicity scale (i.e. no observable sedation).

Sodium selenate does not reduce early seizure activity following kainic acid-induced status epilepticus or fluid percussion injury

To investigate whether the sodium selenate infusions, which commenced following the initial diazepam injection 4 h following the onset of kainic acid-induced status epilepticus and immediately after the FPI injury, reduced the early post-insult seizure activity (which could therefore modify the long term epileptic condition), we acquired continuous video EEG recordings in separate cohorts of rats. Following the first diazepam injection post-kainic acid induced status epilepticus, all rats had at least some recurrent seizure activity recorded in the first 48 h, but this was not significantly different between the selenate versus saline treated rats (mean 2.67 ± 0.58 versus 2.17 ± 0.58 h, n = 7 per group, P = 0.53 Student t-test). Following the FPI injury, 4/6 rats (66.7%) in the selenite-treated group and 1/6 rats (16.7%) in the saline-treated group had seizure activity recorded in the first 48 h (P = 0.24, Fisher’s Exact Test), with no significant difference in the mean total time in seizure activity between the groups (6.6 ± 3.0 mins, n = 6 per group, P = 0.06 Student t-test).

Sodium selenate treatment upregulates PP2A activity and increases the PR55 expression in chronic acquired epilepsy models

To investigate whether sodium selenate influenced the decreased PP2A activity and PR55 levels we had observed in the models of chronic acquired epilepsy, we assayed these biochemical outcomes in limbic regions of sodium selenate-treated and sodium chloride-treated rats. For amygdala kindled rats, we found that sodium selenate significantly increased PP2A activity compared with sodium chloride-treated rats in the amygdala, hippocampus, and cortex (Fig. 5A). We also analysed the expression of PR55 in these same brain regions, and found that sodium selenate treatment significantly increased the ratio of PR55 to GAPDH compared to sodium chloride-treated rats. However, we found no effects of sodium selenate on the expression of the PP2Ac subunit, suggesting the effect was selective for PR55 (Fig. 5B).

We also performed post-mortem analyses in tissue from the post status epilepticus rats after the drug washout period. Similar to the kindling experiments, we found that PP2A activity was significantly increased in limbic brain regions in post status epilepticus rats previously treated with sodium selenate compared to those previously treated with sodium chloride (Fig. 5C). Also, the ratio of PR55 to GAPDH was increased by sodium selenate treatment in these brain areas (Fig. 5D).

We have previously demonstrated similar changes in the brains of post-FPI rats, with selenate treated rats showing an increase in PR55 immunoreactivity and PP2A of 20–40% in the injured cortex compared with post-FPI rats treated with saline (Shultz et al., 2015). Taken together, these studies suggest that loss of PP2A activity, induced by decreases in PR55 levels, promotes epileptogenesis, and that sodium selenate retards epileptogenesis in chronic acquired epilepsy models via attenuating the decrease in PP2A activity and PR55 levels.

Sodium selenate decreases the phosphorylation of tau in chronic acquired epilepsy models

As PP2A is reported to reduce phosphorylation of tau (Benecib et al., 2000; Iqbal et al., 2009), we next investigated the effects of sodium selenate treatment on the phosphorylation of tau in chronic acquired epilepsy models.
Figure 5 Sodium selenate increased PP2A activity and PR55 in rat chronic acquired epilepsy models. (A) In the amygdala kindling model, sodium selenate treatment (n = 4) significantly increased PP2A activity in amygdala, hippocampus and cortex, compared with sodium chloride treatment (n = 4). (B) Sodium selenate treatment (n = 8) also significantly increased the ratio of PR55 immunoreactivity to GAPDH immunoreactivity in amygdala, hippocampus and cortex, compared with sodium chloride treatment (n = 8), but had no significantly effects on PP2Ac level in these brain area following kindling. (C) In the post status epilepticus model, sodium selenate treatment (n = 4) significantly increased PP2A activity in amygdala, hippocampus and cortex, compared with sodium chloride treatment. (D) Also in the post status epilepticus, sodium selenate treatment (n = 8) significantly increased the ratio of PR55 immunoreactivity to GAPDH immunoreactivity in amygdala, hippocampus and cortex, compared with sodium chloride treatment (n = 6), but had no significantly effects on PP2Ac level in these brain area. The data were expressed as mean ± SD (**P < 0.01). IR = immunoreactivity.
Figure 6  Sodium selenate decreased phosphorylation of tau in rat models of chronic acquired epilepsy. (A) In the amygdala kindling rat model, sodium selenate treatment (n = 8) significantly decreased the ratio of pS198 immunoreactivity and pS262 immunoreactivity to Tau-5 immunoreactivity in amygdala, hippocampus and cortex, compared with sodium chloride treatment (n = 8), but the total tau, the ratio of Tau-5 immunoreactivity to GAPHD immunoreactivity was not affected in these brain area. (B) In the post status epilepticus model, sodium selenate treatment (n = 8) significantly decreased the ratio of pS198 immunoreactivity and pS262 immunoreactivity to Tau-5 immunoreactivity in amygdala, hippocampus and cortex compared with sodium chloride treatment (n = 8), but the total tau, the ratio of Tau-5 immunoreactivity to GAPHD immunoreactivity was not affected in these brain area. The data were expressed as mean ± SD (***P < 0.01). IR = immunoreactivity.
phosphorylation of tau in chronic acquired epilepsy models. We found that selenate significantly decreased the ratio of pS198 and pS262 immunoreactivity to Tau-5 immunoreactivity in amygdala, hippocampus and cortex in kindled rats (Fig. 6A), compared with sodium chloride treatment. In the post status epilepticus model, we also found that the ratios of pS198 and pS262 immunoreactivity to Tau-5 immunoreactivity were significantly decreased in the rats previously treated with sodium selenate compared to the sodium chloride treated rats (Fig. 6B). However, the ratios of Tau-5 immunoreactivity to GAPDH immunoreactivity were not significantly influenced in both chronic acquired epilepsy models by sodium selenate (Fig. 6A and B). We have previously demonstrated similar effects of sodium selenate treatment on p-tau in the brains of post-FPI rats, with a decrease of ∼100% in the pS198 and pS262 immunoreactivity to Tau-5 immunoreactivity in the injured cortex of rats treated with sodium selenate compared to vehicle treated post-FPI rats (Shultz et al., 2015). These results suggest that p-tau is associated with limbic epileptogenesis, and that selenate retards epileptogenesis via inhibiting the formation of p-tau but not influencing total tau levels during disease development.

The expression levels of PR55 and p-tau in the amygdala and hippocampus are related to epilepsy severity in post-kainic acid status epilepticus rats

To investigate whether the molecular measures of expression of PR55 and p-tau in the brain, which were shown to be affected by the epileptogenic insults and to be mitigated by the selenate treatment, were related to the epilepsy severity measures we correlated PR55 expression and activity, and the pS198/Tau-5 immunoreactivity and pS262/Tau-5 immunoreactivity, with the seizure frequency and seizure duration on the pre-and post-drug washout video-EEG analysis in the post-kainic acid status epilepticus rats (Table 1). A number of these correlations were significant: the PR55 expression in the amygdala and hippocampus was negatively correlated with seizure duration and frequency in the post-washout video-EEG recordings, indicating that the animals with a higher PR55 activity (which selenate is acting to boost) expressed less and shorter seizures. While the p-tau expression (i.e. pS198/Tau-5 immunoreactivity and pS262/Tau-5 immunoreactivity) in the amygdala and hippocampus was positively correlated with seizure duration and frequency in the post-washout video-EEG recordings, indicating that the animals with a lower relative p-tau expression (which selenate is acting to reduce) expressed less and shorter seizures.

Sodium selenate reduces neurodegeneration following an epileptogenic brain insult

Hippocampal atrophy and ventricular enlargement is common in temporal lobe epilepsy patients (Briellmann et al., 2002), and is also seen in post status epilepticus and post-FPI rat models of epilepsy (Jupp et al., 2012; Shultz et al., 2013). Thus, to investigate whether sodium selenate could prevent these pathologies, we conducted MRI-based volumetric analysis. We found that post status epilepticus rats treated with sodium chloride had reduced hippocampal volumes (Fig. 7A) and larger lateral ventricles (Fig. 7A) compared to sham groups, whereas the post status epilepticus rats treated with sodium selenate did not significantly differ from shams (Fig. 7A).

The integrity of the corpus callosum, a major white matter tract that is found to be damaged in patients with...
epilepsy (Günbey et al., 2011), was also assessed with fractional anisotropy maps generated from diffusion-weighted MRI, which assesses white matter integrity. We found that post status epilepticus rats treated with sodium chloride had decreased fractional anisotropy in the corpus callosum relative to the sham groups, while the post status epilepticus rats treated with selenate did not (Fig. 7B). We also used MRS to investigate the effects of selenate on a number of brain metabolites. The N-acetyl aspartate/creatine ratio is an indicator of neuronal health (Ashford et al., 2010), which can be measured by MRS. The N-acetyl aspartate/creatine in post status epilepticus rats treated with sodium chloride was significantly lower compared with sham injured rats, whereas the post status epilepticus rats treated with sodium selenate did not significantly differ from shams (Fig. 7C). We also assessed the myo-inositol/creatine ratio which is associated with glial activation (Brand et al., 1993). We found that post status epilepticus rats treated with sodium chloride had significantly increased myo-inositol/creatine levels compared to the sham groups, suggesting elevated glial activation, while the post status epilepticus rats treated with sodium selenate did not (Fig. 7C).

In previously published work we have demonstrated similar neuroprotective effects of sodium selenate treatment using MRI on rats post-FPI with a significant mitigation of the reduction in volume loss in the injured cerebral cortex and corpus callosum, the enlargement of the ipsilateral lateral ventricle, and the decrease in fractional anisotropy in the corpus callosum, in selenate treated as compared to saline treated post-FPI rats at 12 weeks (Shultz et al., 2015). Together, these results suggest that sodium selenate treatment reduces brain damage following an epileptogenic brain insult.

**Discussion**

During epileptogenesis a series of molecular and cellular events alter the balance between inhibition and excitation in the brain, increasing neuronal network hyperexcitability, and ultimately resulting in the occurrence of spontaneous seizures (Aroniadou-Anderjaska et al., 2008; Pitkänen and Lukasiuk, 2011). Epileptogenesis is still poorly understood from a neurobiological point of view, and there are no treatments that have been established in patients to effectively mitigate the process (Loscher and Brandt, 2010; Galanopoulou et al., 2012). Amygdala kindling, post status epilepticus and post-FPI are widely used rodent models of limbic epileptogenesis in research (Morimoto et al., 2004; Loscher and Brandt, 2010). While amygdala kindling has its limitations as a model to test anti-epileptogenic treatment, in particular that the animals rarely manifest spontaneous recurrent seizures and that it is difficult to different anti-seizure effects from anti-epileptogenesis (i.e. a drug that suppresses seizures may also slow the rate of kindling), it is a practical and efficient screening model. However, post status epileptogenic and post-FPI are ‘true chronic epilepsy’ models in which the animals manifest spontaneous recurrent seizures, and there is a temporal separation between the inducing epileptogenic insult and the chronic epilepsy that can be used to distinguish anti-epileptogenic from anti-seizure effects. Using these models, we investigated for changes in PP2A and tau during limbic epileptogenesis, and subsequently tested the effects of an activator of PP2A, sodium selenate, on this process.

We found that PP2A activity and PR55 expression were significantly reduced in limbic brain regions (i.e. amygdala, hippocampus and cortex) in each of the chronic acquired epilepsy rat models, while levels of the enzymatic catalytic domain, PP2Ac, was not affected. Consistent with the decrease in PP2A activity, p-tau was significantly increased in these brain regions in the chronic acquired epilepsy rat models. Notably, in other recently published research we showed similar changes in PP2A and p-tau in the FPI rat model (Shultz et al., 2015). We also found that in the post status epilepticus model, and in our previous study in the FPI model, that MRI-detected abnormalities indicative of brain damage, such as enlarged ventricles, hippocampal atrophy, reduced fractional anisotropy in the corpus callosum, decreased N-acetyl aspartate/creatine ratio, and increased myo-inositol/creatine ratio, all of which are similar to neuropathological changes observed in chronic epilepsy patients, were mitigated by treating with sodium selenate following the brain insult. Importantly, we provide evidence here for the first time that sodium selenate, an activator of PP2A, slowed the progression of epileptogenesis and mitigated the associated changes in PP2A, PR55 and p-tau in all three chronic acquired epilepsy models examined. Furthermore, these therapeutic effects are maintained after sodium selenate washout, indicating a sustained disease-modifying effect beyond any acute seizure suppressant effect the drug may have (Jones et al., 2012). Sodium selenate has a short half-life *in vivo* [1.2 to 2.9 h in humans (Corcoran et al., 2010b) and likely shorter in rodents]. The wash-out period of 2 weeks was chosen in our studies so that multiple half-lives of selenate would have elapsed, and therefore it is unlikely that there was any acute anti-seizure effect of selenate persisting to confound the post-washout seizure results, although drug effects can sometimes persist beyond that expected from the pharmacokinetic half-life. Furthermore, we cannot definitely establish from the experiments reported here whether the selenate treatment permanently modified the epileptogenesis, or merely delayed it. However, no duration of follow-up could definitively establish this, because no matter how long the time interval at which the recording was undertaken post the epileptogenic insult it would not exclude that after this time the animals would not ultimately progress to the same epilepsy state as the untreated animals. Nonetheless, our data do demonstrate that the selenate treatment has a biological effect to modify the epileptogenic process. Together, these results suggest that downregulation of PP2A activity following an epileptogenic
Figure 7 Post-status epilepticus induces brain damage and selenate protects it. (A) T2-weighted MRI images showed that hippocampal and lateral ventricle volumes do not differ between post status epilepticus (SE) rats treated with sodium selenate (n = 6) and sham groups (n = 6), whereas post status epilepticus rats treated with sodium chloride (n = 5) have reduced hippocampal volumes and enlarged ventricles compared to sham groups. (B) Fractional anisotropy maps showed that the corpus callosum does not differ between post status epilepticus rats treated with sodium selenate (n = 6) and sham groups (n = 6), whereas post status epilepticus rats treated with sodium chloride (n = 5) have reduced fractional anisotropy compared to sham groups. (C) MRS spectra measure N-acetyl aspartate (NAA), myo-inositol (mlNS), and creatine (continued)
brain insult causes hyperphosphorylation of tau, which promotes epileptogenesis, and that upregulation of PP2A activity via increasing PR55 levels through treatment with sodium selenate attenuates epileptogenesis and associated brain damage.

Our group has previously demonstrated that sodium selenate treatment suppresses seizures in a variety of rodent models (Jones et al., 2012). It is therefore possible that an acute anti-seizure effect of selenate treatment reduced the amount of early seizure activity following the kainic acid-induced status epilepticus or the FPI, resulting in a less severe initial insult, and therefore reduced the long term development of epilepsy and brain damage. However, using continuous video-EEG recording for the first 3 days post-kainic acid status epilepticus and FPI, we found that while many of the animals did experience recurrent seizure activity in the first 48 h post-insult, including following FPI, there were no significant differences between the selenite- and saline-treated rats in the number of rats experiencing early seizures, nor in the mean total time in electrographic seizure activity. In fact, following both types of insults there was a non-significant trend for the selenate groups to have experienced more time in early seizure activity. This indicates that the difference in long term epilepsy and imaging outcomes seen in the selenite-treated rats is unlikely to be due to mitigation of the initial insult by selenate.

PP2A is involved in a variety of cell signalling pathways from development to neurodegeneration. The different compositions of multi-subunits, which generate more than 70 different isoforms of PP2A holoenzymes, provide many possibilities to regulate the location, activity and specific of PP2A holoenzymes. The regulatory B subunit regulates the substrate specificity of the heterocomplex (Janssens and Goris, 2001). PR55, one family of regulatory B subunits, binds to the core enzyme that is composed of a catalytic subunit and a regulatory A subunit, and is an essential component for PP2A to dephosphorylate p-tau (Janssens and Goris, 2001; Janssens et al., 2008; Xu et al., 2008; Shi, 2009). A decrease in PR55 expression levels has been associated with a reduction in PP2A activity in neurodegenerative conditions, such as Alzheimer’s disease (Sontag et al., 2008). Our group has reported that the level of PR55 is decreased in epileptogenesis and that sodium selenate increases PP2Ac-PR55 isoform activity through stabilizing this complex (Corcoran et al., 2010a). Here, we found that sodium selenate retards epileptogenesis, activates PP2A activity and increases PR55 levels. These data suggest that targeting PR55 could be a novel therapeutic approach for epileptogenesis, via specifically increasing PP2Ac-PR55 isoform activity, but not other isoforms of PP2A.

Downregulation of PP2A activity is believed to promote an accumulation of hyperphosphorylated tau in Alzheimer’s disease (Iqbal et al., 2009), and a growing body of work suggests that common pathological processes may be involved in the development of neurodegenerative conditions such as Alzheimer’s disease and epilepsy (Palop and Mucke, 2009). The risk of seizures is high in patients with Alzheimer’s disease, with an up to 87-fold increase in incidence compared with an age-matched reference population (Mendez et al., 1993; Amatniek et al., 2006). Further, a recent post-mortem study found either mild or moderate (i.e. Braak Stages I/II or III/IV) Alzheimer’s disease neurofibrillary tau pathology in almost 70% of patients with chronic, drug-resistant, epilepsy, with a particular association in those who had a remote history of a traumatic brain injury (Thom et al., 2011). Our present and recent results identify increased phosphorylation of tau on Ser198 and 262 in three chronic acquired epilepsy models, two residues that are pathological in Alzheimer’s disease (Hanger et al., 1998), and whose phosphorylation appears under the control of PP2A (Bennecib et al., 2000; Qian et al., 2009). We also found evidence of brain atrophy in post status epilepticus and FPI rats, which is similar to findings in chronic acquired epilepsy patients, and could be induced by hyperphosphorylated tau. It has also been reported that hyperphosphorylated tau damages neurons in Alzheimer’s disease (Zilka et al., 2008; Morales et al., 2012), and that high levels of hyperphosphorylated tau is associated with decreased hippocampus volume in Alzheimer’s disease (Desikan et al., 2011; Frankó et al., 2012). Taken together, these data suggest that hyperphosphorylated tau could play a common pathological role in both Alzheimer disease and acquired epilepsy. In previous studies, treatment with sodium selenate has been shown to be neuroprotective in mouse models of Alzheimer’s disease, which have increased p-tau expression, and these are effects mediated via actions on PP2A (Corcoran et al., 2010a; van Eersel et al., 2010). In the present experiment, we show that PP2A activity is downregulated and phosphorylation of tau is increased during limbic epileptogenesis, and that sodium selenate retards the epileptogenesis via enhancing PP2A. Our findings suggest that downregulation of PP2A activity could be a common target to ameliorate hyperphosphorylated tau and related pathologies in Alzheimer’s disease and limbic epilepsy. However, while the experiments reported in this paper have demonstrated associations between changes in PP2A and PR55 levels and activity and p-tau and the epilepsy severity measures caused by selenate, we have not...
proven that these are the causative mechanisms for the anti-epileptogenic or neuroprotective effects observed. It is possible other ‘off target’ effects of selenate, or one of its metabolites, could play a role. Future research, possibly involving transgenic animals that have specific components of the PP2A-pta system knocked out, will be required to prove the specific molecular mechanism by which the therapeutic effects of selenate in these chronic epilepsy models are effected.

Other questions to be addressed in future research relate to defining the optimal dose and therapeutic window for the anti-epileptogenic and neuroprotective effect of sodium selenate following a brain injury. The dose of sodium selenate with which the rats were treated in this study (i.e. 1 mg/kg/day by continuous infusion pump) was chosen based on preliminary experiments that established this dose as well-tolerated by rats with long term infusions, and was similar to the doses used in human clinical trials (i.e. up to 90 mg/day for adult patients with prostate cancer or Alzheimer’s disease) (Corcoran et al., 2010b; Malpas et al., submitted for publication). The duration of treatment for the amygdala kindling experiment (4 weeks) was chosen to cover the 3 week period of electrical kindling stimulations (i.e. the epileptogenic period), whereas we chose to start the selenate as soon as possible following the epileptogenic insult in the post-kainic acid status epilepticus and FPI models because of increasing evidence in both animal and humans to indicate that the epileptogenic process commences early following the injury (Dudek and Staley, 2011; Vespa et al., 2016). In our previous publication (Shultz et al., 2015) we demonstrated that PP2A (PR55 subunit) expression and activity, a target of sodium selenate, was significantly decreased as early as 2 h post-FPI (compared to sham injured rats), that this persisted for at least 12 weeks post-injury, and that it was normalized by 12 weeks of sodium selenate treatment (at the same doses as in this current study). In the new results reported here, we have demonstrated that expression of PP2A (PR55 subunit), and its activity is present, but downregulated, at 24 h following the last amygdala kindling stimulation and 12 weeks post-kainic acid status epilepticus compared to control rats, and that these measures are normalized in the rats that received the selenate infusion post-insult (Fig. 2). The treatment duration (i.e. 8 weeks and 12 weeks for the post-kainic acid status epilepticus and the FPI experiments, respectively) was empirically chosen to optimally cover what was judged to be the major period of epileptogenesis. However, this period is difficult to accurately define, and likely extends beyond the time periods of our infusions, but the results of the study demonstrating significant effects on the long term epilepsy endpoints indicate that the treatment periods chosen did represent an important component of the epileptogenic period.

Another question for future research is whether the molecular effects of the epileptogenic brain insults on PP2A, PR55 and p-tau demonstrated in the somatosensory cortex, amygdala and hippocampus, were mitigated by the sodium selenate treatment, are confined to sub-regions of these structures or represent a more global effect. In this study, these regions were dissected en bloc, and homogenized for the molecular analysis, and so it was not possible to investigate for subregion specific changes.

In conclusion, our results suggest that downregulation of PP2A activity, via suppression of PR55 expression, plays a mechanistic role in limbic epileptogenesis, and that restoring PP2A activity with sodium selenate could be a novel anti-epileptogenic therapeutic strategy. Furthermore, PP2A activity and PR55 levels could be valuable biomarkers to monitor epileptogenesis. Sodium selenate has already been demonstrated to be safe and well tolerated in doses up to 90 mg per day in a 6-month phase I trial in humans with prostate cancer (Corcoran et al., 2010b), and more recently at doses of up 30 mg/day for up to 2 years in a phase Ia study for Alzheimer’s disease (Malpas et al., submitted for publication). Therefore, this treatment approach has the potential to be expediently translated into clinical studies aimed at demonstrating its anti-epileptogenic effect in humans, and then ultimately into a transformational treatment for patients who have experienced a potentially epileptic brain insult.

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**Supplementary material**

Supplementary material is available at *Brain* online.

**References**


