J Pharmacol Sci **107**, 213 – 220 (2008)

Journal of Pharmacological Sciences

Full Paper

Zonisamide Suppresses Pain Symptoms of Formalin-Induced Inflammatory and Streptozotocin-Induced Diabetic Neuropathy

Mitsuo Tanabe[1,*], Takuhiro Murakami[1], and Hideki Ono[1]

[1]*Laboratory of CNS Pharmacology, Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan*

Received Feburary 4, 2008; Accepted April 28, 2008

Abstract. We evaluated the effects of zonisamide on inflammatory and neuropathic pain using the mouse formalin test and streptozotocin (STZ)-induced diabetic mice with a reduced withdrawal threshold to mechanical stimuli, respectively. When administered systemically (subcutaneously, s.c.), intracerebroventricularly (i.c.v.) or intrathecally (i.t.) before formalin injection, zonisamide (3 and 10 mg/kg, s.c., 10 and 30 μg, i.c.v., or i.t.) significantly reduced licking/biting behavior during the second phase of the formalin test in a dose-dependent manner. However, zonisamide (30 μg, i.t.) did not affect the second phase of the formalin test when given after the first phase, suggesting that it can prevent the development of injury-induced hyperexcitability of the spinal dorsal horn triggered by the repetitive nociceptive input during the first phase. Moreover, zonisamide administered into the dorsal hindpaw ipsilateral but not contralateral to the formalin injection partly reduced the second phase. Thus it is likely that zonisamide generates analgesic effects in the formalin test via both central and peripheral mechanisms. In mice with STZ-induced diabetes, zonisamide (10 and 30 mg/kg, s.c. or 10 and 30 μg, i.t.) reversed the mechanical hyperexcitability. Our results suggest that zonisamide can be a useful therapeutic agent, presumably for both prevention and reversal of pathophysiologic pain.

Keywords: zonisamide, neuropathic pain, inflammatory pain, formalin, diabetes

Introduction

Because of the limited effectiveness of standard analgesics such as morphine and non-steroidal anti-inflammatory drugs for neuropathic pain, it has been of crucial importance to establish new standards for its treatment (1). As represented by the highly successful application of gabapentin for treatment of several neuropathic conditions, a therapeutic approach using antiepileptic drugs has recently been widely employed (2). Growing understanding of a pathophysiological feature shared by epilepsy and neuropathic pain involving the hyperexcitability of neuronal systems (3) may clarify further clinical applications of antiepileptic drugs for treatment of patients with chronic pain.

As has been supported by recent animal studies employing neuropathic pain models involving either chronic constriction injury (4) or partial ligation of the sciatic nerve (5), zonisamide, which has been developed as a new generation antiepileptic drug (6), is expected to be clinically effective for treatment of neuropathic pain (7). Our previous studies demonstrated that zonisamide produced centrally mediated analgesic effects on thermal and mechanical hypersensitivity in mice after peripheral nerve injury, independently of the descending monoaminergic system (5, 8). So far, multiple modes of action have been reported for zonisamide, including inhibition of sodium channels (9) and T-type calcium channels (9, 10), scavenging of free radicals (11), and blockade of nitric oxide (NO) synthesis (12). The relationships between these actions of zonisamide and its analgesic effects remain mostly unclarified.

Evaluating analgesic effects in various animal models of chronic pain is necessary before zonisamide should be considered as an alternative pharmacological tool for treatment of persistent pain. Hence, we further examined the effectiveness and sites of action of zonisamide in animal models of persistent inflammatory and neuro-

*Corresponding author. mitana@phar.nagoya-cu.ac.jp
Published online in J-STAGE on June 10, 2008 (in advance)
doi: 10.1254/jphs.08032FP

pathic pain. To this end, combined with zonisamide administration systemically, locally into the supraspinal and spinal levels or peripherally, persistent nociceptive behaviors elicited by subcutaneous (s.c.) injection of formalin into the plantar surface of the hindpaw and streptozotocin (STZ)-induced diabetic mechanical hypersensitivity were assessed. Subcutaneous injection of formalin elicits biphasic nociceptive behaviors, and the second phase includes central sensitization triggered by nociceptive inputs arriving during the first phase (13). Diabetic animals show increased spontaneous activity as well as hyper-responsiveness of afferent Aδ- and C-fibers (14 – 16), which may contribute to plastic changes in the spinal dorsal horn. We found that zonisamide produced analgesic effects also in these sensitized animal models, suggesting that it would probably be directly applicable for treatment of patients with various chronic pain states.

Materials and Methods

All experimental protocols were approved by the Animal Care and Use Committee of Nagoya City University and were conducted in accordance with the guidelines of the National Institutes of Health and The Japanese Pharmacological Society.

Behavioral testing after formalin injection

In the formalin test, 3 – 4-week-old, male ddY-strain mice were used. Mice were placed individually in a clear plastic chamber and allowed to acclimate to their environment at least for 30 min before testing. A mirror was situated behind the chamber to allow an unobstructed view of the animals' paws. The mice were gently restrained and 20 μl of 1% formaldehyde (2.7% formalin in distilled water) was injected s.c. into the plantar surface of the left hindpaw using a 27-gauge needle. After formalin injection, each mouse was returned to the testing chamber, and the incidence of formalin-induced nociceptive behavior characterized by licking/biting of the affected paw was measured for 50 min. Either zonisamide or vehicle was administered before or after formalin injection (see *Drug administration* in Materials and Methods section). Formalin-induced nociceptive behavior is biphasic, with the first and second phases recorded during 0 – 10 and 10 – 40 min after injection of formalin, respectively. Time spent performing the licking/biting behavior in each 5-min block was recorded continuously during the first and second phases.

Diabetic neuropathy

Three- to 4-week-old male ddY-strain mice were injected with STZ (200 mg/kg, intraperitoneally) dissolved in saline after a 15-h fast. All animals were housed in solid floored cages with a deep layer of paper chips that was changed daily. A 12:12 h light-dark cycle was used, and the animals were allowed free access to sufficient food and water. The blood glucose level was measured 1 day before and 2 weeks after STZ injection by measuring the glucose concentration in a blood sample obtained by tail prick using an Accu-Chek blood glucose monitoring system (Roche Diagnostics, Indianapolis, IN, USA). The mice were defined as diabetic when their blood glucose concentration exceeded 350 mg/dl. Mechanical hypersensitivity was assessed 2 weeks after STZ administration.

Assessment of mechanical hypersensitivity

Mice were placed in individual transparent Perspex cubicles with a wire mesh bottom, and a series of calibrated von Frey filaments (Semmes-Weinstein monofilaments; Stoelting, Wood Dale, IL, USA) was used to determine the 50% likelihood of a paw withdrawal response (50% threshold) by the up-down method of Dixon (17). Eight von Frey filaments, with approximately equal logarithmic incremental bending forces, were chosen (von Frey number: 2.36, 2.44, 2.83, 3.22, 3.61, 3.84, 4.08, and 4.17; equivalent to 0.02, 0.03, 0.07, 0.17, 0.41, 0.69, 1.20, and 1.48 g force, respectively). Testing was initiated with the 0.17-g hair, and each hair was applied perpendicularly to the plantar surface of the hindpaw, with sufficient force to bend the filament, for 3 – 4 s. Lifting of the paw indicated a positive response and prompted the use of the next weaker (i.e., lighter) filament. Absence of a paw withdrawal response prompted the use of the next stronger (i.e., heavier) filament. This paradigm was continued until four measurements had been obtained after an initial change in behavior, or until four consecutive positive scores (score of 0.01 g) or five negative scores (score of 1.5 g) had been obtained. The resulting scores were used to calculate the 50% threshold (18). In diabetic mice, testing was performed on both the right and left hindpaws of each animal [–30 min and immediately before (time zero), 15, 30, 60, 90, and 120 min after drug administration], and the mean of the two 50% threshold values was used for analysis. In the study presented here, a 50% threshold of 0.2 g in the von Frey test 2 weeks after STZ administration was considered to indicate development of mechanical hypersensitivity.

Drug administration

Zonisamide was donated by Dainippon Sumitomo Pharma (Osaka). For systemic administration, zonisamide

was dissolved in 0.9% saline and administered s.c. 15 min before formalin injection in a volume of 0.1 ml/10 g body weight. For intracerebroventricular (i.c.v.) administration, zonisamide was dissolved in dimethylsulfoxide (DMSO, 2% solution in distilled water) and administered 10 min before formalin injection in a volume of 10 μl via a disposable 27-gauge needle, which was inserted into the lateral ventricle (19). For intrathecal (i.t.) injection, zonisamide was dissolved in DMSO (20% solution in distilled water) and administered 7 min before or after formalin injection in a volume of 5 μl via a disposable 27-gauge needle, which was inserted into the subarachnoid space through the intervertebral foramen between L5 and L6 according to the method described by Hylden and Wilcox (20). When injected locally into the dorsal hindpaw, zonisamide was dissolved in 0.9% saline and administered 7 min before formalin injection in a volume of 15 μl.

Statistical analyses

All data are expressed as the mean ± S.E.M. The effects of zonisamide on nociceptive behavior and nociceptive threshold in the formalin and von Frey tests, respectively, were evaluated with respect to time; the time of administration of formalin and zonisamide in the formalin and von Frey tests, respectively, was defined as time zero. Two-tailed non-parametric multiple comparisons with Bonferroni correction following the Kruskal-Wallis test (21) were used for comparisons between the control and zonisamide-treated groups. The Mann-Whitney *U*-test was used for comparisons between two groups. Differences at $P<0.05$ (two-tailed) were considered significant.

Results

Effects of systemically, intracerebroventricularly and intrathecally administered zonisamide on nociceptive behavior in the formalin test

When administered s.c., zonisamide (3 and 10 mg /kg) reduced licking/biting behavior in both the first and second phases of the formalin test. In particular, its analgesic effect on the second phase of formalin-induced nociceptive behavior was potent and dose-dependent (Fig. 1A). When zonisamide was injected i.c.v. or i.t., it was dissolved with the aid of DMSO because of its poor solubility in saline. As shown in Fig. 1B, mice pretreated i.c.v. with 2% DMSO in a volume of 10 μl exhibited less nociceptive behavior after formalin injection compared with those pretreated with saline. Nevertheless, it was evident that zonisamide (10 and 30 μg in 2% DMSO, i.c.v.) produced a dose-dependent analgesic effect during the second phase of the formalin test. In contrast, i.t. injection of 20% DMSO in a volume of 5 μl alone did not influence the subsequent formalin-induced nociceptive behavior. Here again, zonisamide (10 and 30 μg in 20% DMSO, i.t.) produced dose-dependent inhibition of the second phase of formalin-induced nociceptive behavior (Fig. 1C). Together, it appears that both supraspinal and spinal sites contribute largely to the analgesic effects of systemically administered zonisamide on formalin-induced nociceptive behavior.

Effects of intrathecal administration of zonisamide after induction of the first phase in the formalin test

As demonstrated above, zonisamide injected i.t. before formalin injection selectively reduced the second phase of nociceptive behavior. We then explored whether the first phase is requisite for the spinal analgesic effect of zonisamide on the second phase in the formalin test. To this end, zonisamide was administered i.t. after induction of the first phase following formalin injection. Because 20% DMSO was used as a solvent, mice exhibited less nociceptive behavior in the second phase, which, however, was not affected by zonisamide (Fig. 2; 30 μg, i.t.).

Effects of local injection of zonisamide into the dorsal hindpaw in the formalin test

To explore possible peripheral mediation in the analgesic effect of systemically injected zonisamide on formalin-induced nociceptive behavior, it was then locally injected into the dorsal hindpaw. As shown in Fig. 3A, zonisamide (10 and 30 μg) administered into the left hindpaw ipsilateral to the formalin injection exhibited a less potent but dose-dependent inhibition of the second phase. By contrast, zonisamide (30 μg) administered into the right hindpaw contralateral to the formalin injection did not affect formalin-induced nociceptive behaviors (Fig. 3B), revealing that the analgesic effect of zonisamide administered into the left hindpaw was generated at the injection site but not secondarily at other sites, including the central nervous system. Together, the analgesic effect of systemically administered zonisamide in the formalin test appears to be partially mediated peripherally.

Effects of systemically, intracerebroventricularly, and intrathecally administered zonisamide on mechanical hypersensitivity in mice developing diabetic neuropathy

Fifty-seven mice developing diabetic mechanical hypersensitivity 2 weeks after STZ administration were used. Their mean blood glucose concentrations and 50% thresholds before and 2 weeks after STZ administration were 163.5 ± 2.7 mg/dl and 0.500 ± 0.021 g and 572.4 ± 6.0 mg/dl and 0.104 ± 0.005 g, respectively.

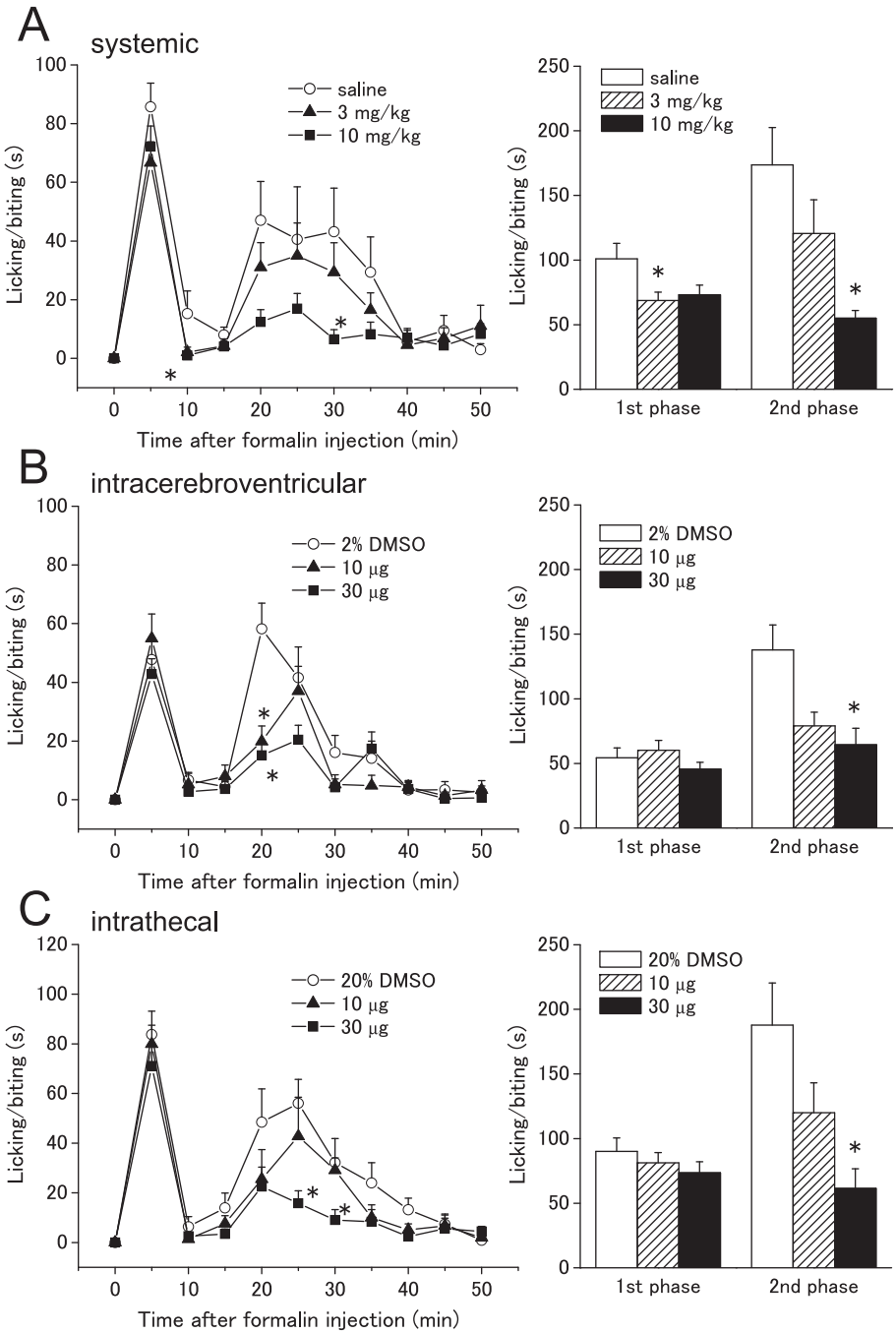

Fig. 1. Subcutaneously (s.c.), intracerebroventricularly (i.c.v.), and intrathecally (i.t.) administered zonisamide reduces formalin-induced nociceptive behavior. Zonisamide (A, 3 and 10 mg/kg, s.c.; B, 10 and 30 μg, i.c.v.; C, 10 and 30 μg. i.t.) was administered 15 min (A), 10 min (B), or 7 min (C) before formalin injection (formalin administered at time zero), and the formalin-induced nociceptive behavior characterized by licking/biting of the affected paw was measured for 50 min. Time-course graphs showing licking/biting behavior (s) in consecutive 5-min blocks and columns showing total licking/biting behavior (s) during the first (0 – 10 min) and second (10 – 40 min) phase. Each point or column represents the mean ± S.E.M. for 10 separate mice. The asterisks indicate data points for which a significant difference between the vehicle control (clear circles or columns) and zonisamide-treated groups (solid triangles and squares or hatched and solid columns) was observed, as determined by two-tailed non-parametric multiple comparisons with Bonferroni correction following the Kruskal-Wallis test (two comparisons in three groups, $*P<0.05$).

Either s.c. or i.t. injection of zonisamide (10 and 30 mg/kg, s.c. or 10 and 30 μg, i.t.) reduced mechanical hypersensitivity dose-dependently and significantly, and zonisamide at each higher dose reversed the 50% threshold almost to the level obtained before STZ administration (Fig. 4: A and C). Although i.c.v.-injected zonisamide (10 and 30 μg) tended to reduce mechanical hypersensitivity, its effect did not reach statistical significance (Fig. 4B).

Discussion

Recent animal studies demonstrating pain relief by the antiepileptic drug zonisamide in neuropathic pain models involving either chronic constriction injury (4) or partial ligation of the sciatic nerve (5) have suggested its therapeutic effectiveness in patients with neuropathic pain. Moreover, as we showed in the present study, zonisamide reduced formalin-induced nociceptive behavior as well as mechanical hypersensitivity due to

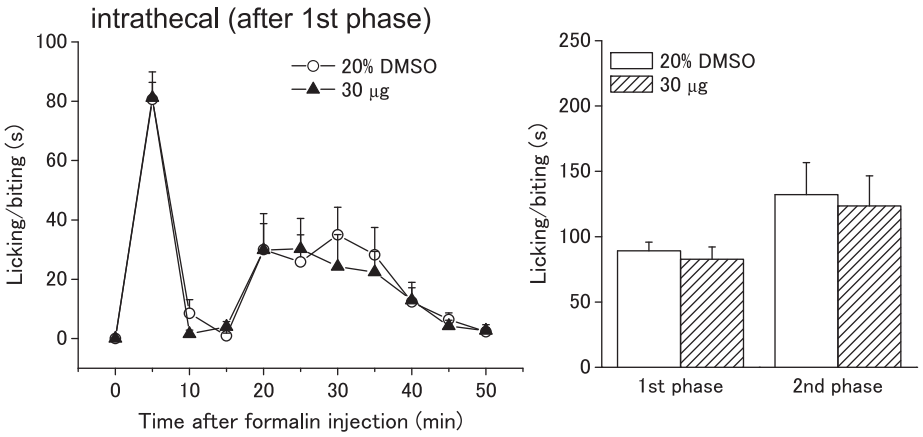

Fig. 2. No analgesic effects of intrathecally (i.t.) administered zonisamide after induction of the first phase in the formalin test. Zonisamide (30 μg) was administered 7 min after formalin injection (formalin administered at time zero), and the formalin-induced nociceptive behavior characterized by licking/biting of the affected paw was measured for 50 min. A time-course graph showing licking/biting behavior (s) in consecutive 5-min blocks and columns showing total licking/biting behavior (s) during the first (0 – 10 min) and second (10 – 40 min) phase. Each point or column represents the mean ± S.E.M. for 10 separate mice.

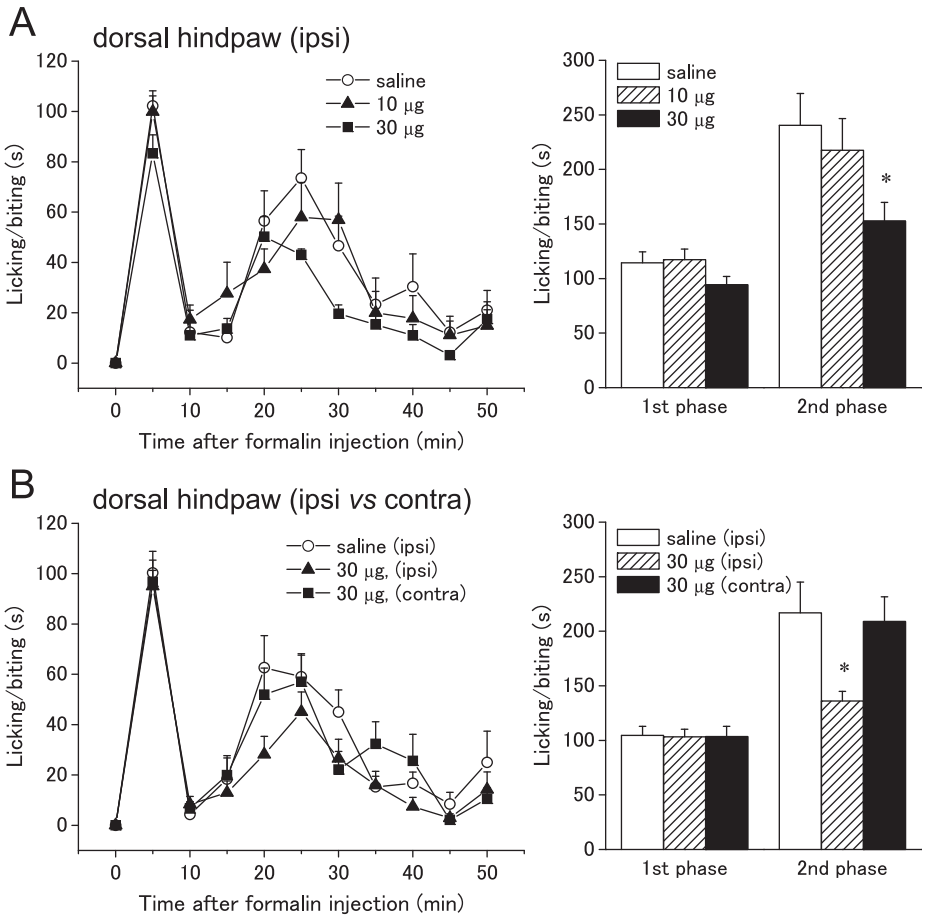

Fig. 3. Effects of intraplantar injection of zonisamide in the formalin test. Zonisamide was injected into the dorsal hindpaw 7 min before formalin injection (formalin administered at time zero), and the formalin-induced nociceptive behavior characterized by licking/biting of the affected paw was measured for 50 min. Time-course graphs showing licking/biting behavior (s) in consecutive 5-min blocks and columns showing total licking/biting behavior (s) during the first (0 – 10 min) and second (10 – 40 min) phase. A: Zonisamide (10 and 30 μg) produced analgesic effects when administered into the left hindpaw ipsilateral (ipsi) to the formalin injection. B: Zonisamide (30 μg) generated no analgesic effects when administered into the right hindpaw contralateral (contra) to the formalin injection. Each point or column represents the mean ± S.E.M. for 10 or 11 separate mice. The asterisks indicate data points for which a significant difference between the vehicle control (clear circles or columns) and zonisamide-treated groups (solid triangles and squares or hatched and solid columns) was observed, as determined by two-tailed non-parametric multiple comparisons with Bonferroni correction following the Kruskal-Wallis test (two comparisons in three groups, *P<0.05).

diabetic neuropathy. Thus, accumulating experimental evidence suggests that zonisamide is an alternative pharmacological tool for treatment of persistent pain that is largely refractory to standard analgesics such as morphine (1).

Since systemically administered zonisamide can penetrate the blood-brain barrier and therefore reach the central nervous system (22), it is most likely that zonisamide produces centrally mediated analgesic effects in the formalin test and in diabetic mice developing mechanical hypersensitivity. Indeed, the effects of systemically administered zonisamide in reducing both formalin-induced nociceptive behavior and diabetic mechanical hypersensitivity were largely mimicked when it was injected directly into the supraspinal and/or spinal region. Together with our previous study reveal-

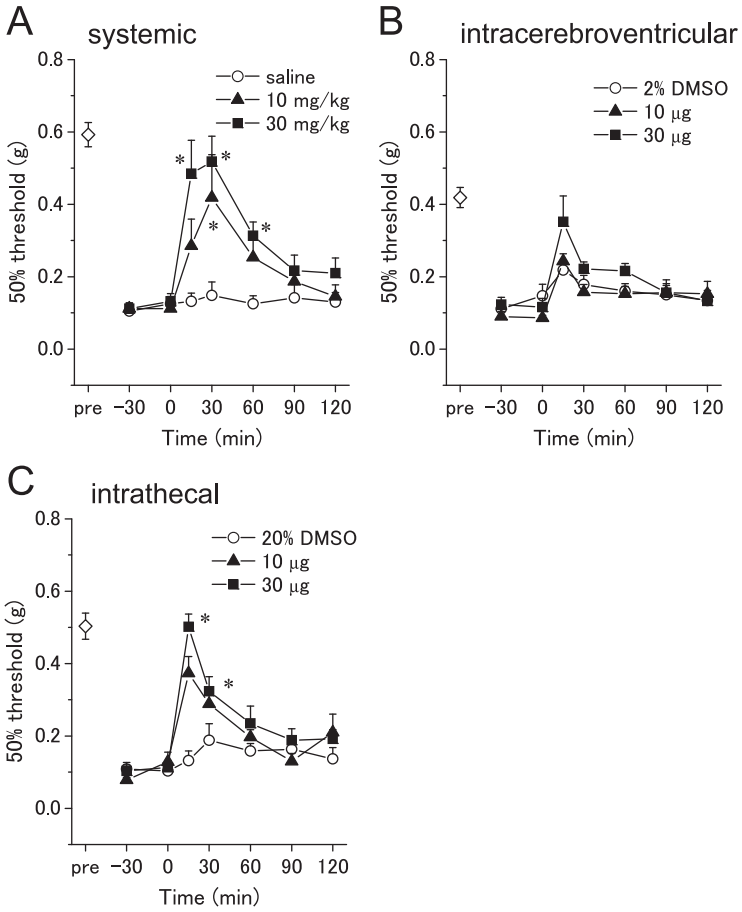

Fig. 4. Subcutaneously (s.c.), intracerebroventricularly (i.c.v.), and intrathecally (i.t.) administered zonisamide reverses the reduced withdrawal threshold to mechanical stimuli developing in streptozotocin (STZ)-injected diabetic mice. STZ was injected into 3- to 4-week-old mice, and mechanical hypersensitivity was assessed 2 weeks later by the von Frey test. Zonisamide (A, 10 and 30 mg/kg, s.c.; B, 10 and 30 μg, i.c.v.; C, 10 and 30 μg. i.t.) was administered at time zero. Each point represents the mean ± S.E.M. for 6 or 7 separate mice. Ordinates: mean 50% thresholds (von Frey test). Abscissae: 2 weeks before (pre: before STZ administration) and time in minutes after zonisamide administration. The clear diamond in each graph indicates the mean of pooled 50% thresholds obtained before STZ administration in the three groups of mice. The asterisks indicate data points for which a significant difference between the vehicle control (clear circles) and drug-treated groups (solid triangles and squares) was observed, as determined by two-tailed non-parametric multiple comparisons with Bonferroni correction following the Kruskal-Wallis test (two comparisons in three groups, $*P<0.05$).

ing its centrally mediated analgesic effect on thermal and mechanical hypersensitivity in mice with partial sciatic nerve ligation (5), it is most likely that the supraspinal and spinal structures are of primary importance when analyzing the analgesic effects of systemically administered zonisamide.

Repetitive pain signals arriving at the spinal cord elicit short- and/or long-lasting increases in the excitability of primary sensory afferent terminals and spinal dorsal horn neurons (23, 24), which contribute to enhanced efficacy of synaptic transmission in the spinal dorsal horn in chronic pain states. Cellular mechanisms of short- and/or long-lasting plasticity and sensitization may include alterations in the function and/or expression of various receptors and ion channels that play a crucial role in the determination of membrane potential. Subcutaneous formalin injection elicits biphasic nociceptive behaviors, and central sensitization induced by nociceptive inputs arriving during the first phase largely contributes to the second phase (13). It is noteworthy that zonisamide was ineffective at ameliorating the second phase of the formalin test when injected spinally following the first phase, suggesting that it prevents the development of plasticity or central sensitization triggered by the first phase but not the persistent pain under sensitized conditions, at least in the formalin test. In contrast, i.t.-injected zonisamide reverses the thermal and mechanical hypersensitivity that develops after partial sciatic nerve ligation (5) and diabetic mechanical hypersensitivity, as shown in this study, suggesting that it generates analgesic effects on sensitized chronic pain. Plausibly, the mechanisms underlying plasticity or central sensitization may differ between the second phase of formalin-induced nociceptive behavior and chronic pathophysiologic pain after peripheral nerve injury (25). Previous studies have demonstrated similar analgesic properties of the NMDA-receptor blocker MK-801 and the local anesthetic lidocaine. Intrathecal treatment with MK-801 (26, 27) and lidocaine (28) before formalin injection, but not after the first phase, reduces the second phase. Moreover, spinally delivered MK-801 (29, 30, but see 31) or lidocaine (32) reverses neuropathic symptoms. Since MK-801 (33) and lidocaine (34) also produce preemptive analgesia, as is often expected by the effectiveness in the formalin test, the possibility arises that zonisamide can produce preemptive analgesia. This remains to be further addressed in a future study. Thus,

zonisamide can be useful for both preventing and reversing pathophysiologic pain.

We also explored a possible peripheral contribution to the analgesic effect of zonisamide in the formalin test. In fact, local peripheral injection of the anticonvulsant gabapentin ameliorates nociceptive behavior in the formalin test (35). Moreover, we previously demonstrated that zonisamide produced analgesic effects on acute nociception via peripheral mechanisms including a use-dependent block of Na^+ channels (36). Since local injection of zonisamide into the dorsal hindpaw ipsilateral to the formalin injection caused dose-dependent inhibition of the second phase without significant effects on the first phase, this peripheral action appears to contribute to the analgesic effect of systemically administered zonisamide assessed in the formalin test.

Considering that zonisamide blocks sodium channels (9) and T-type calcium channels (9, 10), it is possible that these multiple actions on ion channels contribute to the observed analgesic effect in the formalin test and in diabetic mice developing mechanical hypersensitivity. In our preliminary study, the sodium channel blocker mexiletine exhibited marked reduction of the first phase as well as the second phase in the formalin test (data not shown), consistent with the study by Blackburn-Munro et al. (37). They also demonstrated that lamotrigine and carbamazepine, both of which also block sodium channels, preferentially reduced the second phase in the formalin test. These differences of sodium channel blockers in reducing formalin-induced nociceptive behavior have been deduced to reflect both their use-dependence and selective binding properties to voltage-activated sodium channels (37). Thus, the sodium channel–blocking action of zonisamide plausibly participates in the centrally and peripherally mediated analgesic effect observed in the formalin test. Moreover, painful diabetic neuropathy is associated with functional changes in tetrodotoxin-sensitive and -resistant sodium channels and increases in their currents in dorsal root ganglion neurons (38, 39). Hence, zonisamide may block sodium channels centrally at the spinal cord and peripherally along the primary afferent fibers, leading to the analgesic effect on mechanical hypersensitivity in diabetic mice. Recent experimental evidence demonstrates that spinal T-type calcium channels are involved in the pain sensitization process (40) and the first and second phases of formalin-induced nociceptive behavior (41), suggesting that the T-type calcium channel–blocking action of zonisamide seems to be less related to the spinal analgesic effects demonstrated in this study.

Our results confirm that zonisamide also has a supraspinal site of action to produce its analgesic effects in the formalin test and in diabetic mice. In a recent study using mice after peripheral nerve injury, we showed that supraspinally administered zonisamide does not activate the descending noradrenergic pain inhibitory system (8). Moreover, we have to take into consideration that supraspinally injected zonisamide does not produce any antinociceptive effects in normal naïve mice (5). This strongly suggests that some plastic changes may take place supraspinally to enable zonisamide to inhibit ascending pain signals or affect the descending pathways that do not involve the noradrenergic projection, and this issue remains to be further addressed.

In conclusion, the present results indicate that zonisamide effectively generates analgesic effects on persistent pain including inflammatory and neuropathic pain, as shown in the formalin test and in diabetic mice developing mechanical hypersensitivity. Together with our previous study demonstrating its effectiveness for neuropathic pain after peripheral nerve injury (5), we propose that zonisamide should be considered as an alternative pharmacological tool for treatment of persistent pain that is largely refractory to standard analgesics such as morphine.

References

1 Arner S, Meyerson BA. Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain. 1988;33:11–23.

2 Rogawski MA, Löscher W. The neurobiology of antiepileptic drugs for the treatment of nonepileptic conditions. Nature Medicine. 2004;10:685–692.

3 Ruscheweyh R, Sandkühler J. Epileptiform activity in rat spinal dorsal horn in vitro has common features with neuropathic pain. Pain. 2003;105:327–338.

4 Hord AH, Denson DD, Chalfoun AG, Azevedo MI. The effect of systemic zonisamide (Zonegran™) on thermal hyperalgesia and mechanical allodynia in rats with an experimental mononeuropathy. Anesth Analg. 2003;96:1700–1706.

5 Tanabe M, Sakaue A, Takasu K, Honda M, Ono H. Centrally mediated antihyperalgesic and antiallodynic effects of zonisamide following partial nerve injury in the mouse. Naunyn Schmiedebergs Arch Pharmacol. 2005;372:107–114.

6 Baulac M. Introduction to zonisamide. Epilepsy Res. 2006;68 Suppl 2:S3–S9.

7 Pappagallo M. Newer antiepileptic drugs: possible uses in the treatment of neuropathic pain and migraine. Clin Ther. 2003;25: 2506–2538.

8 Tanabe M, Takeuchi Y, Ono H. The supraspinally mediated analgesic effects of zonisamide in mice after peripheral nerve injury are independent of the descending monoaminergic system. J Pharmacol Sci. 2007;104:335–340.

9 Masuda Y, Ishizaki M, Shimizu M. Zonisamide: pharmacology and clinical efficacy in epilepsy. CNS Drug Rev. 1998;4:341–360.

10 Suzuki S, Kawakami K, Nishimura S, Watanabe Y, Yagi K, Seino M, et al. Zonisamide blocks T-type calcium channel in cultured neurons of rat cerebral cortex. Epilepsy Res. 1992; 12:21–27.
11 Mori A, Noda Y, Packer L. The anticonvulsant zonisamide scavenges free radicals. Epilepsy Res. 1998;30:153–158.
12 Noda Y, Mori A, Packer L. Zonisamide inhibits nitric oxide synthase activity induced by N-methyl-D-aspartate and buthionine sulfoximine in the rat hippocampus. Res Commun Mol Pathol Pharmacol. 1999;105:23–33.
13 Coderre TJ, Vaccarino AL, Melzack R. Central nervous system plasticity in the tonic pain response to subcutaneous formalin injection. Brain Res. 1990;535:155–158.
14 Khan GM, Chen SR, Pan HL. Role of primary afferent nerves in allodynia caused by diabetic neuropathy in rats. Neuroscience. 2002;114:291–299.
15 Suzuki Y, Sato J, Kawanishi M, Mizumura K. Lowered response threshold and increased responsiveness to mechanical stimulation of cutaneous nociceptive fibers in streptozotocin-diabetic rat skin in vitro – correlates of mechanical allodynia and hyperalgesia observed in the early stage of diabetes. Neurosci Res. 2002;43:171–178.
16 Chen X, Levine JD. Hyper-responsivity in a subset of C-fiber nociceptors in a model of painful diabetic neuropathy in the rat. Neuroscience. 2001;102:185–192.
17 Dixon WJ. Efficient analysis of experimental observations. Annu Rev Pharmacol Toxicol. 1980;20:441–462.
18 Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods. 1994;53:55–63.
19 Haley TJ, McCormick WG. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br J Pharmacol Chemother. 1957;12:12–15.
20 Hylden JL, Wilcox GL. Intrathecal morphine in mice: a new technique. Eur J Pharmacol. 1980;67:313–316.
21 Glantz SA. Alternatives to analysis of variance and the t test based on ranks. In: Jeffers JD, Englis MR, editors. Primer of Biostatistics. 3rd ed. New York: McGraw-Hill; 1992. p. 320–371.
22 Masuda Y, Utsui Y, Shiraishi Y, Karasawa T, Yoshida K, Shimizu M. Relationships between plasma concentrations of diphenylhydantoin, phenobarbital, carbamazepine, and 3-sulfamoylmethyl-1,2-benzisoxazole (AD-810), a new anticonvulsant agent, and their anticonvulsant or neurotoxic effects in experimental animals. Epilepsia. 1979;2:623–633.
23 Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science. 2000;288:1765–1769.
24 Yang L, Zhang FX, Huang F, Lu YJ, Li GD, Bao L, et al. Peripheral nerve injury induces trans-synaptic modification of channels, receptors and signal pathways in rat dorsal spinal cord. Eur J Neurosci. 2004;19:871–883.
25 Yashpal K, Fisher K, Chabot JG, Coderre TJ. Differential effects of NMDA and group I mGluR antagonists on both nociception and spinal cord protein kinase C translocation in the formalin test and a model of neuropathic pain in rats. Pain. 2001;94:17–29.
26 Coderre TJ, Melzack R. The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. J Neurosci. 1992;12:3665–3670.
27 Yamamoto T, Yaksh TL. Comparison of the antinociceptive effects of pre- and posttreatment with intrathecal morphine and MK801, an NMDA antagonist, on the formalin test in the rat. Anesthesiology. 1992;77:757–763.
28 Yashpal K, Mason P, McKenna JE, Sharma SK, Henry JL, Coderre TJ. Comparison of the effects of treatment with intrathecal lidocaine given before and after formalin on both nociception and Fos expression in the spinal cord dorsal horn. Anesthesiology. 1998;88:157–164.
29 Yamamoto T, Yaksh TL. Spinal pharmacology of thermal hyperesthesia induced by constriction injury of sciatic nerve. Excitatory amino acid antagonists. Pain. 1992;49:121–128.
30 Chaplan SR, Malmberg AB, Yaksh TL. Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat. J Pharmacol Exp Ther. 1997;280:829–838.
31 Erichsen HK, Blackburn-Munro G. Pharmacological characterisation of the spared nerve injury model of neuropathic pain. Pain. 2002;98:151–161.
32 Ma W, Du W, Eisenach JC. Intrathecal lidocaine reverses tactile allodynia caused by nerve injuries and potentiates the antiallodynic effect of the COX inhibitor ketorolac. Anesthesiology. 2003;98:203–208.
33 Wei H, Pertovaara A. Influence of preemptive treatment with MK-801, an N-methyl-D-aspartate receptor antagonist, on development of neuropathic symptoms induced by spinal nerve ligation in the rat. Anesthesiology. 1999;91:313–316.
34 Dahl JB, Brennum J, Arendt-Nielsen L, Jensen TS, Kehlet H. The effect of pre-versus postinjury infiltration with lidocaine on thermal and mechanical hyperalgesia after heat injury to the skin. Pain. 1993;53:43–51.
35 Carlton SM, Zhou S. Attenuation of formalin-induced nociceptive behaviors following local peripheral injection of gabapentin. Pain. 1998;76:201–207.
36 Sakaue A, Honda M, Tanabe M, Ono H. Antinociceptive effects of sodium channel-blocking agents on acute pain in mice. J Pharmacol Sci. 2004;95:181–188.
37 Blackburn-Munro G, Ibsen N, Erichsen HK. A comparison of the anti-nociceptive effects of voltage-activated Na^+ channel blockers in the formalin test. Eur J Pharmacol. 2002;445:231–238.
38 Hong S, Morrow TJ, Paulson PE, Isom LL, Wiley JW. Early painful diabetic neuropathy is associated with differential changes in tetrodotoxin-sensitive and -resistant sodium channels in dorsal root ganglion neurons in the rat. J Biol Chem. 2004;279:29341–29350.
39 Hong S, Wiley JW. Altered expression and function of sodium channels in large DRG neurons and myelinated A-fibers in early diabetic neuropathy in the rat. Biochem Biophys Res Commun. 2006;339:652–660.
40 Ikeda H, Heinke B, Ruscheweyh R, Sandkühler J. Synaptic plasticity in spinal lamina I projection neurons that mediate hyperalgesia. Science. 2003;299:1237–1240.
41 Cheng JK, Lin CS, Chen CC, Yang JR, Chiou LC. Effects of intrathecal injection of T-type calcium channel blockers in the rat formalin test. Behav Pharmacol. 2007;18:1–8.