ELSEVIER

Pharmacology, Biochemistry and Behavior 68 (2001) 7–12

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Effect of immobilization stress on anticonvulsant actions and pharmacokinetics of zonisamide in mice

Yasuhiko Hashimoto, Katsuya Suemaru, Takahiko Yamamoto, Kenya Kawakami, Hiroaki Araki, Yutaka Gomita*

Department of Hospital Pharmacy, Okayama University Medical School, 2-5-1, Shikata-cho, Okayama 700-8558, Japan

Received 10 September 1999; received in revised form 31 March 2000; accepted 17 July 2000

Abstract

The effects of immobilization stress on anticonvulsant actions and pharmacokinetics of zonisamide were investigated in mice. Oral administrations of zonisamide (10, 20, and 50 mg/kg) dose-dependently reduced incidence of tonic extension (TE) induced by maximal electroshock seizure (MES). Immobilization stress for 2 h immediately after the administration of zonisamide further enhanced the anticonvulsive actions of it. On the other hand, the serum zonisamide concentrations in stressed group were lower during the first 30 min after the administration compared with that in nonstressed control group. Thereafter, there were no significant differences in the serum concentrations between two groups. The brain zonisamide concentration and the concentration ratio of brain/serum at 2 h after administration of zonisamide (50 mg/kg) were significantly higher in stressed group, rather than that in the nonstressed control group without changing the serum concentration. These results suggest that immobilization stress enhances anticonvulsant actions of zonisamide, and that increases of brain zonisamide concentration by immobilization stress may be related with this phenomenon.

Keywords: Immobilization stress; Zonisamide; Anticonvulsant action; Serum concentration; Brain concentration

1. Introduction

In clinical, the seizure frequency in epileptic patients is often exacerbated by emotional stress, such as change of circumstance (Neufeld et al., 1994), increasing fatigue (Grant, 1985), and sleeplessness (Konishi et al., 1992). Under such stressed conditions, the epileptic patients are treated with additional antiepileptic drugs or antianxiety drugs having anticonvulsive actions. In general, the variation in a drug's effect may be due to changes in the sensitivity of the site of drug action and/or the pharmacokinetics. Our previous study suggested that the pharmacokinetics of theophylline, nicorandil, and isosorbide dinitrate were markedly altered following various stressed condition, such as immobilization stress, footshock stress, and psychological stress in rats (Okazaki et al., 1995; Yamori et al., 1991). These phenomena are attributed to change of process, such as absorption, distribution volume, excretion, and metabolism of drugs. However, the relationship between the anticonvulsant action of antiepileptic drugs and their brain concentrations in animals exposed to stress still remains obscure.

Zonisamide is a sulfonamide derivative that is effective in treating patients with generalized tonic–clonic seizures, secondary generalized seizures, complex partial seizures, and simple partial seizures (Sackellares et al., 1985; Seino et al., 1991). The mechanism of anticonvulsant action of zonisamide is the blockade of sustained repetitive firing through an action via voltage-sensitive sodium channel, reduction voltage-dependent calcium channels, and suppression and slow-wave discharge. It also has been demonstrated that zonisamide effectively treats electroconvulsive seizure in rodents (Masuda et al., 1980). Thus, in the present study, we investigated that the effects of immobilization stress on anticonvulsive actions of zonisamide to maximal electroshock seizure (MES), and examined the pharmacokinetics in serum and brain under the stress condition in mice.

* Corresponding author.

0091-3057/01/$ – see front matter
PII: S0091-3057(00)00433-0

2. Materials and methods

2.1. Animals

Male ddY strain mice (6 weeks of age) were obtained from Japan SLC. An adaptational period (minimum of 1 week) was allowed before the experiments. The animals were housed in five per plastic-walled cages ($30 \times 20 \times 15$ cm), and in a temperature-controlled environment ($22 \pm 2°C$), under a 12-h light/dark cycle (lights on at 0700 h). Food and water were given ad libitum, except for 12 h before and during the experiment, to prevent absorption and gastric emptying rate of drug from gastric contents.

2.2. Drug and drug administration

Zonisamide sodium obtained from Dainippon Pharmaceutical (Osaka, Japan) was suspended in 0.5% sodium carboxymethylcellulose. It was administered orally in a volume of 0.1 ml/10 g body weight using gastric tube. Drug dose was expressed in terms of the free base.

2.3. Procedure for experiments

In pharmacodynamics study, mice were divided into two groups, immobilization stress and nonexposed control groups. They were administered zonisamide orally at doses of 10, 20, and 50 mg/kg, and then animals were applied to MES 2 h after administration of zonisamide.

In pharmacokinetics study, we conducted three experiments. At first, chronological changes of serum zonisamide concentrations after administration at a dose of 50 mg/kg in control and immobilized groups were examined. Mice were decapitated and blood samples were collected 0.25, 0.5, 1, 2, 4, 6, and 8 h after administration of zonisamide. Second, we measured concentrations of zonisamide in serum and brain at 2 h after administration at doses of 10, 20, and 50 mg/kg. Finally, effect of immobilization stress on concentrations of zonisamide in serum and brain were also examined in mice 2 h after oral administration of zonisamide at 50 mg/kg.

2.4. Loading of immobilization stress and MES

Immediately after the oral administration of zonisamide (10, 20, and 50 mg/kg) or vehicle, mice were immobilized with metal wire for 2 h or returned to their home cages. The animals were applied a 60-Hz AC current of 50 mA, for 0.2 s, through corneal electrodes using Woodbury and Davenport's apparatus. The incidence of tonic hindlimb extension (TE) following maximal electroshock was measured.

2.5. Determination of zonisamide concentrations in serum and brain

Serum samples were obtained after centrifugation of blood at 3000 *g* for 10 min. For the determination of brain zonisamide concentration, whole brain was homogenated in 5 ml physiological saline and centrifuged at 3000 *g* for 10 min, and the supernatant was centrifuged again at 10,000 g for 20 min. A total of 20 μl of serum or 200 μl of the supernatant of brain samples plus 200 μg of *N*,*N*-dimethyl zonisamide, as the internal standard, were passed through a Bond Elut cartridge (C18), which had previously been washed with 2 ml of 99.5% methanol and 2 ml of distilled water. After washing with 1 ml of distilled water and 1 ml of 8% methanol, the substances to be determined were eluted with 200 μl of 99.5% methanol. A 30-μl aliquot of the elute was injected into a high-performance liquid chromatography (HPLC) system with UV spectrophotometric detection. The HPLC system consisted of a pump (model 510, Waters), a reversed phase column LiChroCART, superspher RP(e) 4 μm), autosampler (WISP, 710B, Waters), and UV detector (Lambda-Max Model 418, Waters). The detection of zonisamide was performed at 246 nm. The mobile phase was 40% methanol and the flow rate was 1 ml/min.

2.6. Statistical analysis

Incidence of tonic extensor induced by MES was analyzed by χ^2 test. Zonisamide concentration of serum and brain was

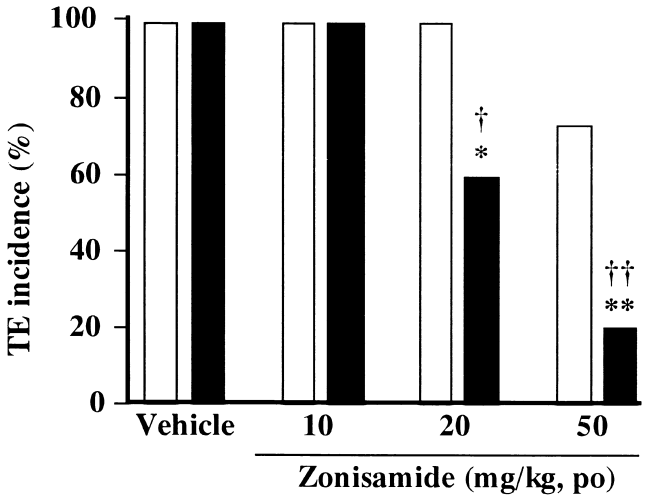

Fig. 1. Effect of immobilization stress on incidence of tonic extensor induced by maximal electroshock in mice treated with zonisamide. Mice were immobilized (closed column) for 2 h immediately or returned to home cage (open column) after oral administration of zonisamide (10, 20, and 50 mg/kg) or vehicle. Each column represents incidence of tonic extensor after maximal electroshock ($n = 10-15$ mice per group). $^{*}P < .05$, $^{**}P < .01$ significantly different from immobilized vehicle group, $^{\dagger}P < .05$, $^{\dagger\dagger}P < .01$ significantly different from nonimmobilized control group at same dose.

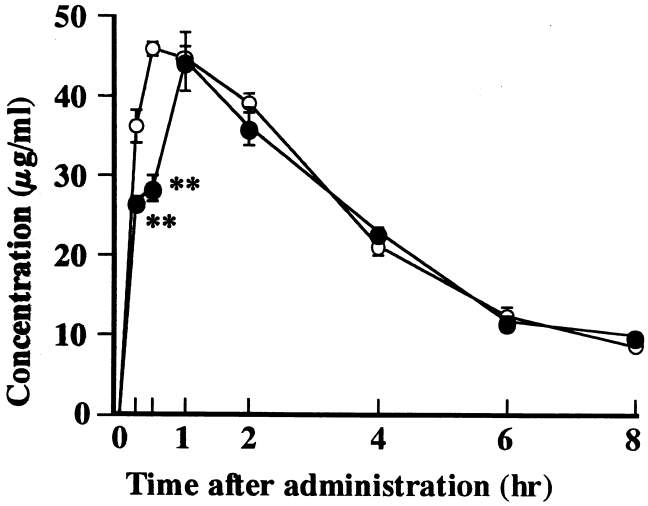

Fig. 2. Time course of the changes in serum zonisamide concentration after the administration in mice loaded (closed circle) or nonloaded immobilization stress (open circle). Mice were immobilized for 2 h immediately after oral administration of zonisamide at 50 mg/kg. Each point represents the mean ± S.E.M. of five mice. * $P < .05$, ** $P < .01$ significantly different from nonimmobilized control.

evaluated by Student's *t* test. Probability values less than .05 were considered to show a significant difference.

3. Results

3.1. Effect of immobilization stress on anticonvulsant action of zonisamide

Fig. 1 shows incidence of MES-induced TE in mice loaded or nonloaded with immobilization stress for 2 h after administration of zonisamide. The incidence of TE in the stress nonloaded group was slightly but not significantly suppressed by zonisamide at 50 mg/kg (73.3%). In the stress loaded group, zonisamide, at doses of 20 and 50 mg/kg, suppressed the incidence of TE dose-dependently and significantly ($P < .05$ and $P < .01$, respectively). The inhibitory effect of zonisamide on MES-induced TE was more marked in immobilized group compared with non-immobilized control group, and there were significant differences in doses of 20 and 50 mg/kg ($P < .05$ and $P < .01$, respectively).

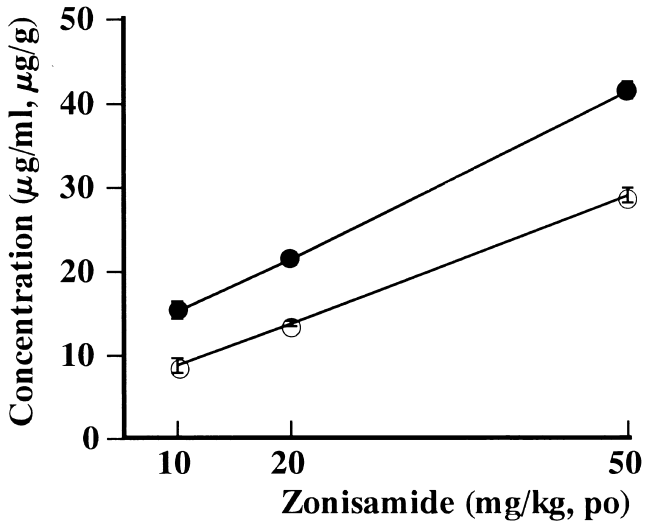

Fig. 3. Serum (open circle) and brain (closed circle) concentrations of zonisamide at 2 h after the administration in mice. Various doses of zonisamide were administered orally. Each point represents the mean ± S.E.M. of five mice.

Table 1
Effect of immobilization stress on serum zonisamide concentrations in mice

Zonisamide doses (mg/kg)	Serum concentration (μg/ml)	
	Control	Immobilization
10	5.59 ± 0.34	4.55 ± 0.17
20	9.46 ± 0.22	8.88 ± 0.37
50	34.29 ± 1.23	34.41 ± 1.47

Mice were immobilized for 2 h immediately after oral administration of zonisamide. Each value represents the mean ± S.E.M. ($n = 10–15$).

3.2. Effect of immobilization stress on pharmacokinetics of zonisamide

Fig. 2 shows the effect of immobilization stress on serum concentration of zonisamide. When nonimmobilized mice were given 50 mg/kg of zonisamide orally, the serum concentrations reached a maximum value of approximately 46.1 μg/ml after 30 min, followed by a gradual decrease to 8.9 μg/ml at 8 h after administration. The serum concentrations in immobilized group were significantly lower at 15 and 30 min after administration of zonisamide, but there was no significant difference at any time.

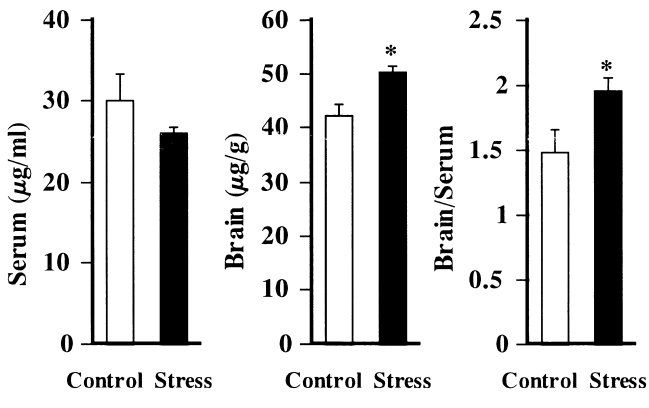

Fig. 4. Effect of immobilization stress on serum and brain concentrations of zonisamide. Mice were immobilized for 2 h immediately after oral administration of zonisamide at 50 mg/kg. Each column represents the mean ± S.E.M. of five mice. * $P < .05$ significantly different from nonimmobilized control.

3.3. Effect of immobilization stress on brain zonisamide concentration

The serum and brain concentrations of zonisamide at 2 h after the administration were increased in proportion to the larger dose of zonisamide (10, 20, and 50 mg/kg, po) with high correlation (serum: $r = .999$, brain: $r = .999$; Fig. 3). Loading of immobilization stress for 2 h had any significant effect on the serum concentrations of zonisamide at three doses (Table 1). However, when zonisamide was given at a dose of 50 mg/kg, brain zonisamide concentration and the concentration ratio of brain/serum were significantly higher in immobilized group compared with those in nonimmobilized group ($P < .05$, respectively), without changing serum zonisamide concentration (Fig. 4).

4. Discussion

It was reported that experimental stress, such as immobilization stress (Oliverio et al., 1983), cold water stress (Soubrie et al., 1980), restrain stress (De-Lima and Rae, 1988), footshock stress (Drugan et al., 1985; Soubrie et al., 1980), revolving drum stress (Goldberg and Salama, 1970a), and emotional stress (Soubrie et al., 1980) have protective effect against convulsion induced by electroconvulsive shock, bicuculline, picrotoxin, and pentylenetetrazol in rat and mice. The protective effects against electroconvulsive shock were shown in increase of electrical seizure threshold (Oliverio et al., 1983) or prolonged seizure latency (De-Lima and Rae, 1991). Furthermore, it was demonstrated that the protective effects of stress are associated with endogenous opioids (Pavone et al., 1986; Shavit et al., 1984) and catecholamines (Goldberg and Salama, 1970b) in the central nervous system. In the present study, immobilization stress did not affect the incidence of convulsion by maximal electroshock in mice untreated with zonisamide. However, immobilization stress markedly enhanced anticonvulsant action of zonisamide in mice.

It has been reported that, when rats are given sub-anticonvulsant doses of phenobarbitone and diphenylhydantoin, restraint stress potentiates the anticonvulsive actions against MES-induced seizures (Bhattacharya and Bhattacharya, 1982). Diphenylhydantoin, phenobarbitone, and zonisamide have anticonvulsive effects via its Na channel-blocking action, GABA receptor, and T-type Ca and Na channel-blocking action, respectively (Honda, 1984; Macdonald and Kelly, 1995; Nicoll et al., 1975; Suzuki et al., 1992). Thus, these findings and our results indicate that these antiepileptic drugs, which possess different pharmacological mechanisms, show anticonvulsant actions under stress condition. Experimental stress, including immobilization stress, can release a number of central neurotransmitters and hormones including GABA, ACTH, and endogenous opioids, which possess anticonvulsant action (Baram and Schultz, 1995; Matsumoto et al., 1987). Thereby, the possibility exists that these neurotransmitters and hormone are related with the enhancement of anticonvulsive actions of zonisamide.

Previous studies indicated that loading of stress, such as acoustic stress, immobilization stress, cold-restrain stress, and wrap-restrain stress, delay gastric emptying and decrease gastrointestinal motility (Gue et al., 1989; Koo et al., 1986). We have already reported that immobilization stress immediately after administration of theophylline, but not prior to the drug administration, decreases the plasma concentrations of it during the absorption phase by inhibition of its absorption from the gastrointestinal tract (Okazaki et al., 1995). Thus, in this study, we examined the effect of immobilization stress when mice were immobilized immediately after zonisamide. The results of this study showed that immobilization stress decreases serum zonisamide concentrations at 0.25 and 0.5 h after the administration, this change was maybe due to inhibition of absorption of the drug. However, there was no significant difference in serum zonisamide concentrations between stressed and nonstressed groups at 2 h after administration, when immobilization stress enhanced the anticonvulsive effect of zonisamide. On the other hand, Nagatomo et al. (1997) has reported that chronic alcohol intake reduces anticonvulsant action of zonisamide in the EL mouse, and that relates decreases in brain zonisamide concentration but not the serum concentration. In the present study, immobilization stress increases brain zonisamide concentration without changing the serum concentrations, suggesting involvement in enhanced anticonvulsive effect of zonisamide by immobilization stress.

Zonisamide has a long half-time, and equilibrates across the blood–brain barrier in the course of a single transcapillary transit but not carrier-mediated mechanisms (Cornford and Landon, 1985). Nishiguchi et al. (1992) reported that zonisamide concentration ratio of brain/serum exceed 1 after oral administration of zonisamide 5, 20, and 80 mg/kg. These findings also support our observation that the concentration of zonisamide in brain was higher than in serum.

Immobilization stress and forced-swimming stress have been reported to increase blood–brain barrier permeability to Evans blue and ^{131}I–sodium tracers in rats and mice (Sharma and Dey, 1986; Sharma et al., 1995). It demonstrated that enhancement of blood–brain barrier permeability during loading of immobilization stress was mediated by serotonin (Sharma and Dey, 1986) and noradrenaline (Borges et al., 1994; Sarmento et al., 1991), which release the stress conditions in the central nervous system. Actually, pyridostigmine, which does not easily penetrate the brain, is reported to inhibit brain acetylcholinesterase in mice exposed to forced-swimming stress (Azevedo and Sarmento, 1997). Furthermore, experimental stress, including immobilization (Buchel et al., 1972), has been reported to potentiate barbiturate-induced hypnosis (Drugan et al., 1992) and increase brain penetration of barbiturates in rats (Wei and Wilson, 1971).

Clinically, the seizure frequency in epileptic patients is often exacerbated by comparatively long-lasting stress, such

as change of circumstance, increasing fatigue, and sleeplessness (Honda, 1984). Experimental animal studies indicated that REM sleep deprivation for 12–140 h is more susceptible to electroshock- and pentylenetetrazol-induced convulsions in rats (Owen and Bliss, 1970; Vale and Leite, 1988). On the other hand, short-term experimental stress have increased or decreased the seizure threshold dependent on kind of stressor, intensity, and timepoint, given convulsive stimulant after stress. De-Lima and Rae (De-Lima and Rae, 1988) showed that swim stress for 3 min increases latency of pentylenetetrazol-induced the first myoclonic jerk in mice pretreated with *p*-chlorophenylalanine, and Oliverio et al. (1983) reported that loading of immobilization stress for 2 h shows protecting effect against electroconvulsive shock seizure in mice. Interestingly, Deutsch et al. (1990) found that anticonvulsant action of flurazepam against an incremental convulsive shock decreased 24 h after swim stress. In addition, Drugan et al. (1985) reported that electrical tail shock stress exhibits protective effect against bicuculline-induced seizure immediately after the exposure to the stress, but that the effect is not shown 2 h after the stress in rats. The present study showed that the anticonvulsant actions of zonisamide is markedly enhanced by immobilization stress for 2 h, and this phenomenon is partially related to its increase of brain zonisamide concentration without changing the serum concentration. To clarify the effect of stress on the pharmacokinetics of antiepileptic drugs in serum and brain, further studies, relating to the influences of exposure to immobilization stress prior to drug administration and long-term or repeated exposure of stress, are necessary. The therapeutic blood concentration range of zonisamide is narrow; high doses often cause side effects, such as ataxia, drowsiness, and loss of appetite (Kumagai et al., 1991; Shimizu et al., 1996). Thus, these suggest that epileptic patients, who received antiepileptic drugs, warrant further attention not enhanced anticonvulsant of zonisamide, but occurred side effect by increase in brain zonisamide concentration under stress condition.

Acknowledgments

We thank the Dainippon Pharmaceutical (Osaka, Japan) for providing zonisamide.

References

Azevedo I, Sarmento A. Stress and the blood brain barrier. Nat Med 1997;3:253 (letter; comment).

Baram TZ, Schultz L. ACTH does not control neonatal seizures induced by administration of exogenous corticotropin-releasing hormone. Epilepsia 1995;36:174–8.

Bhattacharya SK, Bhattacharya D. Effect of restraint stress on anticonvulsant actions of phenobarbitone and diphenylhydantoin in rats. Indian J Exp Biol 1982;20:406–8.

Borges N, Shi F, Azevedo I, Audus KL. Changes in brain microvessel endothelial cell monolayer permeability induced by adrenergic drugs. Eur J Pharmacol 1994;269:243–8.

Buchel L, Prioux-Guyonneau M, Liblau L, Murawsky M. Influence of restraint on the activity, penetration and metabolism of hexobarbital and barbital in white rats. Therapie 1972;27:609–25.

Cornford EM, Landon KP. Blood–brain barrier transport of CI-912: single-passage equilibration of erythrocyte-borne drug. Ther Drug Monit 1985;7:247–54.

De-Lima TC, Rae GA. Participation of serotonergic mechanisms in the anticonvulsant effect of stress in mice treated with pentylenetetrazol. Braz J Med Biol Res 1988;21:333–5.

De-Lima TC, Rae GA. Effects of cold-restraint and swim stress on convulsions induced by pentylenetetrazol and electroshock: influence of naloxone pretreatment. Pharmacol, Biochem Behav 1991;40:297–300.

Deutsch SI, Rosse RB, Huntzinger JA, Novitzki MR, Mastropaolo J. Profound stress-induced alternation in flurazepam's antiseizure efficacy can be attenuated. Brain Res 1990;520:272–6.

Drugan RC, McIntyre TD, Alpern HP, Maier SF. Coping and seizure susceptibility: control over shock protects against bicuculline-induced seizures. Brain Res 1985;342:9–17.

Drugan RC, Scher DM, Sarabanchong V, Guglielmi AM, Meng I, Chang J, Bloom K, Sylvia S, Holmes P. Controllability and duration of stress alter central nervous system depressant-induced sleep time in rats. Behav Neurosci 1992;106(4):682–9.

Goldberg ME, Salama AI. Effect of alpha-methyltyrosine on stress-induced changes in seizure susceptibility. Eur J Pharmacol 1970a;9:175–82.

Goldberg ME, Salama AI. Relationship of brain dopamine to stress-induced changes in seizure susceptibility. Eur J Pharmacol 1970b;10:333–8.

Grant I. The social environment and neurological disease. Adv Psychosom Med 1985;13:26–48.

Gue M, Peeters T, Depoortere I, Vantrappen G, Bueno L. Stress-induced changes in gastric emptying, postprandial motility, and plasma gut hormone levels in dogs. Gastroenterology 1989;97:1101–7.

Honda T. Amino acid metabolism in the brain with convulsive disorders: 2. The effects of anticonvulsants on convulsions and free amino acid patterns in the brain of EL mouse. Brain Dev 1984;6:22–6.

Konishi T, Naganuma Y, Hongo K, Murakami M, Yamatani M, Okada T. Seizure-inducing factors in the patients with childhood epilepsy. No to Hattatsu (Brain and Development). 1992;24:238–43.

Koo MW, Cho CH, Ogle CW. Effects of cold-restraint stress on gastric ulceration and motility in rats. Pharmacol, Biochem Behav 1986; 25:775–9.

Kumagai N, Seki T, Yamawaki H, Suzuki N, Kimiya S, Yamada T, Hara M, Hashimoto R, Takuma Y, Hirai K. Monotherapy for childhood epilepsies with zonisamide. Jpn J Psychiatry Neurol 1991;45:357–9.

Macdonald RL, Kelly KM. Antiepileptic drug mechanisms of action. Epilepsia 1995;36:S2–S12.

Masuda Y, Karasawa T, Shiraishi Y, Hori M, Yoshida K, Shimizu M. 3-Sulfamoylmethyl-1,2-benzisoxazole, a new type of anticonvulsant drug. Pharmacological profile. Arzneim-Forsch (Drug Res). 1980;30: 477–83.

Matsumoto A, Kumagai T, Takeuchi T, Miyazaki S, Watanabe K. Clinical effects of thyrotropin-releasing hormone for severe epilepsy in childhood: a comparative study with ACTH therapy. Epilepsia 1987;28: 49–55.

Nagatomo I, Akasaki Y, Uchida M, Takigawa M. Alcohol intake decreases brain zonisamide concentration in inbred EL mice. NeuroReport 1997;8:391–4.

Neufeld MY, Sadeh M, Cohn DF, Korczyn AD. Stress and epilepsy: the Gulf War experience. Seizure 1994;3:135–9.

Nicoll RA, Eccles JC, Oshima T, Rubia F. Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. Nature 1975;258: 625–7.

Nishiguchi K, Ohnishi N, Iwakawa S, Yagi J, Nakayama S, Takada S, Nakamura H, Yokoyama T, Okumura K. Pharmacokinetics of zonisamide; saturable distribution into human and rat erythrocytes and into rat brain. J Pharmacobio-Dyn 1992;15:409–15.

Okazaki M, Eto K, Furuno K, Oishi R, Gomita Y. Influences of immobilization and footshock stress on pharmacokinetics of theophylline and caffeine in rats. J Pharm Pharmacol 1995;47:530–3.

Oliverio A, Castellano C, Puglisi-Allegra S. Anticonvulsant effects of stress: role of endogenous opioids. Brain Res 1983;271:193–5.

Owen M, Bliss EL. Sleep loss and cerebral excitability. Am J Physiol 1970;218:171–3.

Pavone F, Castellano C, Oliverio A. Strain-dependent effects of shock-induced release of opioids: dissociation between analgesia and behavioral seizures. Brain Res 1986;366:326–8.

Sackellares JC, Donofrio PD, Wagner JG, Abou-Khalil B, Berent S, Aasved-Hoyt K. Pilot study of zonisamide (1,2-benzisoxazole-3-methanesulfonamide) in patients with refractory partial seizures. Epilepsia 1985;26:206–11.

Sarmento A, Borges N, Azevedo I. Adrenergic influences on the control of blood–brain barrier permeability. Naunyn-Schmiedeberg's Arch Pharmacol 1991;343:633–7.

Seino M, Miyazaki H, Ito T. Zonisamide. Epilepsy Res, Suppl 1991;3: 169–74.

Sharma HS, Dey PK. Influence of long-term immobilization stress on regional blood–brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. J Neurol Sci 1986;72:61–76.

Sharma HS, Westman J, Navarro JC, Dey PK, Nyberg F. Probable involvement of serotonin in the increased permeability of the blood–brain barrier by forced swimming. An experimental study using Evans blue and ^{131}I–sodium tracers in the rat. Behav Brain Res 1995;72:189–96.

Shavit Y, Caldecott-Hazard S, Liebeskind JC. Activating endogenous opioid systems by electroconvulsive shock or footshock stress inhibits recurrent kindled seizures in rats. Brain Res 1984;305:203–7.

Shimizu M, Uno H, Ito T, Masuda Y, Kurokawa M. Research and development of zonisamide, a new type of antiepileptic drug. Yakugaku Zasshi 1996;116:533–47.

Soubrie P, Thiebot MH, Jobert A, Montastruc JL, Hery F, Hamon M. Decreased convulsant potency of picrotoxin and pentetrazol and enhanced [^{3}H]flunitrazepam cortical binding following stressful manipulations in rats. Brain Res 1980;189:505–17.

Suzuki S, Kawakami K, Nishimura S, Watanabe Y, Yagi K, Seino M, Miyamoto K. Zonisamide blocks T-type calcium channel in cultured neurons of rat cerebral cortex. Epilepsy Res 1992;12:21–7.

Vale NB, Leite JR. Decreased susceptibility to local anesthetics-induced convulsions after paradoxical sleep deprivation. Psychopharmacology 1988;94:138–40.

Wei E, Wilson JT. Stress-mediated decrease in liver hexobarbital metabolism: the role of corticosterone and somatotropin. J Pharmacol Exp Ther 1971;177:227–33.

Yamori M, Gomita Y, Oishi R. Influence of footshock stress on pharmacokinetics of nicorandil in rats. Life Sci 1991;48:2065–73.