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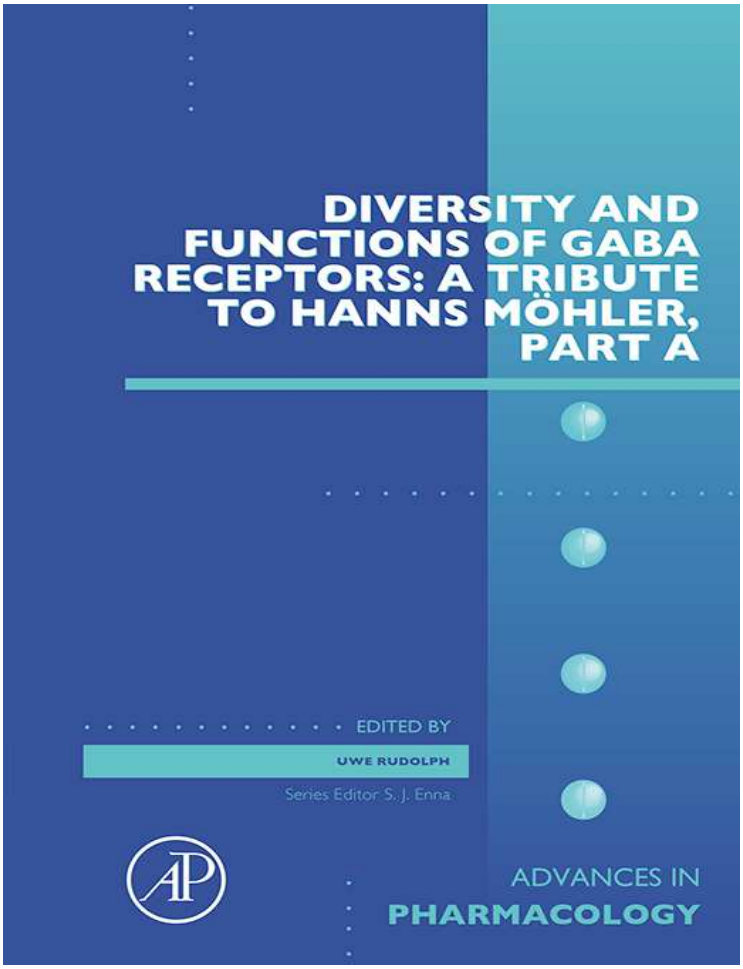

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CHAPTER FIVE

Endozepines

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Contents

Abstract

Since their introduction in the 1960s, benzodiazepines (BZs) remain one of the most commonly prescribed medications, acting as potent sedatives, hypnotics, anxiolytics, anticonvulsants, and muscle relaxants. The primary neural action of BZs and related compounds is augmentation of inhibitory transmission, which occurs through allosteric modulation of the gamma-aminobutyric acid (GABA)-induced current at the gamma-aminobutyric acid receptor ($GABA_AR$). The discovery of the BZ-binding site on $GABA_ARs$ encouraged many to speculate that the brain produces its own endogenous ligands to this site (Costa & Guidotti, 1985). The romanticized quest for endozepines, endogenous ligands to the BZ-binding site, has uncovered a variety of ligands that might fulfill this role, including oleamides (Cravatt et al., 1995), nonpeptidic endozepines (Rothstein et al., 1992), and the protein diazepam-binding inhibitor (DBI) (Costa & Guidotti, 1985). Of these ligands, DBI, and affiliated peptide fragments, is the most extensively studied endozepine. The quest for the "brain's Valium" over the decades has been elusive as mainly negative allosteric modulatory effects have been observed (Alfonso, Le Magueresse, Zuccotti, Khodosevich, & Monyer, 2012; Costa & Guidotti, 1985), but recent evidence is accumulating that DBI displays regionally discrete endogenous positive modulation of GABA transmission through activation of the BZ receptor (Christian et al., 2013). Herein, we review the literature on this topic, focusing on identification of the endogenous molecule and its region-specific expression and function.

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ABBREVIATIONS

BZ benzodiazepine
BZR benzodiazepine receptor
CBR central benzodiazepine receptor
CNS central nervous system
DBI diazepam-binding inhibitor
DZP Diazepam, Valium®
FLZ flumazenil
GABA gamma-aminobutyric acid
$GABA_AR$ gamma-aminobutyric acid receptor
PBR peripheral benzodiazepine receptor
PAM positive allosteric modulator
NAM negative allosteric modulator
ODN octadecaneuropeptide
TTN triakontatetraneuropeptide

1. INTRODUCTION

In 1977, two separate groups utilized radiolabeled diazepam (DZP) binding to brain extracts to identify benzodiazepine receptors (BZRs) in the central nervous system (CNS) (Braestrup & Squires, 1977; Möhler & Okada, 1977; see chapter "The Legacy of the Benzodiazepine Receptor: From Flumazenil to Enhancing Cognition in Down Syndrome and Social Interaction in Autism" by H. Mohler, in this volume). This occurred shortly after identification of endogenous ligands acting on opiate receptors (Hughes et al., 1975), termed endorphins. These findings along with a number of studies demonstrating innate BZ-like physiological activity led investigators to hypothesize that the brain might produce endogenous BZR ligands or endozepines (Costa & Guidotti, 1985; Iversen, 1977). The hunt for endozepines has proven to be exceptionally challenging due to their complex pharmacological and physiological activities. With an erratic history spanning more than three decades, studies in pursuit of these mysterious endozepines and their abounding physiological functions persist.

The molecular mechanism of BZ activity was first indicated by the discovery of that BZs influence GABA function (Costa, Guidotti, & Mao, 1975; Haefely et al., 1975). Later, the purified BZR protein complex was shown to contain binding sites for both GABA and BZs (Schoch, Häring, Takacs, Stähli, & Möhler, 1984; Schoch & Möhler, 1983; Sigel & Barnard, 1984), suggesting that BZs and GABA bind to the same

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receptor. Heterologous expression of recombinant gamma-aminobutyric acid receptors ($GABA_AR$s) revealed that BZs bind to an integral allosteric modulatory site (the central benzodiazepine receptor, CBR) located on the $GABA_AR$ and, once bound, modulate the GABA induced chloride current by modifying the apparent GABA-binding affinity (Seeburg et al., 1990). In the remainder of this chapter, we largely focus on this BZR, the so-called CBR—the pharmacophore that directly modulates GABA function. A distinct binding site not affiliated with $GABA_AR$ binding, the peripheral benzodiazepine receptor (PBR), will be discussed below. BZ-binding site ligands, such as the BZ Diazepam (DZP), that enhance the actions of GABA are classified as CBR agonists or positive allosteric modulators (PAMs). Ligands that bind to the BZ-binding site and reduce the actions of GABA, such as beta-Carbolines, are known as CBR-inverse agonists or negative allosteric modulators (NAMs). Additionally, ligands such as Flumazenil (FLZ) and similar compounds (Hunkeler et al., 1981) bind the BZ-binding site and inhibit the effects of both NAMs and PAMs, but they have no intrinsic effect on the actions of GABA and are considered as BZ antagonists.

Initial attempts to identify endozepines relied on radioligand-binding assays in which isolated brain extracts were shown to displace ^{3}H-BZs from brain membranes. Using this method, several putative endozepines were identified, yet evidence for physiological modulation by these ligands has generally lagged behind, in some cases for decades. Recent studies breathe new life into the unremitting search for endozepines and their role in regulation of GABA transmission.

2. PHYSIOLOGICAL EVIDENCE OF ENDOZEPINES

The synthesis of RO 15-1788 (or flumazenil, FLZ), the first known BZ antagonist (Hunkeler et al., 1981; Ramerstorfer, Furtmüller, Vogel, Huck, & Sieghart, 2010), facilitated a large body of research supporting the hypothesis that endogenous ligands to the BZ-binding site exist and are functionally relevant *in vitro* and *in vivo*. While FLZ has been a valuable tool for identification of physiological BZ actions, its use for this purpose has limitations. For example, it has been shown that FLZ can exert PAM effects in heterologously expressed $GABA_AR$s, especially at high concentrations (Ramerstorfer et al., 2010). Importantly, FLZ has never been shown to have NAM effects on heterologously expressed $GABA_AR$s, suggesting that any

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NAM effects of FLZ on GABA-mediated inhibition are most likely due to antagonism of an endogenous ligand.

In vitro studies have suggested a number of circuits in which endozepines are constitutively expressed and physiologically active, as evidenced by suppressive effects of FLZ on GABA-mediated inhibition, mainly through a decrease in response duration. For example, FLZ suppresses IPSP(C)s in hippocampus (King, Knox, Dingledine, 1985; Krespan, Springfield, Haas, & Geller, 1984) and in neocortical neuronal cultures (Vicini et al., 1986). FLZ has been shown to suppress inhibition in dentate gyrus granule cells in the pilocarpine model of temporal lobe epilepsy (Leroy, Poisbeau, Keller, & Nehlig, 2004) and to suppress inhibition in layer II/III neocortical pyramidal neurons (Ali & Thomson, 2008). Long-term potentiation of inhibitory synapses in hippocampal CA1 area was associated with an increase in IPSC amplitude that was suppressed by FLZ (Xu & Sastry, 2005). Most recently, it has been shown that FLZ suppresses synaptic inhibition neurons of the thalamic reticular nucleus (nRt, Christian et al., 2013), indicating the presence of an endozepine in this nucleus. Together, these studies with FLZ strongly support the presence of endogenous PAM activity in several distinct brain regions, indicating this is a broadly implemented endogenous modulatory mechanism in the CNS.

A number of interesting clinical findings are consistent with FLZ antagonism of endozepine function. For example, FLZ treatment can induce panic attacks in patients with panic disorder but not in healthy controls (Nutt, Glue, Lawson, & Wilson, 1990). It can also precipitate greater panic response in women with premenstrual dysphoric disorder compared to controls (Le Mellédo, Van Driel, Coupland, Lott, & Jhangri, 2000) and to reverse stupor associated with hepatic encephalopathy (Als-Nielsen, Gluud, & Gluud, 2004; Baraldi et al., 2009). Together these studies suggest a physiological role for an accumulation of endozepines in the extracellular space, i.e., a physiological buildup, in regulating anxiety/panic. In addition, serum levels of substances that inhibit FLZ binding to rat cerebellar membranes were reported to increase twofold during delivery by spontaneous labor, an effect not seen in patients undergoing cesarean section (Facchinetti, Avallone, Modugno, & Baraldi, 2006), suggesting that physiological states such as labor might cause endozepine buildup.

The relevance of these findings to human epilepsy patients remains for the moment unknown, as evidence for endozepine actions in epilepsy patients is inconclusive. FLZ can provoke seizures in patients, but at least some patients were likely receiving BZ treatment (Spivey, 1992). For

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example, in a series of 67 patients undergoing evaluation prior to epilepsy surgery (Schulze-Bonhage & Elger, 2000), seizures were provoked in 8 (12%)—all had previously been treated with BZs. Case reports suggest worsening of seizures by FLZ in infants and in the elderly (McDuffee & Tobias, 1995; Thomas, Lebrun, & Chatel, 1993). The reported effects of FLZ on seizures in animal studies are also mixed. In rats younger than 2 weeks, FLZ worsens minor motor seizures induced by PTZ (Rathouská, Kubová, Mares, & Vorlícek, 1993). Notably in high-dose convulsant models, either suppression of seizure activity (Kaijima, Le Gal La Salle, & Rossier, 1983) or no effect (Hunkeler et al., 1981) has been reported. In the GAERS model of genetic spontaneous absence epilepsy, FLZ had concentration-dependent effects, with low doses suppressing spike-wave discharges (SWDs) and higher doses enhancing them (Marescaux et al., 1984). In addition, a genetic mutation (R43Q) in the human γ2 subunit associated with both familial absence and febrile seizures was shown to abolish (Wallace et al., 2001) or to reduce (Bowser et al., 2002) *in vitro* sensitivity of specific $GABA_A$Rs to DZP. The mechanism for increased seizure activity related to receptor insensitivity to DZP, an exogenous ligand, is not yet known. One provocative hypothesis is that the mutation renders the receptors insensitive to a naturally occurring endogenous BZ. Since an endogenous BZ would likely have antiseizure properties, mutations in the receptor which prevent binding of the endogenous BZ would be expected to cause seizures. However, receptor trafficking is also affected by this mutation (Kang & Macdonald, 2004; Sancar & Czajkowski, 2004) and could contribute to the seizure activity, so the role of endozepines in seizures related to the γ2R43Q mutation remains controversial.

FLZ has also been reported to reverse idiopathic recurrent stupor (Rothstein et al., 1992), although in recent years, it has become evident that at least some patients who responded to FLZ had surreptitious BZ usage (Granot, Berkovic, Patterson, Hopwood, & Mackenzie, 2004) and this field remains controversial (Cortelli et al., 2005). In a recent study, FLZ normalized vigilance in a well-characterized group of patients with hypersomnia. A peptidergic PAM activity was found in cerebrospinal fluid (CSF) of these patients (Rye et al., 2012). However, the PAM did not interact with a potentiation by the BZ midazolam and partly persisted in α1(H101R), $GABA_A$Rs with a point mutation rendering them BZ insensitive, indicating that it may not be a classical BZ-mimicking agent (Rye et al., 2012). The identity of this CSF PAM and its role in the pathophysiology of hypersomnia remain unknown.

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3. CANDIDATE ENDOZEPINES

Efforts to uncover endogenous ligands for the BZ-binding site have led to identification of several putative endozepines (Costa & Guidotti, 1985). These molecules have included peptides, fatty acid derivatives, and small organic molecules including purine metabolites and naturally occurring BZs. A number of studies describe isolation of naturally occurring BZs (e.g. molecules with a benzene ring fused to a diazepine ring) including DZP and nordiazepam from animal brains (Medina, Peña, Piva, Paladini, & De Robertis, 1988; Rothstein et al., 1992; Sangameswaran & de Blas, 1985; Sangameswaran, Fales, Friedrich & De Blas, 1986; Unseld, Krishna, Fischer, & Klotz, 1989), but interpretations of these findings were limited due to the inability to discriminate between endogenous and exogenous BZs and possible contamination. In 1990, Unsled et al. observed the presence of BZs in human brain tissue specimens banked before the initial reports of BZ synthesis in the 1950s, essentially excluding contamination with synthetically derived BZs as an explanation at least in those cases (Unseld, Fischer, Rothemund, & Klotz, 1990). However, natural BZs have been found in plants, plant products, and soil (Unseld et al., 1990, 1989; Wildmann et al., 1987, 1988) suggesting ingestion of exogenous naturally occurring BZs as an explanation for the presence of these compounds in brain tissue.

Fatty acids and other small organic molecules have also been proposed as putative endozepines. Oleamides isolated from sleep-deprived animals have been shown to have hypnotic effects (Cravatt et al., 1995). These effects depend on the expression of the $GABA_AR$ ß3 subunit (Laposky, Homanics, Basile, & Mendelson, 2001), but direct evidence for these compounds binding to the BZ-binding site and activating the $GABA_AR$ is lacking. Other small molecule candidate ligands including inosine, hypoxanthine, and nicotinamide have low affinity for the $GABA_AR$ BZ-binding site and are present in low concentrations in the brain and thus are unlikely to represent the natural physiological relevant endozepines (Asano & Spector, 1979; Bold, Gardner, & Walker, 1985; Lapin, 1980; Tallman, Paul, Skolnick, & Gallager, 1980).

4. DIAZEPAM-BINDING INHIBITOR

The most widely studied endozepine to date is diazepam-binding inhibitor (DBI). This 10 kDa protein was originally isolated and purified from

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rat brain based on its ability to displace exogenous BZs (DZP) from whole brain membrane preps (Guidotti et al., 1983). DBI is highly conserved across eukaryotic species from yeast to mammals (Gray, Glaister, Seeburg, Guidotti, & Costa, 1986; Lihrmann et al., 1994; Mocchetti, Einstein, & Brosius, 1986; Owens, Sinha, Sikela, & Hahn, 1989). Early studies of DBI confirmed its expression in the brain—both in neurons and astrocytes (Alho et al., 1985; Alho, Bovolin, Jenkins, Guidotti, & Costa, 1989; Alho, Harjuntausta, Schultz, Pelto-Huikko, & Bovolin, 1991). However, concerns about its role as a neuromodulator were raised when it was determined that DBI is identical to acyl-CoA-binding protein (Knudsen, 1991), a well-characterized cytosolic protein with a primary role in fatty acid metabolism (Mogensen, Schulenberg, Hansen, Spener, & Knudsen, 1987). Furthermore, it was unclear how a cytosolic protein could interact with an extracellular BZ-binding site on $GABA_A$Rs in the intact brain. Consequently, interest in DBI as a modulator of GABA signaling waned over the following decade.

Subsequent studies of the social amoeba *Dictyostelium discoidium*, however, have demonstrated that the homologue of DBI in this organism is secreted through an unconventional pathway and, after activation by proteolytic cleavage in the extracellular space, binds to and activates a cell surface receptor as part of the pathway for starvation-induced sporulation (Manjithaya, Anjard, Loomis, & Subramani, 2010). As earlier immunlocalization assays demonstrated strong expression of DBI in astrocytes, studies on release of DBI in the mammalian brain have focused on astrocytes and the roles they might play in DBI signaling. Indeed, cultured astrocytes from rat brain have been found to readily secrete DBI through an unconventional pathway that can be induced by autophagy, similar to that described for secretion by *Dictyostelium* (Loomis, Behrens, Williams, & Anjard, 2010). Several means of DBI secretion have been demonstrated in astrocyte cultures, indicating that DBI release is a common downstream consequence of several distinct signal pathways. For example, treatment with steroid hormones (Loomis et al., 2010), PAC1-R ligands (Masmoudi et al., 2003), urotensin II (Jarry et al., 2010), elevated K^+ (Ferrarese et al., 1987; Qian, Bilderback, & Barmack, 2008), and ß-amyloid (Tokay et al., 2008) induces secretion, while somatostatin and $GABA_B$ receptor activation inhibit release (Masmoudi et al., 2005). Experiments with brain slices performed under conditions in which astrocytes were metabolically poisoned by the aconitase inhibitor fluorocitrate (Christian & Huguenard, 2013b) failed to demonstrate a DBI-dependent endozepine activity in nRt, suggesting that astrocytes are a primary source of DBI-dependent endozepines.

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Ten different DBI transcript variants have been reported, with region-specific expression (Ludewig, Klapper, Wabitsch, Döring, & Nitz, 2011; Ludewig, Nitz, Klapper, & Döring, 2011; Nitz, Kruse, Klapper, & Döring, 2011), all of which have an alternative promoter or first exon differentiating the proteins at the 5′-end. DBI promoters display multiple sites for transcription factors, including AP-1/2, SP-1, ETF, Y-box-binding protein, CTF/NF-1, C/EBP, HNF-3, SRE-like sequence, GREs, and PPREs. The role for different transcripts in the different putative functions of DBI (fatty acid metabolism and modulation of $GABA_AR$ signaling) remains unclear.

DBI has a number of putative endoprotease sites and several cleavage products including triakontatetraneuropeptide (TTN, DBI(17–50)), octadecaneuropeptide (ODN, DBI(33–50)), and octapeptide (OP, DBI (43–50)) (Ferrero, Santi, Conti-Tronconi, Costa, & Guidotti, 1986) which have been identified in rat and rhesus monkey CNS (Alho et al., 1989, 1991; Slobodyansky, Kurriger, & Kultas-Ilinsky, 1992) and all demonstrate the ability to displace BZs and modulate inhibition via allosteric modulations of the $GABA_AR$. The majority of evidence for the action of DBI peptides at $GABA_ARs$ suggests negative allosteric modulation. Indeed, exogenous application of DBI reduces synaptic inhibition in cultured neurons (Bormann, 1991; Costa & Guidotti, 1991). Recently, a NAM effect of ODN was demonstrated on GABA-mediated currents from progenitor cells of the subventricular zone. Activation of $GABA_ARs$ on neural progenitors favors their differentiation to neuroblasts while ODN reduces GABA-evoked currents and increases proliferation, suggesting a natural role for ODN in the regulation of this critical developmental process (Alfonso, Le Magueresse, Zuccotti, Khodosevich, & Monyer, 2012). By contrast, Christian et al. have provided evidence that DBI expression is necessary for PAM endozepine activity in thalamic reticular nucleus (nRt). Animals devoid of DBI gene product did not show FLZ effects, while viral overexpression of DBI rescued the response. These data showed that *dbi* gene and products were required for the thalamic endozepine activity (Christian et al., 2013). This study further demonstrated that endozepine expression in the thalamus was nucleus specific. FLZ effects on synaptic inhibition were only observed in nRt, but not in relay nuclei such as the ventrobasal (VB) complex of VPL/VPM. Sniffer patch biosensors, made up of out-side out membrane patches obtained from VPL/VPM relay neuron membranes (Christian & Huguenard, 2013a), were able to detect endozepine actions, as shown by FLZ effect, when the patches were placed within nRt, but

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not in VB. This result had two important conclusions, as follows: (1) the endozepine response was not dependent on the specific $GABA_AR$ composition in nRt, $\alpha3\beta3\gamma2$ (Pirker, Schwarzer, Wieselthaler, Sieghart, & Sperk, 2000), as they were detected with patches from relay neurons, which express a quite distinct receptor composition: $\alpha1\beta2\gamma2$, and (2) the endozepines were constitutively expressed in the extracellular space, site of BZ binding on $GABA_ARs$, only in nRt, pointing to a nucleus-specific DBI processing path/way that locally secretes DBI to the extracellular space where it is presumably cleaved to the final PAM product (Christian et al., 2013). Thus, DBI appears to be capable of both PAM and NAM effects on $GABA_AR$ signaling. It is not yet known if specific peptide ligand fragments or subunit-specific $GABA_ARs$ mediate these opposing actions of DBI.

Behavioral studies of DBI peptides activity have revealed a complex picture. For example, ODN and fragments were reported to suppress PTZ-induced seizures in rats and audiogenic seizures in dba/2j mice, with both effects blocked by FLZ (Garcia de Mateos-Verchere, Leprince, Tonon, Vaudry, & Costentin, 1999). Notably, maximal electrical shock-induced seizures were not affected. Based on a U-shaped ODN dose–response relationship for PTZ seizures, these authors suggested that ODN itself may be an inverse agonist (i.e., NAM), perhaps at unique $GABA_AR$ subunit combinations, and these actions would compete with an agonistic (i.e., PAM) proteolytic fragment of ODN. At high ODN doses then the antagonist effect of the parent compound would dominate through competitive interaction at the BZ-binding site. Another study demonstrated proconvulsive effects of ODN when injected into the brain (Ferrero et al., 1986). Intracerebroventricular injections of DBI, ODN, and TTN into rats induced anxiogenic activity via the BZ-binding site located on $GABA_ARs$ and therefore were antagonized by FLZ (Slobodyansky, Berkovich, Bovolin, & Wambebe, 1990; Slobodyansky, Guidotti, Wambebe, Berkovich, & Costa, 1989). Inhibition within nRt is proposed to regulate absence seizure generation (Huntsman, Porcello, Homanics, DeLorey, & Huguenard, 1999; Schofield, Kleiman-Weiner, Rudolph, & Huguenard, 2009) and recent studies suggest the existence of a DBI-related peptide that serves a natural seizure-regulating function in the nRt (Christian et al., 2013). Consistent with this was the finding that $\alpha3H126R$ mice, devoid of endozepine sensitivity in nRt neurons, experienced more intense seizures that presumably resulted from lack of this endogenous, adaptive, regulation (Christian et al., 2013). Although DBI peptide(s) play a role in circuit excitability

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and network synchronization, the mechanisms by which DBI peptide(s) regulate seizure activity either constitutively or in an activity-dependent fashion at this point remain unclear.

It is important to note that DBI peptides can modulate GABA-mediated currents through an alternate pathway distinct from binding to the BZ-binding site on $GABA_A$Rs. Whereas DBI and ODN were originally found to bind the BZ-binding site on $GABA_A$Rs, it was later discovered that DBI and TTN can bind a second BZR, the so-called peripheral benzodiazepine receptor (PBR) (Slobodyansky et al., 1990, 1989). The PBR, also known as translocator protein, is a cholesterol transporter located in outer mitochondrial membranes found ubiquitously in non-neuronal and neuronal tissue (Verma & Snyder, 1989; Gavish, Katz, Bar-Ami, & Weizman, 1992). DBI (and fragments) binding to the PBR stimulates cholesterol transport into mitochondria, increasing the concentration of this metabolite which is rate limiting for neurosteroid synthesis (Costa et al., 1994; Korneyev et al., 1993). Neurosteroids bind to an allosteric site on the $GABA_A$R, distinct from the BZ-binding site, and potentiate synaptic and extrasynaptic $GABA_A$R function (Puia, Vicini, Seeburg, & Costa, 1991; Porcello, Huntsman, Mihalek, Homanics, & Huguenard, 2003; see chapter "Inhibitory Neurosteroids and the $GABA_A$ Receptor" by T. Smart, in this volume). Therefore, DBI peptides are capable of binding to and directly modulating $GABA_A$R-mediated phasic (synaptic) inhibition and indirectly (via neurosteroid synthesis) modulating $GABA_A$R-mediated tonic (extrasynaptic) inhibition; thus providing multiple distinct and potentially cooperative means of adaptive inhibitory control in the brain.

5. CONCLUSION

The search for endozepines began over 30 years ago, and recent studies have identified clear DZP-like, PAM actions, yet several key unanswered questions remain. Are the naturally occurring BZs found in the CNS exclusively from an endogenous source such as DBI, or might there also be contributions from the environment, for example, from dietary sources? In either case, what are the processing pathways that produce and/or modify such ligands? Regarding DBI, what mechanisms serve to mediate nucleus-specific secretion, as is the case in the thalamus where DBI/BZ effects were only observed in the ventral thalamus (nRt) but not dorsal thalamus (VB)? How can a single DBI gene encode products with both PAM and NAM effects on the $GABA_A$R? Two possible explanations for the

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opposing activity of DBI are that (1) DBI peptide activity at $GABA_AR$s varies depending on a specific peptide fragment that binds and/or (2) that DBI peptide(s) activity varies depending on the specific $GABA_AR$ subunit composition. To characterize binding and activity specificity of the individual DBI peptide products at distinct $GABA_AR$s, several key questions must be answered. For example, which of the various neuronal factors that regulate the intricate expression, processing, and release of DBI peptide fragments are involved in producing peptides capable of $GABA_AR$ modulation? Which other posttranslational modifications are required for DBI peptides to form an active PAM or NAM? Altogether, the studies of idiopathic recurrent stupor suggest an unidentified $GABA_AR$ PAM(s) still remains. Accordingly, what is their identity and how are they produced, secreted, and/or metabolized?

This chapter, along with previous studies, attends to the role of endozepine activity on synaptic (phasic) inhibition. The majority of extrasynaptic $GABA_AR$s mediating extrasynaptic (tonic) inhibition contain the δ subunit, rendering them insensitive to BZs (Nusser & Mody, 2002). However, there is a growing body of evidence that tonic inhibition, in some cells, is mediated via α5 and γ2 subunit-containing receptors capable of BZ modulation (Jo et al., 2011) and, therefore, may be sensitive to direct endozepine modulation. Furthermore, endozepine activation of the PBR stimulates the synthesis of neurosteroids, known to affect α4 or α6 and δ subunit-containing extrasynaptic tonic receptors (Stell, Brickley, Tang, Farrant, & Mody, 2003), providing a potential pathway for endozepines to indirectly regulate tonic inhibition. The capacity to which endozepines directly or indirectly modulate $GABA_AR$-mediated tonic inhibition is a compelling question; however, it has yet to be addressed experimentally.

BZs are one of the most commonly prescribed medications for a variety of psychiatric and neurological disorders. There is substantial evidence that endozepines also play a critical role in many of these disorders, for example, by providing an endogenous mechanism for regulation of anxiety and seizures. Answers to the remaining questions will further our understanding of the complex inhibitory mechanisms in neuronal processing of the healthy brain as well as $GABA_AR$-related disorders and could enable site-directed drug design for such disorders.

CONFLICT OF INTEREST

None.

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