



# THAR SHE BLOWS! The Search for the Great Sperm Whale of Carbamazepine Resistance

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## Polyamine Modulation of Anticonvulsant Drug Response: A Potential Mechanism Contributing to Pharmacoresistance in Chronic Epilepsy

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Despite the development of numerous novel anticonvulsant drugs, ~30% of patients with epilepsy remain refractory to antiepileptic drugs (AEDs). Many established and novel AEDs reduce hyperexcitability via voltage- and use-dependent inhibition of voltage-gated Na<sup>+</sup> channels. For the widely used anticonvulsant carbamazepine (CBZ), use-dependent block of Na<sup>+</sup> channels is significantly reduced both in experimental and human epilepsies. However, the molecular underpinnings of this potential cellular mechanism for pharmacoresistance have remained enigmatic. Here, we describe the mechanism that leads to the emergence of CBZ-resistant Na<sup>+</sup> channels. We focused on the endogenous polyamine system, which powerfully modulates Na<sup>+</sup> channels in a use-dependent manner. We had shown previously that the intracellular polyamine spermine is reduced in chronic epilepsy, resulting in increased persistent Na<sup>+</sup> currents. Because both spermine and CBZ bind use-dependently in spatial proximity within the Na<sup>+</sup> channel pore, we hypothesized that spermine loss might also be related to diminished CBZ response. Using the pilocarpine model of refractory epilepsy in male rats and whole-cell patch-clamp recordings, we first replicated the reduction of use-dependent block by CBZ in chronically epileptic animals. We then substituted intracellular spermine via the patch pipette in different concentrations. Under these conditions, we found that exogenous spermine significantly rescues use-dependent block of Na<sup>+</sup> channels by CBZ. These findings indicate that an unexpected modulatory mechanism, depletion of intracellular polyamines, leads both to increased persistent Na<sup>+</sup> currents and to diminished CBZ sensitivity of Na<sup>+</sup> channels. These findings could lead to novel strategies for overcoming pharmacoresistant epilepsy that target the polyamine system. Significance Statement: Pharmacoresistant epilepsy affects ~18 million people worldwide, and intense efforts have therefore been undertaken to uncover the underlying molecular and cellular mechanisms. One of the key known candidate mechanisms of pharmacoresistance has been a loss of use-dependent Na<sup>+</sup> channel block by the anticonvulsant CBZ, both in human and experimental epilepsies. Despite intense scrutiny, the molecular mechanisms underlying this phenomenon have not been elucidated. We now show that a loss of intracellular spermine in chronic epilepsy is a major causative factor leading to the development of CBZ-resistant Na<sup>+</sup> currents. This finding can be exploited both for the screening of anticonvulsants in expression systems and for novel strategies to overcome pharmacoresistance that target the polyamine system.

## Commentary

Approximately 30% of individuals with epilepsy are resistant to antiseizure drugs;<sup>1,2</sup> however, the mechanisms underlying pharmacoresistance remain elusive. Many antiseizure drugs, such as carbamazepine (CBZ), function by decreasing voltage-gated Na<sup>+</sup> channel activity with the goal of reducing aberrant neuronal activity. Na<sup>+</sup> channels have complex biophysical and pharmacological properties and are known to be altered in many pathological states, including epilepsy.<sup>3</sup> At their most basic level, Na<sup>+</sup> channels are essential to drive neuronal activity via their generation of action potentials. They

also show use-dependent inactivation, meaning they remain inactivated for some period of time following their activation. This biophysical property of Na<sup>+</sup> channels allows for temporally discrete transfer of information during neuronal activity by facilitating brief neuronal activation. Interestingly, Na<sup>+</sup> channel use-dependent block can become disrupted in the epileptic brain.<sup>4</sup> Loss of use-dependent block could facilitate repeated pathological firing of action potentials at inappropriately high frequencies, potentially consistent with seizure generation. This begs the question, do changes in Na<sup>+</sup> biophysical properties or in their use-dependent block play a role in



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pharmacoresistant epilepsy? If so, can they be therapeutically targeted to improve patient treatment options and overcome drug-resistant epilepsy?

Previous work has implicated the polyamine, spermine, in the modulation of Na<sup>+</sup> channel activity. Depletion of the polyamine spermine occurs in pharmacoresistant chronic epilepsy increasing the persistent Na<sup>+</sup> current.<sup>5</sup> Polyamines interact with the intracellular face of voltage-gated Na<sup>+</sup> channels, in order to block the selectivity filter in a use-dependent manner.<sup>6</sup> To investigate the association between spermine modulation of Na<sup>+</sup> channel activity and pharmacoresistance, the Beck Lab at the University of Bonn asked whether depletion in spermine levels (i.e. an increase in Na<sup>+</sup> current) is related to CBZ efficacy in a rat model of temporal lobe epilepsy. To answer this question, they examined CBZ efficacy together with experimental modulation of spermine levels in dissociated hippocampal dentate gyrus cells (DGCs) from chronically epileptic rats. First, they were able to replicate previous work from Remy et al<sup>4</sup> showing that while CBZ effectively decreases I<sub>Na</sub> conductance in DGCs from sham animals, it has little effect in chronically epileptic animals. Also in line with Remy et al,<sup>4</sup> carbamazepine did not have differential effects on steady state I<sub>Na</sub> activation and inactivation between sham and post status epilepticus (post-SE) mice.

The authors then examined the messenger RNA (mRNA) expression of several enzymes involved in spermine metabolism. In line with a previous study by Royeck et al,<sup>5</sup> showing that spermine levels decrease following SE, they found that 2 enzymes, spermidine/spermine N(1)-acetyltransferase (SSAT) and ornithine decarboxylase (ODC), were increased post-SE relative to sham animals. The increase in SSAT, a spermine degradation enzyme, may be a factor in the spermine depletion shown by Royeck et al.<sup>5</sup> Interestingly, ODC, a spermine synthesis enzyme, was also increased post-SE. Perhaps the increase in ODC is a way for DGCs to compensate for the depletion of spermine and/or an increase in SSAT; however, this has yet to be tested.

Next, the effects of increasing intracellular levels of spermine on voltage-gated Na<sup>+</sup> channel steady state activation, inactivation, and recovery from inactivation were tested. The greatest effects were seen in sham animals, where 100 μM and 1 mM of spermine decreased the recovery from fast inactivation, consistent with spermine's ability to limit Na<sup>+</sup> channel activity. Additionally, high levels of spermine shifted the voltage dependence of activation and inactivation to more hyperpolarizing potentials in sham animals, mimicking the effects of CBZ. Interestingly, none of these effects of spermine were seen in epileptic animals. While this study is focused on the mechanism of pharmacoresistance in epilepsy, rather than mechanism of epilepsy itself, these data suggest that increasing intracellular spermine levels alone is not sufficient to restore the use-dependent block of Na<sup>+</sup> channels. Based on these results, the authors hypothesized that the addition of spermine may reverse the loss of CBZ efficacy seen in rats post-SE. Indeed, in DGCs from epileptic rats, both 100 μM and 1 mM of spermine increased CBZ efficacy. Interestingly, the reverse effect was

seen in the sham animals. This suggests that the loss of intracellular spermine in epileptic animals plays a role in CBZ resistance and that increasing spermine can restore CBZ's effects on use-dependent inactivation of Na<sup>+</sup> channels. This is an exciting finding that has clear implications for developing novel antiseizure drugs and identifying biomarkers for pharmacoresistance.

A few caveats of this study should be noted. First, the authors focus strictly on pharmacoresistance without delving too deeply into the interactions between CBZ and spermine on a molecular level or into how pharmacoresistance becomes established during the progression of epilepsy. We hope this talented group will pursue these questions in the future. Second, the authors used a wide range of times post-SE for electrophysiological experiments (i.e. 29-110 days). Perhaps they would be able to better elucidate the association between spermine depletion, chronic epilepsy, and pharmacoresistance by examining multiple, discrete time windows during the progression of epileptogenesis, similar to the manner in which mRNA expression of spermine enzymes was tested. Finally, the study does not attempt to use *in vivo* manipulation of spermine to better understand how the suppression fast voltage-gated Na<sup>+</sup> channels play a role in epileptogenesis or whether these changes are crucial to pharmacoresistance in chronic epilepsy. These caveats aside, this study demonstrates an exciting link between spermine levels and CBZ pharmacoresistance.

This study, providing a link between polyamines and CBZ pharmacoresistance, suggests a number of subsequent exciting experiments. It would be interesting to see how increasing spermine levels in whole animal models, versus dissociated DGCs, would affect chronic epilepsy and resistance to CBZ. Since increasing spermine in DGCs results in increased CBZ efficacy, a reasonable hypothesis would be that CBZ would then be able to decrease seizure frequency in chronically epileptic animals. Taking this one step further, detecting spermine levels in the blood of individuals with epilepsy may allow for physicians to develop more personalized treatment plans for patients to attenuate seizures. For example, if an individual with epilepsy has decreased levels of spermine, the physician may decide to use a treatment other than CBZ for their epilepsy since it would not be as efficacious in those individuals. Recent work has developed techniques to detect spermine levels in blood and urine, which could be translated to humans.<sup>7</sup> Additionally, this work opens the possibility of modulating spermine levels in patients to make current drugs, such as CBZ, effective in those with drug-resistant epilepsy. For example, high-throughput screening approaches could be developed to identify mechanisms that increase neuronal spermine levels, thereby enhancing CBZ efficacy. Identifying mechanisms of pharmacoresistance and targeted modulation of those systems may make existing pharmacotherapies more efficacious for patients utilizing available antiseizure medications.



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