In Search of the Ever-Elusive Positive Endozepine

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In this issue of *Neuron*, Christian et al. (2013) provide functional evidence for positive endozepines (positive allosteric modulators of $GABA_A$Rs) within the thalamic reticular nucleus. These molecules are encoded by the *Dbi* gene and modulate thalamocortical oscillations.

Since their initial discovery over 50 years ago, benzodiazepines have become one of the most commonly prescribed medications in the fields of Psychiatry and Neurology. Thanks to their ease of administration (orally), potency, efficacy, and low toxicity, benzodiazepines are widely used as anti-anxiety, anticonvulsant, sedative, and muscle-relaxing agents. One mechanism by which these medications mediate their effect involves increasing the duration of inhibitory postsynaptic currents (IPSCs) through $GABA_A$Rs, thereby enhancing inhibitory synaptic transmission (Mody et al., 1994). Biochemical studies have revealed the presence of a benzodiazepine binding site, termed the benzodiazepine receptor (BR), within $GABA_A$Rs to which benzodiazepines can bind and mediate their pharmacologic effects (Braestrup and Squires, 1977; Möhler and Okada, 1977).

It turns out that benzodiazepines are not the only molecule able to bind to the BR within $GABA_A$Rs. In fact, a diversity of small molecules can bind this site and produce a wide array of effects. Classically, these effects are divided into three categories: (1) positive allosteric modulators (PAMs) like the traditional benzodiazepines that enhance GABAR-mediated function; (2) negative allosteric modulators (NAMs), such as beta-carbolines, that reduce GABAR-mediated function; and (3) antagonists, such as flumazenil, that block the actions of both PAMs and NAMs by competing with them for access to the BR (Braestrup et al., 1980; Hunkeler et al., 1981; Mody et al., 1994).

The discovery of the BR within $GABA_A$Rs led to the hypothesis that the CNS produces endogenous molecules that bind to this site and serve as allosteric modulators of $GABA_A$Rs—molecules that have been referred to as "endozepines" (Iversen, 1977). This hypothesis in turn led to the discovery of a 10 kDa protein termed diazepam binding inhibitor (DBI), also known as acyl-CoA binding protein (Knudsen, 1991). Elimination of the gene encoding this protein has been linked to negative allosteric modulatory effects on $GABA_A$Rs, one consequence of which is to promote neurogenesis postnatally in the subventricular zone (Alfonso et al., 2012). This success in identification of endogenous NAMs notwithstanding, discovery of endogenous PAMs has proven more challenging. Antagonists of the BR reduce GABA-mediated IPSCs recorded from acutely isolated hippocampal slices and cultured cortical neurons (King et al., 1985; Vicini et al., 1986). These findings are consistent with the presence of an endogenous PAM. However, these results could also be explained by negative modulatory effects of these compounds on $GABA_A$Rs, thus precluding a definitive conclusion.

In this issue of *Neuron*, Christian et al. (2013) continue the search for an endogenous PAM. Christian et al. (2013) focus their search within a single thalamic nucleus—the reticular nucleus (nRT). The nRT plays a critical gating role in oscillatory firing between thalamic and cortical circuits (Steriade et al., 1993). Synaptic inhibition intrinsic to nRT functions to control these oscillations and a reduction of such inhibition manifests as epileptiform oscillations that promote absence seizures (Sohal and Huguenard, 2003). Interestingly, benzodiazepines can suppress these thalamocortical oscillations by enhancing inhibition within nRT (Sohal et al., 2003). Furthermore, humans with a mutation of the γ2 subunit of $GABA_A$Rs that disrupts the BR commonly develop absence seizures (Wallace et al., 2001). Together, these observations led Christian et al. (2013) to hypothesize that a PAM of $GABA_A$Rs resides within the nRT and that it functions to enhance synaptic inhibition, thereby limiting thalamocortical oscillations.

In pursuit of this hypothesis, several key findings emerged. First, Christian et al. (2013) studied mutant animals with a point mutation of the α3 subunit of $GABA_A$R (α3(H126R)) which disrupts the BR. Whole-cell recordings from neurons within nRT revealed reduced duration of both spontaneous ISPCs (sIPSCs) and evoked IPSCs (eIPSCs) in slices from mutant animals compared to wild-type controls. Responses of outside-out patches from WT and mutant nRT cells to laser-evoked GABA uncaging were similar, arguing that differences in GABA affinity, chloride conductance, or $GABA_A$R expression did not account for the differences observed in IPSCs. Moreover, a BR antagonist reduced duration of IPSCs in nRT cells of slices of wild-type but not mutant animals. These findings are consistent with the presence of an endogenous PAM within nRT of wild-type mice.

Christian et al. (2013) provide additional convincing evidence of a PAM residing within nRT by examining an adjacent thalamic nucleus—the ventrobasal (VB) nucleus. In contrast to neurons within nRT, a BR antagonist had no effect on the duration of IPSCs of neurons within VB. Might this be due to differences in

the nature of $GABA_A$Rs in the two nuclei? Or might PAM activity be present within nRT but not VB? To distinguish these possibilities, Christian et al. (2013) performed an elegant series of experiments combining "sniffer patches" with GABA uncaging. Outside-out membrane patches containing $GABA_A$Rs were obtained from VB cells and then placed into either VB or nRT within thalamic slices. Moving the patches from VB to nRT resulted in an increased duration of the GABA response within nRT compared to VB. These results exclude the possibility that differences in composition of $GABA_A$Rs are sufficient to account for the different responses to the BR antagonist in VB compared to nRT. Instead the results provide powerful support for the presence of a PAM within nRT.

In search of the molecular identity of the PAM, Christian et al. (2013) explore DBI—a protein that is highly expressed in nRT and has previously been shown to bind the BR of $GABA_A$Rs. Using a mouse lacking a 400 kb region of chromosome 1 (*nm1054*) containing the *Dbi* gene plus several others, Christian et al. (2013) detect a reduction of sIPSC duration in the mutant animal compared to wild-type controls. These findings are similar to those observed in mice with a disrupted BR (α3(H126R)). Importantly, the reduced IPSC duration was rescued by viral expression of *Dbi*, demonstrating that loss of this gene in particular is sufficient to account for the reduced IPSC duration. These findings provide strong evidence that the *Dbi* gene encodes the endogenous PAM within nRT.

The fact that inhibition within nRT plays a critical role in regulating thalamic oscillations led Christian et al. (2013) to query whether the reduced duration of IPSCs within nRT neurons might be associated with enhanced sensitivity to absence seizures in a chemoconvulsant model. Indeed, enhanced sensitivity to chemoconvulsant-induced seizures was detected in mice lacking the *Dbi* gene (*nm1054* mice). Similarly, mice with a disrupted BR in their $GABA_A$Rs (α3(H126R)) exhibited prolonged epileptiform activity in response to the chemoconvulsant. These findings are consistent with the proposal that an endogenous PAM within nRT, specifically encoded by the *Dbi* gene, reduces susceptibility to absence seizures by enhancing $GABA_A$R function.

In sum, this lovely series of experiments establishes the presence of a PAM of $GABA_A$R function that acts through the BR. Additionally, this work narrows the molecular identity of this PAM to a product of a single gene—*Dbi*; that said, unanswered questions persist. Christian et al. (2013)'s immunohistochemical study reveals expression of the *Dbi* gene within both VB and nRT, yet PAM effects are detectable only within nRT. Moreover, in contrast to the PAM effects linked to the *Dbi* gene in the present study, NAM effects have been linked to the *Dbi* gene in work by Alfonso et al. (2012). How can products of the same gene function as both NAMs and PAMs (Figure 1)? Do distinct peptides derived from the *Dbi* gene mediate these opposing effects? Or might distinct post-translational modifications of the same peptide result in opposing effects? And from what cells are these peptides released and how?

Answers to these questions promise to inform how $GABA_A$Rs function in the healthy nervous system. Additionally, as demonstrated in this present study, disordered function of PAMs and/or NAMs may contribute to some diseases of the nervous system. As such, these PAMs and NAMs may provide novel targets for new classes of pharmacological agents that modulate $GABA_A$R function similar to benzodiazepines but ideally without the tolerance and dependence associated with chronic benozdiazpine use.

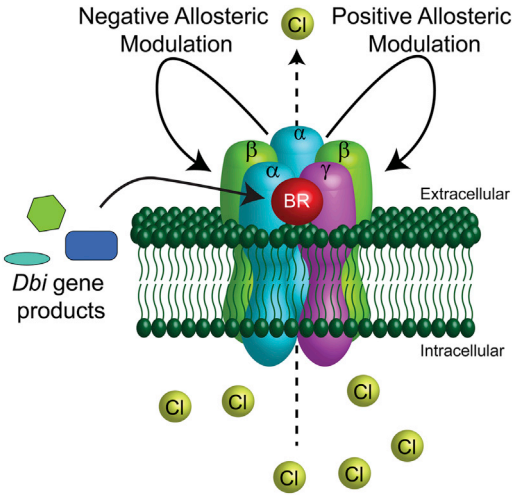

Figure 1. *Dbi* Gene Products Can Bind to the BR of $GABA_A$Rs and Positively or Negatively Modulate GABA-Mediated Function

Following the work of Christian et al. (2013), there is now evidence that the various products arising from the *Dbi* gene can bind to the BR of $GABA_A$Rs and act as both PAMs and NAMs in the CNS. The specific function (either positive or negative) appears to be brain region specific. Understanding how these opposing effects arise from the same gene will shed light on $GABA_A$R function in health and disease.

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