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BW1003C87, phenytoin and carbamazepine elevate seizure threshold in the rat amygdala-kindling model of epilepsy

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Abstract

We examined the anticonvulsant effects of BW1003C87 (5-(2,3,5-trichlorophenyl)-2,4-diaminopyrimidine ethane sulphonic acid), which is structurally related to the new antiepileptic drug, lamotrigine, and compared its effects to those of the conventional antiepileptic drugs, phenytoin and carbamazopine, using the rat amygdala-kindling model of epilepsy. BW1003C87 (2.5–10 mg/kg, i.p.) had potent and long-lasting (48 h after single administration) effects on amygdala-kindled seizures. The effects of BW1003C87 were completely reversed when the stimulus intensity was increased to 2 or 3 times the threshold determined. Since the same effects on seizure threshold were obtained for phenytoin and carbamazepine in the present study and for lamotrigine in our previous study, we propose that the principal mechanism of these antiepileptic drugs, which act primarily on voltage-sensitive Na^+ channels, is significant elevation of the seizure threshold in epileptogenic foci and that BW1003C87 has a profile similar to that of these drugs. © 1997 Elsevier Science B.V.

Keywords: BW1003C87; Phenytoin; Carbamazopine; Anticonvulsant effects; Amygdala-kindling; Seizure threshold

1. Introduction

BW1003C87 (5-(2,3,5-trichlorophenyl)-2,4-diaminopyrimidine ethane sulphonic acid) is structurally related to the new antiepileptic drug, lamotrigine. Lamotrigine has been reported to have potent antiepileptic effects in human epilepsy (Pellock, 1994; Brodie et al., 1995). We have recently demonstrated that lamotrigine has unique anticonvulsant profiles in the kindling model (Otsuki et al., 1995), which is accepted as the best animal model of temporal lobe epilepsy (Sato et al., 1990).

Since lamotrigine acts on type IIA voltage-sensitive Na^+ channels (Cheung et al., 1992; Lang et al., 1992; Xie et al., 1995) and reduces veratrine-induced excessive glutamate release (Leach et al., 1986), lamotrigine and BW1003C87 have been expected to have neuroprotective effects against ischemic brain damage. In fact, several recent studies have reported that BW1003C87 reduces glutamate release and protects neurons in the rat hippocampus and striatum following transient ischemia (Meldrum et al., 1992; Lekieffre and Meldrum, 1993; Gaspary et al., 1994). These findings in turn suggest that BW1003C87 should be useful as an antiepileptic drug.

In the present study, we examined the time-dependent and dose-dependent anticonvulsant effects of BW1003C87 on amygdala-kindled seizures in rats. Furthermore, the effect of BW1003C87 on the seizure threshold was compared with the effects of the conventional antiepileptic drugs, phenytoin and carbamazepine.

2. Materials and methods

2.1. Kindling procedures

Details of the kindling procedures were as described in our previous report (Morimoto et al., 1997). Male Sprague–Dawley rats weighing 250–350 g at the time of surgery were used. All rats were individually housed with ad libitum access to food and water. Under pentobarbital

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anesthesia (50 mg/kg), a tripolar electrode was stereotaxically implanted into the left amygdala (2.6 mm posterior and 4.9 mm lateral from bregma and 7.8 mm below the dura), using the coordinate assignments of the Paxinos and Watson (1986) brain atlas. The electrode consisted of 3 twisted stainless-steel wires (0.2 mm diameter) insulated with a Teflon coating. In addition, a screw electrode was placed in the right frontal skull for electroencephalogram (EEG) recording.

After a recovery period of 1 week, all rats received kindling stimulation to the amygdala once daily, which consisted of 2 s trains of 100 Hz monophasic square pulses at the intensity of the afterdischarge threshold until at least 5 consecutive generalized seizures (stage 4 or 5 seizures using the classification of Racine (1978)) appeared.

The generalized seizure-triggering threshold was determined for each rat by the application of trains of stimuli with intensity increasing by 50 μA steps, starting with 50 μA, at intervals of 15 min. After stable stage 5 seizures had been induced by stimulation at the determined generalized seizure-triggering threshold intensity, the pharmacological experiments were performed.

2.2. Pharmacological experiments

BW1003C87 (supplied by Wellcome Research Labs) was dissolved in 5% glucose (2 ml/kg). Phenytoin and carbamazepine (Sigma) were dissolved in 70% propylene glycol and 2% Tween 80 (2 ml/kg). All drugs and vehicles were administered intraperitoneally (i.p.).

2.3. Assessment of anticonvulsant effects

2.3.1. Time- and dose-dependent effects

In amygdala-kindled rats ($N = 5$), the amygdala was stimulated with the determined generalized seizure-triggering threshold intensity at 4, 24, 48, 72, 96, 120, 144 and 168 h after i.p. administration of BW1003C87 at 2.5, 5.0 or 10 mg/kg. The amygdala-kindled seizure stage (measured by the classification of Racine (1978)) and afterdischarge duration were compared with those at 24 h before drug administration. The sequence of drug dosages was randomly assigned. The interval between dose trials was at least 2 weeks.

Since the maximal anticonvulsant effects of BW1003C87 appeared 4 h after i.p. administration in this experiment, we chose 4 h as the post-injection time for the testing of BW1003C87 in subsequent experiments.

2.3.2. Effects on seizure thresholds

In amygdala-kindled rats ($N = 5$), electrical stimulation at 2 or 3 times the determined generalized seizure-triggering threshold intensity was delivered 4 h after i.p. administration of 5 mg/kg BW1003C87 and reversal of seizure stage and afterdischarge duration was compared with those obtained with generalized seizure-triggering threshold stimulation.

In addition, another group of amygdala-kindled rats ($N = 5$) was used to test the effects of phenytoin and carbamazepine on seizure threshold. 30 min after i.p. administration of phenytoin (180 mg/kg) or carbamazepine (40 mg/kg), electrical stimulation at the intensity of generalized seizure-triggering threshold or 2 or 3 times generalized seizure-triggering threshold was delivered to the amygdala and reversal of anticonvulsant effects was measured. The drug doses and post-injection time selected were those found maximally effective in our previous studies (Otsuki et al., 1995).

2.4. Statistics

All values are expressed as means ± S.E.M. Seizure stage scores were analysed by the two-tailed Wilcoxon's signed-rank test, and afterdischarge durations by the two-tailed Student's *t*-test.

3. Results

3.1. Time- and dose-dependent effects

BW1003C87 had long-lasting and potent anticonvulsant effects on amygdala-kindled seizures following single i.p. administration (Fig. 1 and Table 1). For example, 4 h after administration of a high dose of BW1003C87 (10 mg/kg), complete abolition of both seizure and afterdischarge induction was observed in 4 of 5 rats, while the remaining rat exhibited stage 2 seizure with brief afterdischarge. Significant anticonvulsant effects on seizure stage were still observed at 24 and 48 h, but had completely disappeared by 168 h after administration.

These anticonvulsant effects were clearly dose-dependent. A small dose of BW1003C87 (2.5 mg/kg) had weak and insignificant anticonvulsant effects at 4 h, while a moderate dose (5 mg/kg) had potent and significant effects at 4 and 24 h after administration.

The anticonvulsant effects of BW1003C87 appeared to be all-or-none in character: the numbers of amygdala-kindled rats which exhibited complete suppression of kindled seizures were 1/5 (2.5 mg/kg, 4 h), 4/5 (5 mg/kg, 4 h) and 5/5 (10 mg/kg, 4 h).

3.2. Effects on seizure threshold

After treatment with BW1003C87 at 5 mg/kg in amygdala-kindled rats, when electrical stimulation was delivered at generalized seizure-triggering threshold intensity, the mean seizure stage was reduced to 0.8 (control: 5.0), the

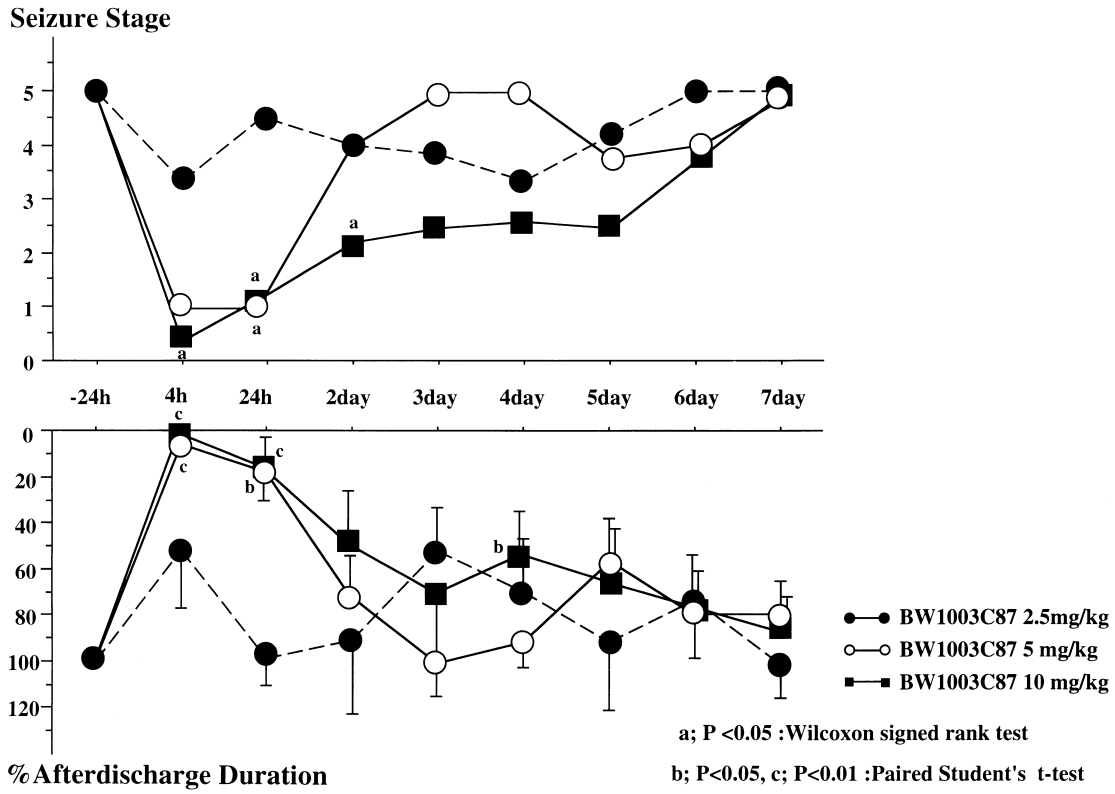

Fig. 1. Time- and dose-dependent anticonvulsant effects of BW1003C87 on amygdala-kindled seizures in rats. BW1003C87 was administered i.p. at various doses and electrical stimulation was delivered to the amygdala 4 h to 7 days after administration. The seizure stage and afterdischarge duration were compared with those 24 h before administration. Note that maximal effects were observed at 4 h and that effects were long-lasting after single administration.

mean after-discharge duration was significantly shortened to 2.2 ± 2.2 s (control: 114.6 ± 10.2 s) and generalized seizures were suppressed in 5 of 5 rats. As stimulus intensity was increased, these anticonvulsant effects were significantly reversed in a stimulus intensity-dependent fashion. When stimulation was delivered at 2 or 3 times generalized seizure-triggering threshold, the means of seizure stage and afterdischarge duration were 3.4 and 4.4 and 54.0 ± 29.7 and 93.2 ± 23.7 s and generalized seizures appeared in 3 and 5 of 5 rats, respectively (Fig. 2 and Table 1). Thus, despite BW1003C87 administration, there were no significant differences in anticonvulsant effects at 3 times generalized seizure-triggering threshold intensity, compared with effects at generalized seizure-triggering threshold.

After treatment with phenytoin and carbamazepine, potent and significant anticonvulsant effects were also observed on amygdala-kindled seizures (seizure stage: respectively, 0.0 and 0.2, afterdischarge duration: 1.4 ± 1.4 and 6.8 ± 2.9 s). Similar to the results for BW1003C87, when stimulus intensity was increased to 2 or 3 times generalized seizure-triggering threshold intensity, the anti-

Table 1
Effects of BW1003C87, phenytoin and carbamazepine on seizure threshold in amygdala-kindled rats

Treatment/stimulus intensity	No. of generalized clonic seizures		
	BW1003C87 (5 mg/kg)	phenytoin (180 mg/kg)	carbamazepine (40 mg/kg)
Vehicle			
GST	5/5	5/5	5/5
Antiepileptic drugs			
GST	0/5	0/5	0/5
2 × GST	2/5	4/5	2/5
3 × GST	5/5	5/5	5/5

GST: generalized seizure-triggering threshold.

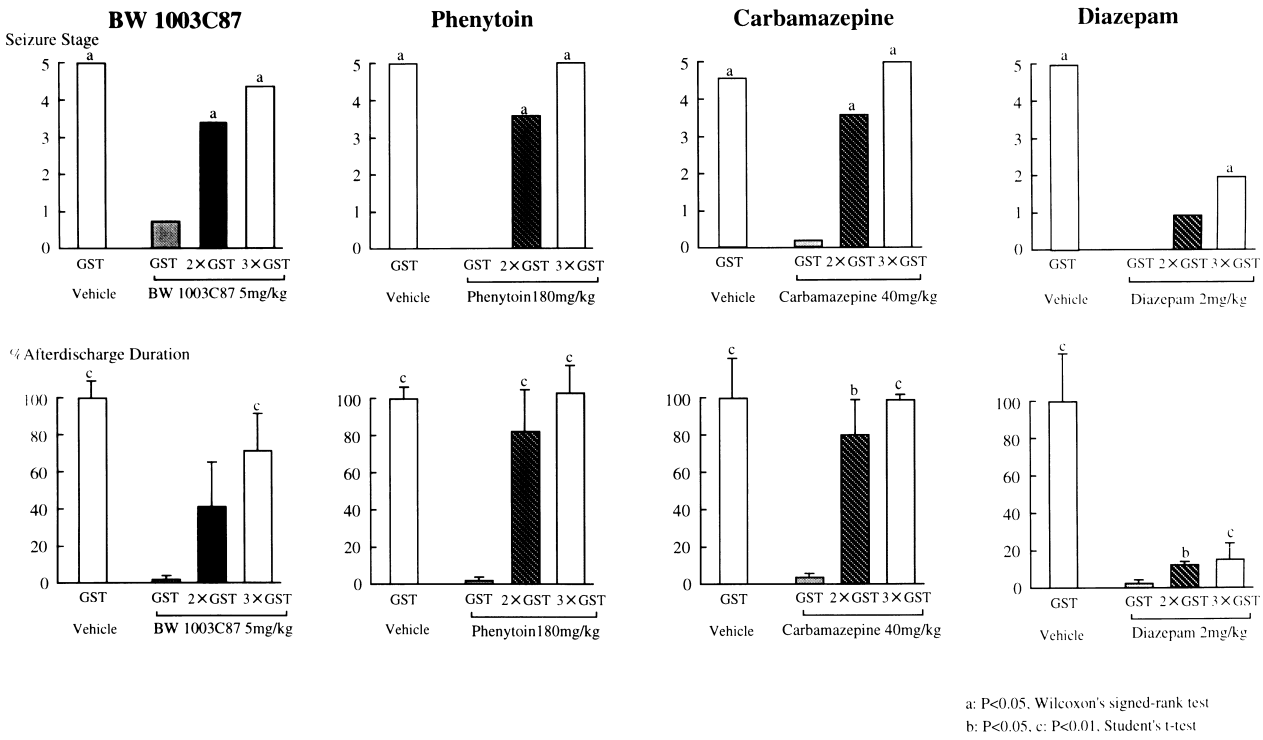

Fig. 2. Effects of BW1003C87, phenytoin, carbamazepine and diazepam on seizure threshold in amygdala-kindled rats. Electrical stimulation at the intensity determined for the generalized seizure-triggering threshold (GST) and 2 or 3 times GST was delivered after i.p. administration of BW1003C87 (5 mg/kg, 4 h), phenytoin (180 mg/kg, 30 min), carbamazepine (40 mg/kg) or diazepam (2 mg/kg, 20 min). Note that the anticonvulsant effects of BW1003C87, phenytoin and carbamazepine on both seizure stage (upper) and afterdischarge duration (bottom) were completely reversed as stimulus intensity was increased. In contrast, only a slight effect was obtained from treatment with diazepam. The results for diazepam are from Morimoto et al., 1987.

convulsant effects of phenytoin and carbamazopine were completely reversed (Fig. 2 and Table 1).

4. Discussion

4.1. Anticonvulsant effects of BW1003C87

The present study clearly demonstrated potent and unique anticonvulsant effects of BW1003C87 in the amygdala-kindling model of temporal lobe epilepsy. Our findings indicate that BW1003C87 has unusually long-lasting effects, since amygdala-kindled seizures were significantly reduced for 48 h and the effects of BW1003C87 did not disappear completely until 168 h after single i.p. administration. We recently found that lamotrigine, a new antiepileptic drug which is structurally related to BW1003C87, also has long-acting anticonvulsant effects (approximately 24 h) on both amygdala- and hippocampal-kindled seizures in rats (Otsuki et al., 1995). The very long biological half-life of lamotrigine (approximately 25 h) was reported in an earlier study (Messenheimer, 1995).

In our previous study (Otsuki et al., 1995), lamotrigine also suppressed kindled seizures in a nearly all-or-none fashion, in which effective doses (20 mg/kg) of lamotrigine completely eliminated amygdala- and hippocampal-kindled seizures and induction of afterdischarge in all rats tested. The anticonvulsant effects of lamotrigine were successfully reversed when the stimulus intensity was increased. Therefore, the profile of anticonvulsant effects of BW1003C87 is almost the same as that of lamotrigine.

4.2. Effects of seizure threshold

We also compared the effects of BW1003C87 on seizure threshold in amygdala-kindled rats with the effects of the conventional antiepileptic drugs, phenytoin and carbamazepine. Interestingly, the anticonvulsant effects of all 3 of these compounds were completely reversed when stimulus intensity was increased, suggesting that the principal mechanism of action of these compounds is elevation of seizure threshold in the kindled epileptogenic focus. In a previous study by Rundfeldt et al. (1990) who used a different method to measure seizure threshold, phenytoin also potently raised the threshold for focal seizures up to about 600% in amygdala-kindled rats.

In contrast, in previous studies of other types of antiepileptic drugs, including diazepam (benzodiazepine agonist, see Fig. 2), tiagabine (selective γ-aminobutyric

acid (GABA) uptake inhibitor) and valproate (GABA metabolism enhancer), anticonvulsant effects on amygdala-kindled seizures were not completely reversed when the stimulus intensity was increased even to 3 times the generalized seizure-triggering threshold, suggesting that these antiepileptic drugs have a weaker effect on seizure threshold than do phenytoin and carbamazopine (Morimoto et al., 1987; Löscher et al., 1993; Morimoto et al., 1997). Furthermore, no effects on seizure threshold were found for 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxaline (NBQX), a selective antagonist of excitatory amino acid receptors (Namba et al., 1993). Taken together, these findings suggest that antiepileptic drugs can be classified into 3 types by their profiles of effect on seizure threshold in the amygdala-kindling model of epilepsy: phenytoin/carbamazopine type, valproate/diazepam type, and NBQX type.

Recently, a number of studies have demonstrated that phenytoin and carbamazepine act primarily at voltage-sensitive Na^+ channels, resulting in the blockade of sustained repetitive neuronal firing (see reviews by Rogawski and Porter, 1990 and Macdonald and Kelly, 1995). BW1003C87 and lamotrigine can be categorized as being of the phenytoin/carbamazepine-type, based on their effects on seizure threshold observed in the kindling model. Although enhancers of GABA/benzodiazepine systems, including diazopam, tiagabine and valproate, elevate seizure threshold at high doses (Morimoto et al., 1987; Löscher et al., 1993; Morimoto et al., 1997), the fundamental profile of this type of antiepileptic drugs is different from that of phenytoin/carbamazopine-type antiepileptic drugs.

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