Intracellular CICR
-associated pharmacological mechanism of action of antiepileptic drugs

Gang Zhu¹, Motohiro Okada², Shukuko Yoshida³ and Sunao Kaneko³

¹Department of Psychiatry, the First Hospital of China Medical University, Shenyang110001, China
²Department of Psychiatry, Division of Neuroscience, Graduate School of Medicine, Mie University, Tsu 514-8507, Japan
³Department of Neuropsychiatry, Graduate School of Medicine, Hirosaki University, Hirosaki 036-8562, Japan

Key words: antiepileptic drugs, carbamazepine, zonisamide, mechanism of action, calcium-induced calcium releasing systems

Published online September 3, 2010

Summary

Carbamazepine (CBZ) and zonisamide (ZNS) are antiepileptic drugs (AEDs) with multiple mechanisms of action, including inhibition of voltage-dependent sodium and calcium channels, enhancement of inhibitory events mediated by GABAergic neurotransmission, and blockade of the glutamatergic neurotransmission in the brain. Recently, the intracellular signaling pathways have been implicated as the new targets of AEDs. Especially, we have investigated the functional importance of Ca²⁺ mobilization, composed of influx through Ca²⁺ channels and output through ryanodine receptor (RyR)- and inositol-triphosphate receptor (IP3R)-sensitive intracellular Ca²⁺-induced Ca²⁺ releasing systems (CICRs), in the pathogenesis of epilepsy and the pharmacological mechanism of AEDs.
In this review, we discuss the actions of CBZ and ZNS on neurotransmitter exocytosis associated with RyR-sensitive CICR. Further studies on the mechanisms of action of AEDs may help to understand the clinical benefits of AEDs in the treatment of epilepsy disorders.

**Introduction**

Epilepsy is a chronic disorder characterized by recurrent seizures [1, 2]. Epilepsy affects up to 1% of the population and causes significant morbidity [3]. Most cases of epilepsy are idiopathic, with suspected polygenic inheritance of susceptibility loci that may be influenced by environmental and developmental factors [3]. Although 20-25% of patients with epilepsy fail to achieve good control with anti-epileptic drugs (AEDs), AED remain the mainstay of therapy at present [2]. The proposed mechanism of action for most AEDs is the ability of the drugs to modulate excitatory and inhibitory neurotransmission through ion channels, neurotransmitter receptors and neurotransmitter metabolism [2]. However, the mechanisms of action that account for their anti-epileptic properties are not fully understood.

Carbamazepine (CBZ) and zonisamide (ZNS) have a wide clinical spectrum of use in the treatment of epileptic disorder [4, 5]. CBZ is used as a first-line drug against both simple and complex partial seizures, and is also effective against generalized tonic clonic seizures [6]. ZNS is effective for the treatment of a wide variety of epileptic seizures [5, 7, 8], mood disorders [9, 10] and Parkinson’s disease [11, 12]. Especially, ZNS has been well established as a major AED against both partial and generalized seizures in Japan [5]. The major mechanisms of antiepileptic actions of CBZ and ZNS are considered to be their inhibitory effects on voltage-gated Na⁺ channel (VGSC) [13, 14], voltage-sensitive Ca²⁺ channel (VSCC) [15-21], and neurotransmitter exocytosis [17-29].

Our previous studies have demonstrated the concentration-dependent effects of CBZ and ZNS on various neurotransmitter releases. At therapeutic relevant concentrations (plasma concentrations conferring antiepileptic effect), CBZ and ZNS enhance the N-type VSCC-associated dopamine (DA), serotonin (5-HT) and acetylcholine (ACh) exocytosis during the resting stage; in contrast, CBZ and ZNS inhibit the P-type VSCC-associated exocytosis during the neuronal hyperexcitable stage [18, 20, 21, 27]. Release of synaptic vesicles containing neurotransmitters is triggered by influx of Ca²⁺ through VSCC and ligand-gated ion channels, as well as output from the intracellular Ca²⁺ store associated with endoplasmic reticulum, namely the Ca²⁺-induced Ca²⁺-release system (CICR) [28-38]. The functional importance of CICRs in the pathogenesis of epileptic seizure and neuronal damage associated with epilepsy has been demonstrated by several studies [39-42]. Two types of CICRs have been identified in neurons: inositol 1,4,5-trisphosphate (IP3) receptor (IP3R)-sensitive store and ryanodine receptor (RyR)-sensitive store [30, 32]. We have recently demonstrated the up-regulation of mRNA expression of RyR in rat hippocampus following kainate-induced seizures [42]. Both
RyR and IP3R inhibitors have shown protective action against seizure-induced neuronal cell death [39, 43]. Furthermore, we have demonstrated the functional action of AEDs on CICR-associated neurotransmitter exocytosis [28, 29, 33]. However, the detailed mechanism remains to be further discussed, and this might be a key to further understand the mechanisms of antiepileptic and neuroprotective actions of AEDs including CBZ and ZNS.

Effects of CBZ and ZNS on glutamate and GABA exocytosis

A number of studies have proposed the "imbalance hypothesis": that epileptic seizures are preceded by an imbalance between excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmissions [2, 18]. To clarify the possible mechanism of the antiepileptic action of AEDs, the concentration-dependent effects of CBZ and ZNS on hippocampal glutamate and GABA releases have been elucidated in rat hippocampus, using in vivo microdialysis method [17, 28, 29].

The hippocampal basal GABA release is tetrodotoxin-sensitive, Ca\(^{2+}\)-dependent and K\(^{+}\)-sensitive, whereas the basal glutamate release is tetrodotoxin-insensitive, Ca\(^{2+}\)-independent but K\(^{+}\)-sensitive [28, 29, 44, 45]. Contrary to basal release, the K\(^{+}\)-evoked releases of GABA and glutamate are tetrodotoxin-sensitive, Ca\(^{2+}\)-dependent and K\(^{+}\)-sensitive [28, 29, 44, 45]. Perfusion with CBZ and ZNS (at therapeutic relevant concentrations) increases extracellular basal GABA level in rat hippocampus, but does not affect the extracellular basal glutamate level (Table 1) [28, 29]. On the other hand, the K\(^{+}\)-evoked releases of glutamate and GABA are inhibited by CBZ and ZNS concentration-dependently (Table 1) [28, 29].

These lines of evidence strongly suggest that under the microdialysis condition, the GABA level in hippocampal perfusate (basal GABA release) is primarily neuronal in origin, whereas the basal glutamate release is not [28, 29, 44, 45]. Contrary to the pattern for basal release, the K\(^{+}\)-evoked releases of GABA and glutamate are both neuronal in origin [28, 29, 44, 45]. CBZ and ZNS might elevate the seizure threshold resulting from the enhancement of inhibitory neurotransmission without affecting the basal glutamate release, during the resting stage; whereas, CBZ and ZNS might prevent the propagation of epileptic hyperactivation resulting from the inhibition of neurotransmission, during the hyperexcitable stage (seizures) [28, 29].

Table 1. Effects of CBZ and ZNS on glutamate and GABA release

<table>
<thead>
<tr>
<th></th>
<th>Basal release</th>
<th>K(^{+})-evoked release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glutamate</td>
<td>GABA</td>
</tr>
<tr>
<td>CBZ</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>ZNS</td>
<td>-</td>
<td>↑</td>
</tr>
</tbody>
</table>

CBZ: carbamazepine, ZNS: zonisamide, ↑: increase, ↓: decrease, -: no effect
Effects of RyR-associated CICR on glutamate and GABA exocytosis

Neurotransmitter release requires the Ca\(^{2+}\)-dependent exocytosis processes. Arriving neuronal activation at presynaptic terminals leads to Ca\(^{2+}\) influx via N-type and P-type VSCCs, which is the trigger for neurotransmitter exocytosis [31, 46]. Pharmacological analyses have demonstrated that the releases of DA, 5-HT, ACh and glutamate are regulated by the functional complexes between VSCCs and SNARE [soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein (SNAP) receptor] [18, 29, 33, 35, 45, 47, 48]. Basal releases of DA, 5-HT, ACh and GABA are regulated predominantly by the N-type VSCC/syntaxin pathway and weakly by the P-type VSCC/synaptobrevin pathway; whereas, basal glutamate release is not regulated by either functional complex [18, 29, 33, 35, 45, 47, 48]. The K\(^+\)-evoked releases of DA, 5-HT, ACh, glutamate and GABA are regulated predominantly by the P-type VSCC/synaptobrevin pathway, and weakly by the N-type VSCC/syntaxin pathway [18, 29, 33, 35, 45, 47, 48].

The Ca\(^{2+}\) influx, which increases intracellular Ca\(^{2+}\) from a basal level of 100 nM to higher than 100 µM [31, 46], activates CICRs [30-32]. CICR regulates a wide variety of neuronal processes including neurotransmitter exocytosis [30-32, 36-38]. However, overload response of CICR prevents neuronal processes and leads to neuronal toxicity [30, 34, 36-38]. Both in vivo and in vitro experiments using CICR inhibitors have demonstrated that the CICRs, including IP3R and RyR, are involved in the exocytosis of DA, 5-HT, glutamate and GABA [28, 29, 33, 36-38]. In particular, our recent studies using in vivo microdialysis have demonstrated that inhibition of RyR reduces K\(^+\)-evoked glutamate and GABA releases without affecting their basal releases [28, 29]. The RyR agonist, ryanodine, affects GABA and glutamate releases in a biphasic concentration-dependent manner [28, 29]. Perfusion with lower concentrations of ryanodine increases both basal and K\(^+\)-evoked GABA releases concentration-dependently; whereas higher concentrations of ryanodine exhibit reduced stimulatory effects on basal and K\(^+\)-evoked GABA releases (Table 2) [28, 29].

<table>
<thead>
<tr>
<th>Table 2. Effects of ryanodine on glutamate and GABA release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal release</td>
</tr>
<tr>
<td>Glutamate</td>
</tr>
<tr>
<td>Lower concentration of ryanodine</td>
</tr>
<tr>
<td>Higher concentration of ryanodine</td>
</tr>
</tbody>
</table>

: increase, ↓ or ↓↓: decrease
The extracellular basal glutamate level is increased by ryanodine concentration-dependently (both lower and higher concentrations); whereas the K+-evoked glutamate release is increased by lower concentration and decreased by high concentration of ryanodine (Table 2) [28, 29]. However, it is noteworthy that the inflection point on the concentration–response curve for ryanodine is lower with GABA release than with glutamate release in both resting and K+-evoked conditions [28, 29]. These observations suggest that RyR tends to be nonfunctional during the resting stage while the potential of RyR-sensitive CICR in neurotransmitter release could conceivably be promoted during the neuronal hyperexcitable stage [28, 29, 33]. The elevation of intracellular Ca²⁺ concentration in the active zone induced by neuronal hyperexcitability and/or overload response of CICR may reduce neurotransmitter exocytosis [28, 29, 33, 34, 36-38]. Therefore, activated RyR enhances the releases of glutamate and GABA, whereas hyperactivation of ryanodine sensitive CICR could lead to an imbalance between GABAergic and glutamatergic transmissions via predominantly breakdown of GABAergic transmission, induced by overload response of Ca²⁺ mobilization [28, 29, 38].

**Effects of CBZ and ZNS on RyR-associated glutamate and GABA exocytosis**

Pretreatment with therapeutic relevant concentrations of CBZ and ZNS inhibits the stimulatory effects of ryanodine on basal glutamate and GABA releases, and abolishes the inflection point on the concentration-response curve of ryanodine for GABA release during the resting stage [28, 29]. Likewise, CBZ and ZNS inhibit the stimulatory effects of ryanodine on the K⁺-evoked glutamate and GABA releases, and abolish the inflection point on the concentration-response curve of ryanodine for K⁺-evoked glutamate and GABA releases during neuronal hyperexcitability [28, 29].

A number of studies propose the “imbalance hypothesis”: that epileptic seizures are preceded by an imbalance between excitatory (relative enhancement) and inhibitory (relative reduction) neurotransmissions [2, 18]. Both glutamate and GABA releases depend on Ca²⁺ mobilization, composed of Ca²⁺ influx via VSCC and output via CICR [28, 29, 38, 45]. However, GABA exocytosis is more sensitive to Ca²⁺ mobilization than glutamate exocytosis [28, 29, 38]. Hyperactivation of Ca²⁺ mobilization could lead to an imbalance between GABAergic and glutamatergic transmission [28, 29, 38]. Either Ca²⁺ or Ca²⁺-associated processes is involved in the induction of epilepsy [39, 43]. Several studies have provided evidence that activation of CICRs contributes to the elevation of intracellular Ca²⁺ associated with neuronal cell damage following epileptic seizures [42, 43, 49]. Both RyR and IP3R antagonists have no effect on the induction or maintenance of epileptiform discharges, but both agents prevent seizure-induced cell death [39, 43]. CBZ and ZNS inhibit the elevation of intracellular free Ca²⁺ induced by neuronal hyperactivation.
Moreover, CBZ and ZNS reduce the RyR associated transmission system, and prevent the breakdown of the neurotransmitter release mechanism induced by hyperactivation of RyR-associated CICR [28, 29, 33].

Based on the above findings, we propose a hypothesis that a possible mechanism of the antiepileptic and neuroprotective actions of ZNS and CBZ may be at least partially due to the abolishment of the dysfunction of RyR-associated neurotransmitter exocytosis via inhibition of overload response of RyR-associated Ca\textsuperscript{2+} mobilization. CICRs might be new targeting sites for antiepileptic and neuroprotective actions of AEDs.

**Conclusion**

In this review, we have discussed the actions of CBZ and ZNS on RyR-associated neurotransmitter exocytosis; that therapeutic relevant concentrations of CBZ and ZNS inhibit the elevation of ryanodine-induced glutamate and GABA releases, and abolish the inflection point in the concentration-response curve for ryanodine on neurotransmitter. Therefore, CBZ and ZNS both protect against the breakdown of the neurotransmitter release mechanism induced by hyperactivation of RyR-associated CICR. These actions of CBZ and ZNS appear to be involved, at least partially, in the mechanisms of antiepileptic and neuroprotective actions of the two agents. Further studies on the mechanisms of action of AEDs may help us to understand the clinical benefits of AEDs in the treatment of epilepsy disorders.

**Acknowledgements**

This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture (18390316 and 18659330); a Grant from the Mitsubishi Pharma Research Foundation; a Grant from the Japan Epilepsy Research Foundation; and a Grant from National Natural Science Foundation of China (30400146).

**References**


[40] Matsumoto M., Nagata E. Type 1 inositol


