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Review Article

Seizure preconditioning and epileptic tolerance: models and mechanisms

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Abstract: One or more brief seizures can serve to activate endogenous protective programmes which render brain regions temporarily less susceptible to damage following an otherwise harmful episode of *status epilepticus* (a prolonged seizure). Epileptic tolerance has been demonstrated using a variety of seizure preconditioning paradigms, including electroconvulsive shocks and low doses of excitotoxins such as kainic acid. The cell and molecular mechanisms underlying the protection are not fully understood but proposed mediators include the transcription factor NfκB, altered ion channel expression, upregulation of growth factors and other protective genes, and suppression of pro-apoptotic Bcl-2 family proteins. Application of microarrays to profile the transcriptome of seizure-preconditioning and tolerance has provided further insights, including roles for chromatin remodeling and evidence that preconditioning generates an anti-excitotoxicity phenotype by reprogramming the transcriptional response to *status epilepticus*. This review summarizes the various animal models of epileptic tolerance, reviews the key effector(s) and the utility of this experimental paradigm for identifying novel targets for neuroprotection and anti-epileptogenesis.

Key words: Apoptosis, epileptogenesis, hippocampal sclerosis, ischemic toleranc, neuroprotection

Tolerance in the brain

It is widely recognized that exposing a tissue or organ to a low intensity of an otherwise harmful stressor temporarily generates a damage-resistant state against a subsequent and otherwise harmful insult. The term "tolerance" was coined to describe the acquisition of this damage-refractory state, with the stressor known as "preconditioning" [1]. This represents an endogenous programme of tissue preservation which likely evolved from mechanisms to cope with restricted substrate supply. Indeed, some of the deduced cell and molecular mechanisms underlying tolerance bear close resemblance to processes activated in hibernating animals which endure long periods of low oxygen/glucose without incurring permanent harm [2-4].

Dahl & Balfour first showed that pre-exposure of rats to anoxia protected brain ATP levels during a second episode [5]. Experiments later by Kitagawa and coworkers were important in both demonstrating damage by global ischemia in gerbils could be reduced by prior brief ischemic events, as well as showing this occurred in a time- and dose-dependent manner [6, 7]. Ischemic tolerance is also long-recognized in the heart [8] and has been exploited clinically to protect tissue prior to surgery (e.g. for heart by-pass) [9]. In the present review, we consider the phenomenon of epileptic tolerance, whereby brief or repeated non-harmful seizures serve to protect the brain against damage from an otherwise harmful prolonged seizure – *status epilepticus* (SE).

Mechanisms of tolerance

A tolerance state can be considered to be acquired through the actions of three components: (1) SENSORS – surface receptors, channels and intracellular enzymes sensitive to changes in oxidation and or substrate state changes. (2) TRANSDUCERS - transcription fac-

tors and post-translational modifiers. (3) EFFECTORS - the molecules and cell components which generate the damage-refractory phenotype [10]. At least two temporal profiles of tolerance exist. Short-term tolerance refers to the protective effect of preconditioning which lasts just a few hours before waning [1]. This is not dependent on new gene synthesis but rather results from rapid biochemical events. In the setting of cerebral ischemia, several biochemical mediators of short-term tolerance have been proposed. These include activation of adenosine A1 receptors and K_{ATP} channels [11], ubiquitin-mediated degradation of the pro-apoptotic Bcl-2 family protein Bim [12] and also short-term restructuring of synapses that appear to disable NMDA-mediated excitotoxicity [13].

Long-term or classical tolerance is the second form. This is dependent on *de novo* protein synthesis for the protection to manifest [14]. Work in ischemia models has suggested several mediators of this form of tolerance, including stress proteins [15] and anti-apoptotic Bcl-2 [16, 17], among others [1, 10]. Remarkably, low-level activation of enzymes associated with effecting apoptotic cell death (caspase-3) has also been shown to mediate ischemic tolerance [18]. In addition to the insights provided by this one gene-at-a-time approach, the mechanisms underlying tolerance have been pursued by microarray profiling. Stenzel-Poore and coworkers showed that the transcriptome of preconditioning, normal prolonged ischemia, and ischemic tolerance share few genes in common [4]. Moreover, the major expressional response in tolerance was gene down-regulation [4]. Together, these data implied that tolerance, like hibernation and certain energy-conservation states (e.g. torpor) comes about by suppression of gene transcription and that preconditioning reprograms the genomic response to ischemia on a scale not previously appreciated [19].

Preconditioning agents

In addition to ischemia itself, a wide variety of stimuli have been shown to be effective at preconditioning against ischemic damage in tolerance paradigms [1, 10]. This includes spreading depression [20], seizures [21], hyperthermia [22, 23], lipopolysaccharide [24-26], a toll-like receptor ligand [27] and certain chemicals which inhibit oxidative phosphorylation [28].

The repertoire of effective tolerance paradigms recently expanded to include post-conditioning. In this scenario, a prolonged ischemic insult is followed by a brief episode of ischemia which also reduces damage; an observation first made in the setting of tolerance in the myocardium [29]. Pignataro and colleagues demonstrated a brief second occlusion of the middle cerebral artery of the mouse after a prolonged occlusion of the same vessel could reduce ischemic damage [30]. Perhaps more surprising still, the preconditioning stimulus may not necessarily need to be applied to the same organ. Remote preconditioning has been demonstrated whereby an ischemic event outside the brain subsequently protected against prolonged focal ischemia within the brain [31]. Collectively these efforts have demonstrated remarkable flexibility in the basic tenet of tolerance, in particular that the nature of the conditioning stimulus does not have to be the same as the subsequent harmful challenge. The effectiveness of such a diverse set of preconditioning agents implies that tolerance is not dependent on a single common pathway or mechanism, although they may converge on a small number of critical effector(s). Nevertheless, the tolerant state may be tailored by the nature of the preconditioning stimulus [19].

Epileptic tolerance

Discovery

Epileptic tolerance was first demonstrated by Kelly & McIntyre, who showed brain damage after SE could be reduced when preceded by a preconditioning seizure [32]. In that study, rats received hippocampal kindling–repeated electrical stimulations until generalized convulsions are elicited – followed by SE induced by systemic kainic acid (KA). The kindled rats displayed dramatic reductions in damage to the piriform cortex, substantia nigra reticulata, as well as the hippocampus [32]. Since that study, several groups have explored different preconditioning paradigms, different SE triggers and different rodent species and strains. Epileptic tolerance shares some common mechanisms with ischemic tolerance, such as a temporal window for protection to manifest as well as a restricted temporal window after which the effect dissipates. In some models, protection appears to be secondary to reduced seizure severity during the challenge phase. Like ischemic tolerance, epileptic tolerance

has some dependency on new protein synthesis [33]. However, important differences are found in the degree of reprogramming to injury that seizure preconditioning generates, and some of the sensors, transducers and effectors are different. Also, the extent of hippocampal (and extra-hippocampal) protection varies between models as might be expected, and has been found to be almost complete in some instances.

Kindling

The study by Kelly & McIntyre showed that repeated electrical stimulation of the hippocampus by implanted electrodes protected against damage in several brain structures from SE induced by intraperitoneal KA [32]. The time period over which this protection lasts was very protracted, lasting at least 28 days. Such kindling-mediated protection against SE has also been demonstrated using related paradigms by other groups [34]. Strong protection of limbic structures including the hippocampus was also observed when the amygdala was the site of kindling and when SE was induced by the cholinergic mimetic pilocarpine [35]. Of note, the hilar region of the hippocampus was not protected against SE by amygdala kindling in this model, which may explain why certain functional deficits were also not prevented (discussed in more detail below).

Electroshocks

Electroconvulsive shocks are used for treating refractory depression and are long-known to induce widespread changes in gene expression and neuronal function [36-38]. Kondratyev et al. found a seven day treatment of rats with minimal electroshocks delivered via orbital (corneal) electrodes significantly reduced limbic damage produced by systemic KA-induced SE [39]. However, changes to the intensity and/or route of electroshock administration can produce very different effects. Andre and coworkers reported enhanced brain damage in maximal electroshock-treated rats delivered via ear-clips when later subjected to SE induced by pilocarpine [35, 40]. Although methodological factors such as intensity of stimulus may underlie these disparate findings, this suggests the electroshock model has a rather narrow window of effectiveness in epileptic tolerance.

Systemic KA

Systemic KA is a widely used, simple and effective means for seizure preconditioning either as a single or repeated dose, usually via an intraperitoneal injection. In rats, this can be achieved by systemic administration of 5 mg/kg KA [41, 42]. Higher KA doses are also effective but pentobarbital must be used to prevent development of overly-prolonged seizures [43-45].

In our laboratory, we use systemic KA to elicit low-grade generalized seizures in mice prior to SE elicited by intra-amygdala KA [33, 46]. At a dose of 15 mg/kg this reduces damage by ~50% [33, 46]. When this dose was halved to 7.5 mg/kg there was no protective effect [46]. The model's strength is also the simplicity of the single preconditioning step and inter-stimulus interval of 24 h which enables high throughput of experiments. However, the extent of hippocampal protection is less than has been achieved when a double-preconditioning paradigm was used. Indeed, Borges et al. achieved nearly total protection against status when repeated preconditioning was employed [45]. In our experience, repeating the preconditioning seizures on a second day did not increase protection so the double-preconditioning paradigm may be dependent on technical features of the SE paradigm or differences between rats and mice.

Centrally-administered excitotoxins

While less common, presumably because of the additional technical demands, preconditioning has also been delivered via central injections of excitotoxins. The Rougier laboratory demonstrated that unilateral intrahippocampal KA in rats substantially protected the contralateral hippocampus from damage caused by intracerebroventricular KA given either 1 or 7 days later [47, 48]. However, this model breaks somewhat from the traditional approach to tolerance in that the preconditioning stimulus is cytotoxic (0.5 µl of 2 nM KA), albeit not to the target region in which tolerance is produced and measured.

Other preconditioning agents

In addition to the models described, seizure preconditioning or epileptic tolerance has been demonstrated using systemic *N*-methyl-D-aspartate against KA-induced SE in mice [49].

The utility of the model is limited to strains which are vulnerable to systemic KA. Ogita et al. used Std-ddY mice but the ability to use C57BL/6 mice is important and this strain develops little or no hippocampal damage after systemic KA except at nearly-lethal doses [50]. Seizures are also not the only means with which to induce tolerance against SE. Cross-tolerance has also been demonstrated, whereby a non-seizure stimulus is used for preconditioning and this effectively reduces damage after SE. This has been shown using an episode of ischemia against KA-induced SE [51], and by injection of lipopolysaccharide ahead of SE evoked by pilocarpine [52].

"Transferability" of tolerance models

One question of interest to our group was whether or not a tolerance paradigm could readily transfer to another mouse strain. This is important because of the above-mentioned strain-specific differences in hippocampal damage following KA [50]. Indeed, C57BL/6 mice which we used in our tolerance model are among the most resistant to the effects of systemic KA [50]. We tested this idea recently by subjecting SJL mice, a damage-vulnerable strain [53], to the same tolerance paradigm. The SJL mice were readily tolerable, showing damage reduction after SE when given i.p. KA 24 h before [54]. Of note, we found we had to use a higher rather than lower dose of KA for preconditioning [54]. These data suggest seizure preconditioning elicits broadly conserved responses supporting the ubiquity of the phenomenon of epileptic tolerance.

How far does protection extend?

The major focus of studies has been on whether or not seizure preconditioning protects the hippocampus against damage following SE. We have found that seizure-preconditioning by systemic KA in SJL mice is most protective against SE-induced damage to the ventral hippocampus [54]. However, several groups have also provided information on extra-hippocampal areas. In general, it appears seizure preconditioning can also protect non-hippocampal structures [35]. However, many groups either did not comment on non-hippocampal regions [43, 46, 47, 49, 52] or reported minimal protection outside of this region [45].

Epileptic tolerance and epileptogenesis

Epileptogenesis is defined as the process whereby a neuronal network develops recurrent epileptic seizures *de novo* or following an insult, and the process whereby seizures become more severe and frequent in chronic epilepsy. [55]. Currently available pharmacological treatments of epilepsy only suppress seizures and have not been found to impact on epileptogenesis. It is controversial as to whether neuroprotection applied at the time of injury (e.g. from SE) influences in a significant way the subsequent development of epilepsy. Certainly, anticonvulsant administration during SE to curtail seizures reduces the subsequent development of epilepsy [56]. Certain seizure-suppressive molecules applied during or after SE can also be anti-epileptogenic [57, 58]. However, reduced cell death by neuroprotection has mainly failed to influence the course of disease expression [59-61]. Is the neuroprotection afforded by epileptic tolerance anti-epileptic? To date, studies which have formerly tested this question have produced contradictory findings. Andre et al. conducted two studies in which kindling was compared to electroshock as the preconditioning agent, ahead of pilocarpine-induced SE [35, 40]. No beneficial effect on epilepsy development was found in rats preconditioned by kindling [35]. In contrast, fewer rats given maximal electroshock developed epilepsy after SE [35, 40]. Of note, the anti-epileptogenic effect of maximal electroshock was associated with more brain damage despite less epilepsy, implying disconnection of certain circuits underlay the anti-epileptic effect [35, 40]. Lipopolysaccharide-induced epileptic tolerance was also recently reported not to alter the course of epileptogenesis [52], although no details of how spontaneous seizures were recorded and quantified were not reported in that study.

Our group undertook an analysis of epilepsy development after intra-amygdala KA-induced SE in mice to determine the effect of seizure preconditioning-mediated neuroprotection [33]. Before embarking on the study, we were concerned about missing subtle anti-epileptogenic effects by intermittent, behaviour-only or "late" monitoring of epilepsy. Therefore we instrumented mice with radiotelemetry units and recorded continuous EEG for 12 days after SE [33]. Using this method, we recorded ~70 % fewer epileptic seizures in tolerance mice compared to sham-preconditioned SE animals. This establishes neuroprotection applied through tolerance can have a strong positive

anti-epileptogenic effect. The difference between the study outcomes may rest with technical and methodological factors. Of note, hilar as well as CA3 neurons are among the protected cell populations in tolerant mice in our model. Certainly, replication of the observations is now needed to strengthen the evidence either for or against tolerance as a strategy for anti-epileptogenesis.

Mediators of epileptic tolerance

Inhibition/dampening of the secondary response to SE

Is the neuroprotection in animal models of epileptic tolerance really equivalent to that in ischemic tolerance? It emerged early on that the duration and/or intensity of SE was often reduced in preconditioned mice [32, 43, 62]. Thus, protection afforded by seizure preconditioning could simply be due to an anti-epileptic effect diminishing the severity of the prolonged challenge (SE). This would constitute secondary protection via seizure-suppressive effects rather than through traditional pathways to tolerance (that is, where protection is observed in spite of having undergone the second insult of equivalent severity). In the original study by Kelly & McIntyre, only 63% of kindled rats went into SE following systemic KA, compared to 97% of the controls [32]. However, severe limbic seizures in the kindled rats actually developed sooner when they occurred and tended to last longer [32]. Thus, there may be bi-directional effects of seizure preconditioning on the electrographic and behavioural response to the subsequent SE. Reduced SE intensity has also been reported by others when kindling was used to precondition [35]. Diminished SE is also observed in several other tolerance models, including when the preconditioning stimulus is intra-hippocampal KA [47, 48]. These nevertheless remain useful models to study tolerance and neuroprotection. However, if we are to identify molecular effectors of tolerance this is obviously a confounding factor. Preconditioning agents which seem to cause minimal or no reduction in the intensity of SE include maximal electroshocks [35], lipopolysaccharide [52] and single-dose systemic KA in mice [46, 54]. In our studies, we recorded EEG during SE in both C57BL/6 and most recently SJL mice after intra-amygdala KA and found it was not different in animals preconditioned by systemic KA [46, 54]. Why certain models are less susceptible to anti-epileptic effects of preconditioning is not understood but the nature of the preconditioning stimulus is probably the main influence. Presumably the preconditioning seizures in some models effectively engage the machinery of tolerance without causing changes to channels and neuronal structures that lead to an anti-epileptic phenotype. Indeed, while pilocarpine-induced SE was strongly suppressed in amygdala-kindled rats, maximal electroshocks had only a small delaying effect on SE onset after pilocarpine [35]. While seizure-suppressive effects of preconditioning remain a complicating factor, several molecular pathways have been identified which may contribute to the manifestation of epileptic tolerance, examples of which are given below:

Adenosine

Adenosine was one of the earliest proposed mediators of ischemic tolerance [11, 63]. The effect is thought to be mediated via A1 receptors, which function to reduce neuronal excitability and inhibit glutamate release via presynaptic effects [64, 65]. Indeed, activating central adenosine A1 receptors or blocking adenosine degradation has powerful anti-convulsant and anti-epileptogenic effects [64, 66]. Blondeau et al. showed that an adenosine receptor agonist could mimic the effect of seizure preconditioning using low-dose systemic KA in rats [41]. Whether this mechanism explains epileptic tolerance *in vivo* remains unproven.

Transcription factors: NfκB and others

Given the requirement for new protein synthesis for tolerance to manifest, it is likely that transcription factors are important contributors. The strongest evidence to date comes from the study by Blondeau and coworkers on nuclear factor κB (NfκB) [42]. A mild seizure elicited by 5 mg/kg systemic KA in rats was found to produce a prolonged increase in NfκB activity in the hippocampus [42]. Importantly, inhibition of NfκB blocked the neuroprotection of tolerance [42]. The specific gene targets of NfκB were not investigated in this study, but may include Bcl-2 which has been implicated in ischemic tolerance [16, 67]. An additional strength of this study was to show NfκB involvement was common to multiple tolerance paradigms [42].

Other transcription factors may also be involved. This includes the nuclear activator protein 1 (AP1) transcription factor. Systemic NMDA activates AP1 in mice at a dose which is effective in generating tolerance against systemic KA [49, 68]. Other transcription factors include c-Jun, which are induced following electroconvulsive seizures [37], another means of inducing epileptic tolerance. Extracellular signal regulating kinase (ERK) and the p38 mitogen-activated protein kinase have also been proposed as mediators of tolerance [44].

Neuropeptide Y

El Bahh et al. noted an increased expression of neuropeptide Y (NPY) in their model of epileptic preconditioning by intrahippocampal KA in rats [47]. NPY has powerful anti-epileptic effects [69] so this seems to be a plausible mediator, particularly in models associated with anti-epileptic effects after preconditioning. Borges et al. also reported NPY increases after preconditioning in rats with systemic KA [45].

Growth factors

Growth factors can exert potent neuroprotective effects against seizures, seizure-induced brain injury, and can also influence epilepsy development [70-72]. Induction of growth factors has been reported in several seizure-preconditioning paradigms. This includes electroshock seizures [36, 38] and following systemic KA [45]. Thus, overexpression of brain-derived neurotrophic factor (BDNF) or a related protein after preconditioning may exert a neuroprotective effect that suppresses neuronal death during SE.

Heat Shock Proteins

A remarkable spatio-temporal overlap has been found for the induction of heat shock proteins (HSPs) with the onset and subsequent waning of the tolerant state after ischemic preconditioning [7, 15, 73]. HSP70 in particular was among the first potentially protective genes to be linked as an effector of ischemic tolerance [7, 15, 73]. Over-expression of several HSPs including HSP70 is protective against seizure-induced neuronal death *in vivo* [74, 75] and HSP70 may be important in epileptic tolerance. As in ischemic preconditioning, seizure-preconditioning induces HSP70 over a time-frame compatible with the acquisition of a tolerant state and therefore may represent a common tolerance effector between paradigms [41].

Bcl-2 family proteins

Early studies of ischemic tolerance also focused on anti-apoptotic Bcl-2 as a potential candidate effector. Bcl-2 is upregulated in several models of ischemic preconditioning, and ischemic tolerance can be blocked by targeting Bcl-2 [16, 67]. The pathway upstream of Bcl-2 may be NfκB, although the cyclic AMP response element-binding protein (CREB) has also been proposed [17]. It is likely that regulation of other Bcl-2 family proteins is also important since pro-apoptotic Bim is degraded after preconditioning and ischemic tolerance can be mimicked, at least *in vitro*, by depleting Bim levels in neurons [17].

Regulation of Bcl-2 family members has been reported in several models of epileptic tolerance. Kondratyev and colleagues reported repeated minimal electroshocks blocked induction of pro-apoptotic Bcl-Xs after SE [39]. Our own group has observed electroshock seizures down-regulate protein levels of pro-apoptotic Bim [76], and also upregulate anti-apoptotic Bcl-w [77]. The effects on Bcl-2 family members are quite specific as Bid, Bad and Bcl-XL show no expressional changes at a protein level after electroshock seizures [76, 77].

Insights from microarray profiling the transcriptional response to preconditioning and tolerance

Transcriptome analysis has been an important source of insight into the cell and molecular mechanisms of preconditioning and tolerance. In the study by Stenzel-Poore and coworkers, microarray profiling revealed that the major transcriptional response to a harmful ischemic episode was gene upregulation, but showed the response after harmful ischemia in previously preconditioned brain was predominantly gene downregulation [4]. This implied the brain can re-programme its response to ischemia when forewarned by preconditioning, generating a hibernation-like state in which energy-expensive cell functions were suppressed to better cope with the crisis [19, 78]. Such insights may eventually be revised in light of challenges introduced by newer technology platforms [79] and knowledge of large-

scale post-transcriptional mRNA silencing by microRNAs [80].

Microarray analyses have helped shape our understanding of the cell and molecular mechanisms of seizure preconditioning and epileptic tolerance. Borges et al. analyzed individual hippocampal subfields for gene expression after seizure preconditioning in rats [45]. They found that gene responses differed between the major subfields, with most changes occurring in the dentate granule cell layer. Most of the commonly regulated genes between the subfields were increased after preconditioning, and this included transcripts for NPY and a sodium channel [45]. There was not marked upregulation of neuroprotective genes. Indeed, prominent processes altered included signalling, tissue structure, neurotransmission and metabolism [45].

In our laboratory, we also used microarrays to study the transcriptome of preconditioning and tolerance. Similar to Borges et al., [45] we only studied microdissected CA3 because of concerns that the genetically heterogeneous hippocampal subfields would be confounders in data interpretation. Because our tolerance model was in the mouse, we could take advantage of the wide coverage afforded by the Affymetrix mouse 430 2.0 chips which detect ~35,000 gene transcripts. We began by profiling the gene changes in the hippocampus after preconditioning seizures alone. We actually found rather small mRNA changes 24 h after preconditioning – just 37 genes regulated 1.8 fold over control, which was reduced to 20 after correction for multiple testing [46]. Nevertheless, the dataset was informative about potential mechanisms. The genes upregulated by preconditioning included an anti-apoptotic gene and a gene involved in chromatin remodelling, certainly plausible candidates in tolerance [81, 82]. We also detected changes to chromatin remodelling and cell cycle genes among those downregulated [46]. Another functional category overrepresented was post-translational modifications involving ubiquitin, which again fits with processes associated with tolerance [12, 13].

We next undertook a study to define the transcriptome in previously preconditioned mice after SE [33]. These studies were probably more informative than the preconditioning-alone analysis about how the tolerance state is acquired. The first finding of note was that

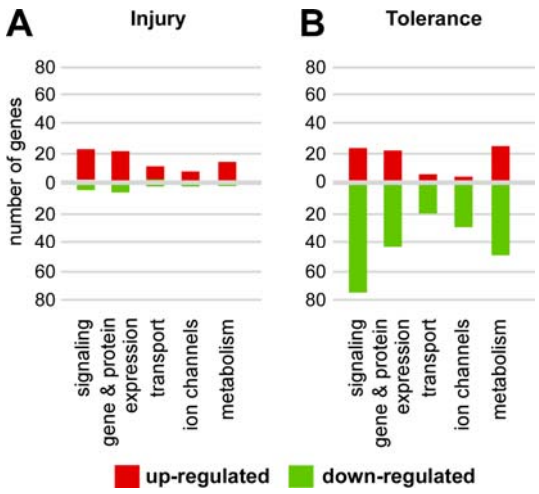

Figure 1. *Hippocampal CA3 transcriptome in epileptic Tolerance.* Mice were subject to *status epilepticus* alone (Injury) or given seizure preconditioning 24 h before *status epilepticus* (tolerance) and mRNA from the CA3 subfield analyzed by microarray 24 h later. Graphs show the number of genes differentially up- (red) and down- (green) regulated between injury and tolerance for key processes. Note majority of differentially regulated genes in tolerance are down-regulated. Data from Jimenez-Mateos *et al.* [33]. Copyright © 2008 Elsevier Inc.

tolerant mice displayed almost 50 % more gene regulation after SE than mice subject to SE alone [33]. When we analyzed the degree of gene overlap between tolerant mice and the SE-only group, we found a 58 % gene share between the conditions [33]. This is much higher than was observed in an equivalent ischemia setting, where overlap between tolerance and ischemia-only was < 20% [4]. Thus, the "reprogramming" effect in epileptic tolerance, at least in this model, is weaker than in ischemia. The third major finding was the molecular phenotype of the differentially expressed genes in tolerance – the core of what is different about the preconditioned brain when subjected to SE. Here, we found 73 % of the differentially expressed genes in tolerance were downregulated compared to control (**Figure 1**). This appears very similar to what was observed in ischemia, where the major transcriptional phenotype was also downregulation. Were the same processes being affected? Not quite. The directional change for genes associated with transport were the same, but whereas ischemic toler-

ance leads to suppressed metabolism, these genes were more upregulated in epileptic tolerance [33]. In fact, the most distinctly suppressed category in epileptic tolerance was for genes associated with calcium signalling [33]. Ion channel and neurotransmitter genes were also highly over-represented among the genes uniquely downregulated in tolerance [33]. Thus, the neuroprotected state in epileptic tolerance may be acquired through generation of an anti-excitotoxicity phenotype rather than the suppressed metabolism and defence state that is the defining phenotype of ischemic tolerance [19].

Where next? A significant portion (30 %) of the differentially down-regulated genes in the tolerance group were unknown [33]. Further mining of these datasets once gene identification is more complete may yield more targets of interest. An obvious question is whether the response has a central "coordinating" mechanism? Given we detected 565 different gene changes in tolerance compared to SE-only, identifying key effectors will be critical to move toward feasible targeting. As to what the targets might be, we do not yet know. However, chromatin remodelling and epigenetic modifications as well as known transcriptional suppressors such as repressor element-1 silencing transcription factor (REST) are attractive candidates for future investigation [83, 84]. If these can be pharmacologically targeted then we have the potential to shift this field beyond an insightful paradigm to study endogenous programmes of neuroprotection toward one with translational relevance.

Final perspectives and future directions

While the field of epileptic tolerance began later than its better-understood ischemic cousin, significant progress has been made. We know epileptic tolerance is broadly conserved both between models and between species. We know that more than one form of epileptic tolerance occurs, with preconditioning protecting either by generating an "anti-epileptic" state or by conventional induction of "neuroprotective" pathways. We do not know all the cell and molecular mediators of tolerance but we foresee differences from ischemic tolerance as well as conserved mechanisms (e.g. gene silencing). Several problems and questions remain. First, while we understand how to elicit preconditioning we probably do not understand enough about the line between insufficient stimulus and the tipping-point of causing actual tissue damage by preconditioning. Second, to what extent is genomic reprogramming – a major mechanism in ischemia - important? What other effectors of tolerance may be out there? Should we borrow more from ischemia and apply to epilepsy, or should we focus just on delineating the mechanisms in epileptic tolerance? Gene silencing may be important but is this achieved by silencers such as REST, epigenetic modifications or novel mechanisms (e.g. microRNAs)? These questions and more will ensure exciting future discoveries in this area of epilepsy research.

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