# Regional Accumulation of $^{14}$C-zonisamide in Rat Brain during Kainic Acid-induced Limbic Seizures

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**ABSTRACT:** ***Background:*** Zonisamide (ZNS) is an antiepileptic drug developed in Japan. Various experimental studies have investigated the effects of ZNS. However, the mechanism of action of ZNS against limbic seizures and secondary generalization is not well-known. We studied ictal regional accumulation of ZNS in the rat brain during kainic acid (KA)-induced limbic status epilepticus. ***Methods:*** Fourteen male Wistar rats underwent a stereotactic operation. For recording the electroencephalogram (EEG), electrodes were placed in the left amygdala (LA), left dorsal hippocampus, and over the left sensorimotor cortex. For microinjection, a stainless steel cannula was also inserted into the LA. Seven days after surgery, rats were anesthetized and a catheter was inserted into the femoral vein. The animals were immobilized and allowed to recover from anesthesia for at least two hours. In eight rats, 1.0μL (1.0μg) of KA was injected into the LA, and 1.0 μL of phosphate buffer solution was injected into the LA in six control rats. Sixty minutes after injection, $^{14}$C-ZNS was administered intravenously, and an autoradiographic study was done. ***Results:*** During limbic status epilepticus, only seizures in the sensorimotor cortex were markedly attenuated a few minutes after $^{14}$C-ZNS administration. Additionally, high uptake of $^{14}$C-ZNS was noted ipsilaterally in the sensorimotor cortex, parietal cortex and thalamus (lateral portion). In control rats, no EEG change was seen, and distribution of $^{14}$C-ZNS was rather homogeneous. ***Conclusions:*** These results suggested that ZNS suppresses secondary generalization of limbic seizures by a direct effect on the cerebral cortex.

**RÉSUMÉ: Accumulation régionale de zonisamide-C$^{14}$ dans le cerveau de rat pendant des convulsions limbiques induites par l'acide kaïnique.** ***Introduction:*** Le zonisamide (ZNS) est un antiépileptique qui a été développé au Japon. Plusieurs études expérimentales ont investigué les effets du ZNS, mais le mécanisme d'action du ZNS contre les convulsions limbiques et la généralisation secondaire demeure mal connu. Nous avons étudié l'accumulation ictale régionale de ZNS dans le cerveau de rats chez qui on a induit un status épilepticus au moyen de l'acide kaïnique (AK). ***Méthodes:*** Quatorze rats Wistar mâles ont subi une chirurgie stéréotaxique. Pour l'enregistrement électroencéphalographique (ÉEG), les électrodes ont été placées dans l'amygdale gauche (AG), l'hippocampe dorsal gauche et le cortex sensitivomoteur gauche. Une canule d'acier inoxydable a été insérée dans l'AG. Sept jours après l'intervention, les rats ont été anesthésiés et un cathéter a été inséré dans la veine fémorale. Les animaux ont été immobilisés et on les a laissés se remettre de l'anesthésie pendant au moins deux heures. Chez huit rats, 1.0 μL (1.0 μg) d'AK a été injecté dans l'AG et 1.0 μL de solution tampon au phosphate a été injecté dans l'AG chez six rats contrôles. Soixante minutes après l'injection, le ZNS-C$^{14}$ a été administré par voie intraveineuse et une étude autoradiographique a été effectuée. ***Résultats:*** Pendant le status épileptius limbique, seules les crises situées dans le cortex sensitivomoteur ont été atténuées de façon importante quelques minutes après l'administration du ZNS-C$^{14}$. De plus, une captation élevée du ZNS-C$^{14}$ a été notée dans le cortex sensitivomoteur, le cortex pariétal et le thalamus (portion latérale) ipsilatéral. Chez les rats contrôles, aucun changement ÉEG n'a été observé et la distribution du ZNS-C$^{14}$ était plutôt homogène. ***Conclusions:*** Ces résultats suggèrent que le ZNS supprime la généralisation secondaire des crises convulsives limbiques par un effet direct sur le cortex cérébral.

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Zonisamide (ZNS; 3-sulfamoylmethyl-1, 2-benzisoxazole), an antiepileptic drug developed in Japan, is used clinically to treat partial as well as generalized seizures.[1,2] While various experimental studies have investigated the effects of ZNS in animal models of epilepsy,[3-7] the mechanism of action of ZNS against limbic seizure and secondary generalization is not well-known.

We studied regional accumulation of ZNS in the rat brain by $^{14}$C-ZNS autoradiography during kainic acid (KA)-induced limbic status epilepticus.[7-14]

## Methods and materials

Fourteen male Wistar rats (250 to 300 g) were prepared for experiments by a stereotactic operation performed with

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intraperitoneal pentobarbital anesthesia (40 mg/kg). For recording the electroencephalogram (EEG), a stainless steel screw was placed in contact with the dura overlying the left sensorimotor cortex (LCx). An additional screw was placed in the frontal sinus as a reference electrode. Bipolar depth electrodes were placed in the LA and the left dorsal hippocampus (LH; coordinates A, 4.0; L, 2.0; and D, 2.5).[15,16] For microinjection, a stainless steel cannula (outer diameter, 0.6 mm) with an inner needle guide (diameter, 0.3 mm) was inserted into the left basolateral nucleus of the amygdala (left amygdala (LA); coordinates A, 5.0; L, 5.0; and D, -3.0). All electrodes as well as the cannula were fixed in place with dental cement. Rats were unrestrained during seven days of recovery from surgery, and were allowed free access to food and water prior to experiments.

Seven days after the operation, rats were anesthetized with 1.5% halothane and a catheter was inserted into the femoral vein. The rats were immobilized from the waist down by a loose-fitting plaster cast and were allowed to recover from anesthesia for at least two hours. The inner guide of the cannula was replaced with an injection needle, and 1.0 μg of KA (Sigma, St Louis, MO) dissolved at a concentration of 1.0 μg/μL in phosphate-buffered saline solution (PBS; 0.2 M at pH 7.4) was injected into the LA in eight rats. The rate of injection was 1.0 μL/min. One microliter of PBS was injected into the LA in six control rats. All procedures were performed under aseptic conditions.

Limbic status epilepticus ensued in the KA-injected rats. Sixty minutes after KA injection, while rats were exhibiting limbic status epilepticus, $^{14}$C-ZNS (6 mg/kg; specific activity, 33.4 μCi/mL) was administered via the femoral vein. Ten minutes after $^{14}$C-ZNS administation, rats were decapitated. Brains were removed immediately and frozen in liquid Freon (-40°C). Consecutive 20 μm thick coronal sections were cut with a cryostat, affixed to glass coverslips, and dried at 60°C. Autoradiograms were prepared by exposing Kodak SB-5 film in a radiographic cassette to dried sections for seven days.

Optical densities in autoradiograms were quantitated with a Macintosh computer using the public domain National Institutes of Health (NIH) Image program (written by Wayne Rasband at the US NIH). Regional accumulation ratios were calculated as optical density of the structure of interest/optical density of the pineal body, which lies outside the blood-brain barrier (BBB). Multiple comparisons for structures of interest were made between ipsilateral side of the control and the others: contralateral side of the control, ipsilateral and contralateral side of KA-injected rats. Bonferroni/Dunn (Dunn's procedure for comparing a control to all other means) method was used for statistical comparisons.

## Results

On the EEG in KA-injected rats, multiple spike discharges initially appeared in the LA 10 to 20 minutes after KA injection (Figure 1a and b), and rapidly propagated to the LH. Sixty minutes after KA injection, the seizure involved the LCx and resulted in limbic status epilepticus (Figure 1c). Behaviorally, the rats showed bilateral facial twitching, mastication, and salivation. A few minutes after $^{14}$C-ZNS administration, the seizures in the LCx were markedly attenuated (Figure 1d). In addition, behavioral seizure manifestations such as facial

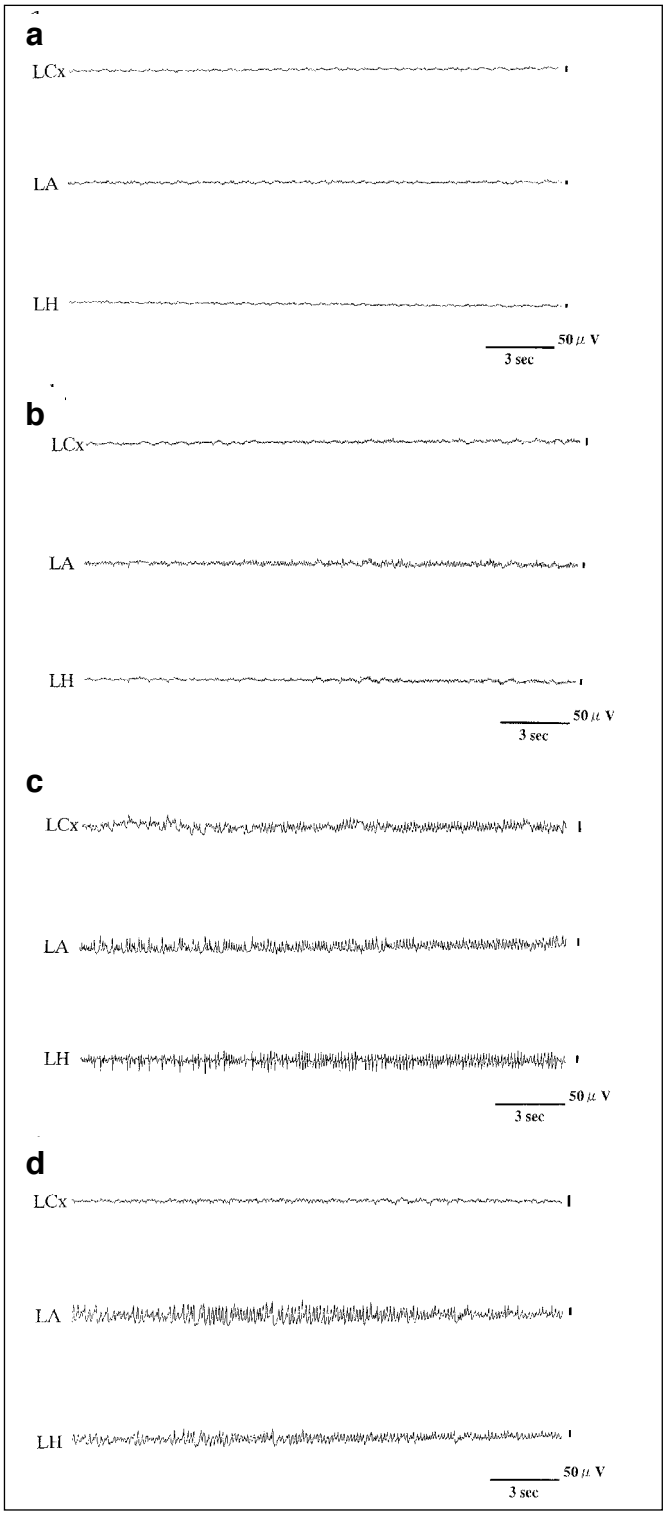


***Figure 1.*** *Serial changes in the electroencephalogram (EEG) after kainic acid injection into the left amygdala (LA).*
*(a) before injection. No abnormality is present.*
*(b) 15 min after injection. Multiple spike discharges appeared in the LA.*
*(c) 60 min after injection. Multiple spike discharges propagated to the left dorsal hippocampus (LH) and left sensorimotor cortex (LCx).*
*(d) 65 min after injection (5 min after $^{14}$C-ZNS administration). In the LCx, the multiple spike discharges disappeared.*



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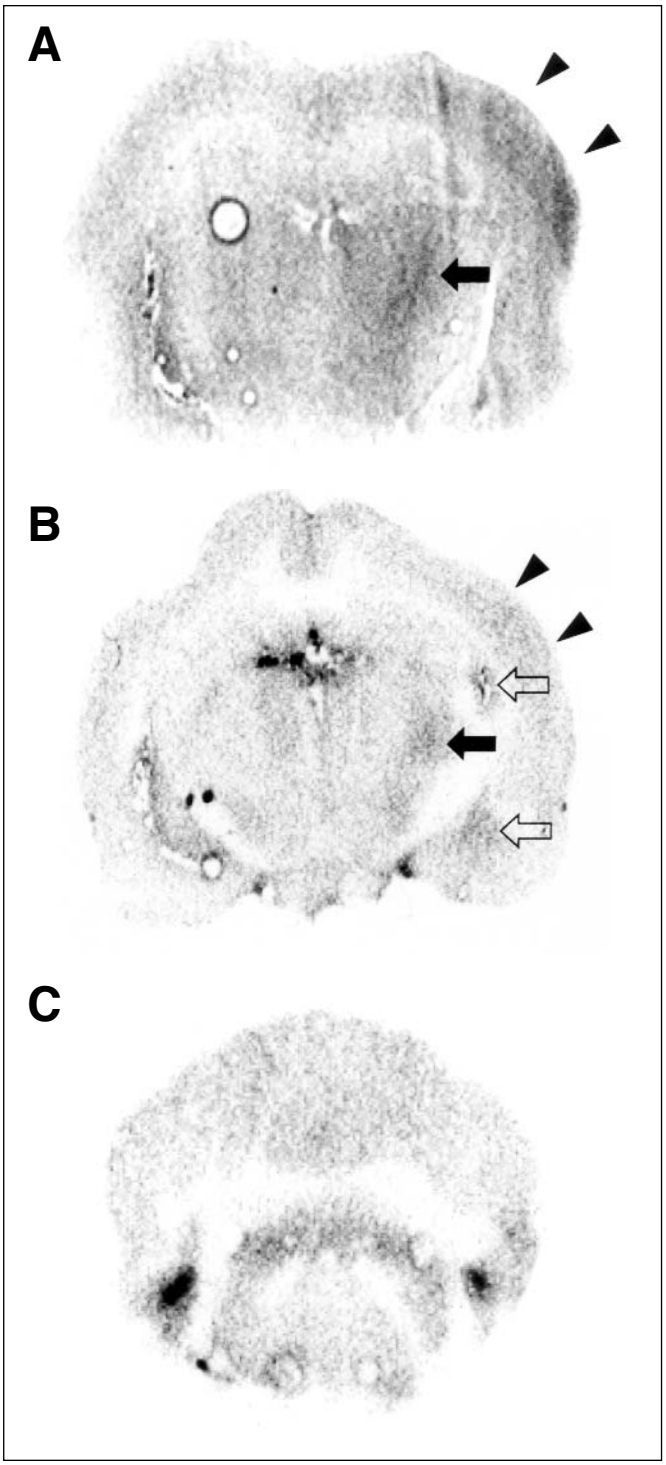


***Figure 2:*** *$^{14}$C-zonisamide (ZNS) autoradiograms in KA-injected rats. High uptake of $^{14}$C-ZNS was seen ipsilaterally in the sensorimotor cortex (arrowheads in A), parietal cortex (arrowheads in B), and thalamus (lateral portion; filled arrow in A and B), while moderate uptake was observed in the hippocampal CA3 region (open arrow in B). In Figure 2C, distribution of $^{14}$C-ZNS in the brainstem and the cerebellum was homogeneous except choroid plexus in the 4th ventricle.*

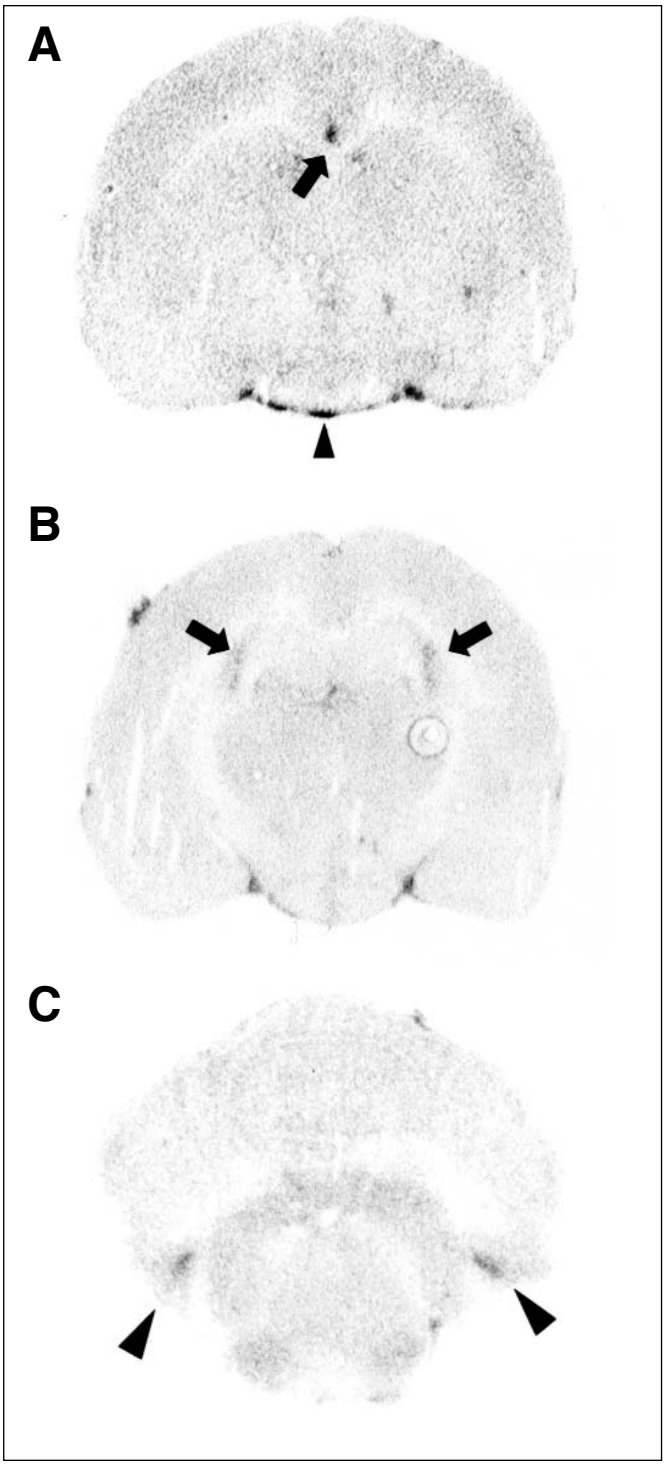


***Figure 3:*** *$^{14}$C-zonisamide (ZNS) autoradiograms in control rats. Distribution of $^{14}$C-ZNS was rather homogeneous, with uptake in the cortex and thalamus greater than that in white matter. Autoradiographic figures demonstrated the regions lying outside the blood-brain barrier; A. Pineal recess of third ventricle (arrow) and median eminence (arrowhead), B. choroid plexus in the lateral ventricle (arrow), C. Choroid plexus in the 4th ventricle (arrowhead).*



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**Table:** Regional accumulation of $^{14}$C-zonisamide in rat brain

| Region | KA (n=8) | | Control (n=6) | |
|---|---|---|---|---|
| | left | right | left | right |
| Cerebral cortex | | | | |
| Frontal | 0.50±0.07 | 0.44±0.06 | 0.44±0.06 | 0.42±0.06 |
| Sensorimotor | 0.71±0.07** | 0.43±0.05 | 0.41±0.07 | 0.40±0.06 |
| Parietal | 0.75±0.04** | 0.47±0.05 | 0.42±0.03 | 0.39±0.04 |
| Occipital | 0.53±0.05 | 0.47±0.04 | 0.44±0.05 | 0.46±0.04 |
| Caudate | 0.40±0.06 | 0.41±0.06 | 0.39±0.05 | 0.40±0.05 |
| Substantia nigra | 0.50±0.06 | 0.48±0.08 | 0.42±0.02 | 0.42±0.01 |
| Amygdala | 0.44±0.09 | 0.42±0.08 | 0.40±0.04 | 0.42±0.04 |
| Hippocampus | | | | |
| CA1 | 0.42±0.08 | 0.40±0.08 | 0.38±0.05 | 0.36±0.04 |
| CA3 | 0.62±0.07* | 0.41±0.07 | 0.42±0.08 | 0.37±0.06 |
| Thalamus | | | | |
| medial portion | 0.49±0.06 | 0.46±0.04 | 0.40±0.05 | ().40±0.06 |
| lateral portion | 0.74±0.07** | 0.44±0.08 | 0.44±0.02 | 0.41±0.04 |
| Hypothalamus | 0.52±0.06 | 0.49±0.07 | 0.39±0.06 | 0.43±0.05 |
| Reticular formation | 0.44±0.09 | 0.44±0.09 | 0.39±0.05 | 0.39±0.03 |
| Cerebellum | | | | |
| cortex | 0.37±0.07 | 0.40±0.10 | 0.37±0.06 | 0.35±0.04 |
| dentate nucleus | 0.39±0.13 | 0.37±0.11 | 0.31±0.05 | 0.30±0.04 |

Regional accumulation ratios were calculated as the optical density of each structure / optical density in the pineal body (outside the blood-brain barrier). Values are mean±SE. **p< 0.01, and *p< 0.05 for multiple comparisons between ipsilateral side of the control and the others: contrarateral side of the control, ipsilateral and contralateral side of the KA-injected rats. Bonferroni / Dunn (Dunn's Procedure for Comparing a Control to All other Means) method was used for statistical comparisons.

twitching and mastication disappeared. In controls, no behavioral or EEG changes were seen.

In autoradiograms of the two groups, distribution of $^{14}$C-ZNS in the cortex and thalamus was greater than that in white matter, and high uptake of $^{14}$C-ZNS was seen in the regions outside the BBB such as pineal body, choroid plexus, and median eminence. In KA-injected rats, high uptake of $^{14}$C-ZNS was observed in the ipsilateral sensorimotor cortex, parietal cortex, and thalamus (lateral portion), while moderate uptake was seen in the hippocampal CA3. (Figure 2A to C; Table, left columns). In controls, distribution of $^{14}$C-ZNS was rather homogeneous. (Figure 3A to C; Table, right columns). Significant differences between the ipsilateral side of the control and the KA-injected rats were found in the sensorimotor cortex ($p<0.01$), parietal cortex ($p<0.01$), thalamus (lateral portion, $p<0.01$), and hippocampal CA3 ($p<0.05$).

## Discussion

In the reports by Matsumoto et al[17] $^{14}$C-ZNS readily crossed the BBB and accumulated in the tissue of the brain. According to these reports, it seems likely that distribution of $^{14}$C-ZNS in the present study represents the accumulation of the tracer in the tissue compartment, not in the vascular space. Tanaka et al[11] demonstrated that local cerebral blood flow (LCBF) in the cerebral cortex did not increase 1-2 hours after KA injection into unilateral amygdala, whereas LCBF of the limbic system increased remarkably. Considering this, if $^{14}$C-ZNS distribution was to represent tracer in the vascular space, high uptake should be found in the limbic system rather than the cerebral cortex. Therefore, it seems that $^{14}$C-ZNS distribution represents tracer accumulation in the tissue compartment. In the kinetics of distribution of $^{14}$C-ZNS, Matsumoto et al[17] found that tissue activity of $^{14}$C-ZNS in the rat brain disappeared by 96 hours.

Accumulation patterns of various anticonvulsants have been studied previously in normal animals.[18-21] Geary et al[18] found $^{14}$C-phenytoin to accumulate in the cerebral cortex, cerebellar cortex, thalamus, and striatum. A report by Pantarotto et al[21] noted high levels of carbamazepine accumulation in the hypothalamus, hippocampus, striatum, thalamus, and cerebral hemispheres 60 minutes after a single injection. As for $^{14}$C-ZNS accumulation in normal rat brain, Mimaki et al[20] reported high uptake in the cerebral cortex and inferior colliculus and moderate uptake in the cerebellar cortex, thalamus, hypothalamus and striatum. In the present study, a homogeneous distribution of $^{14}$C-ZNS was observed in control animals. While this discrepancy is difficult to explain, different methods of analysis or different time points studied might be responsible (10 minutes in our study vs. 5 minutes in that of Mimaki et al).

As for the effects of ZNS in experimental epilepsy models, Masuda et al[6] studied the effect of ZNS on electric shock and chemically induced seizures and speculated that ZNS, like phenytoin and carbamazepine, exerted its anticonvulsant effect mainly by inhibiting spread of seizures. Ito et al[4] reported that in cats, ZNS given at doses ineffective against the thalamic and hippocampal after discharges suppressed cortical focal seizures



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induced by electric stimulation, and that ZNS also suppressed both interictal spikes and secondary generalized seizures induced by cortical application of tungstic acid gel. These authors[4] concluded that ZNS has a direct suppressive effect on the cortical epileptogenic focus. In kindling models, Kamei et al[5] reported that ZNS was effective against neocortical and hippocampal kindling but not amygdalar kindling. However, Hamada et al[3] reported that ZNS retarded the development of amygdalar kindling. In KA-induced amygdalar seizures, Takano et al[7] previously demonstrated that ZNS suppressed seizure propagation but did not suppress the epileptic activity of the amygdala. Thus, despite various studies of the effects of ZNS, the mechanism of its anticonvulsive action is still unclear.

In the present experiments, we investigated regional accumulation of ZNS in the rat brain by an autoradiographic method, and assessed the effects of ZNS on KA-induced limbic seizures[7-14] by EEG monitoring. In the present study, as soon as the seizure attenuation was recognized by $^{14}$C-ZNS administration, $^{14}$C-ZNS accumulation in the brain was studied. $^{14}$C-ZNS accumulated in the ipsilateral sensorimotor cortex and parietal cortex, and focal suppression of epileptic discharges in the sensorimotor cortex was seen a few minutes after intravenous $^{14}$C-ZNS administration. Regional $^{14}$C-ZNS accumulation was also observed in the ipsilateral thalamus (lateral portion) and hippocampal CA3 region.

Blood-brain barrier function is important in interpreting drug distribution findings. In KA-induced amygdalar seizures, $^{14}$C-aminoisobutyric acid autoradiography revealed severe disruption of BBB function only in the hippocampal CA3 region and slight damage in the amygdala on the KA-injected side.[10] Additionally, Tanaka et al[11] studied local cerebral glucose utilization (LCGU) and LCBF autoradiographically using $^{14}$C-2-deoxyglucose and $^{14}$C-iodoantipyrine during KA-induced limbic status epilepticus, demonstrating relative hypoxia due to a high degree of uncoupling of LCGU and LCBF in several limbic structures, especially CA3. However, such uncoupling was not seen in the cerebral cortex. Considering these results,[11] disruption of the BBB is likely to have induced the moderate uptake of $^{14}$C-ZNS in CA3 in our study, but the cortical accumulation was not caused by BBB dysfunction. We speculated that extravascular ZNS was promptly transferred to the secondarily excited zone in the cortex, maintaining a favorable extravascular/intravascular ZNS gradient for further ZNS transport from the intravascular to the extravascular space.

Why ZNS accumulates in the thalamus remains obscure. In KA-induced limbic status epilepticus, Tanaka et al[11] reported increases of LCGU and LCBF in the ventrobasal complex, including the ventroposteromedial nucleus of the thalamus and the ventroposterolateral nucleus of the thalamus, that most likely reflected retrograde input from the sensorimotor cortex to the ventrobasal complex. This mechanism might favor ZNS accumulation in the lateral portion of the thalamus. Further experiments will be required to elucidate this point.

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