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Broad spectrum anticonvulsant activity of BW534U87: possible role of an adenosine-dependent mechanism

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Abstract

The novel putative anticonvulsant drug 1-[2,6-difluorophenyl)-methyl]-1*H*-1,2,3-triazolo[4,5-*c*]) pyridine-4-amine monohydrochloride (BW534U87) effectively reduced seizures induced in rodents by threshold maximal and supramaximal electroshock, electrical kindling, pentylenetetrazole (PTZ) infusion and by vestibular stimulation in the genetically seizure-prone epilepsy-like (EL) mouse. The range of animal seizure models in which BW534U87 was effective is consistent with a broad spectrum anticonvulsant profile. In the EL mouse, the activity of BW534U87 was partially reversed by predosing with the selective adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), suggesting that an adenosine-dependent mechanism contributed to the antiseizure activity of the drug. BW534U87 inhibited rat brain homogenate adenosine deaminase activity, thus, raising the possibility that, by blocking the metabolism of endogenous adenosine by this route, BW534U87 limited seizure activity by promoting the inhibitory tone mediated by endogenous adenosine in the brain. The seizure protection conferred by the selective adenosine deaminase inhibitor *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) in EL mice and mice infused with PTZ confirms that inhibition of adenosine metabolism by deamination is an effective antiseizure strategy in these models.
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1. Introduction

A preliminary investigation into the antiseizure activity of the novel putative anticonvulsant drug 1-[2,6-difluorophenyl)-methyl]-1*H*-1,2,3-triazolo[4,5-*c*]) pyridine-4-amine monohydrochloride (BW534U87) revealed its capacity to protect rats against seizures induced by supramaximal electroshock (Kelley et al., 1995). The efficacy of BW534U87 in this model of generalised tonic clonic seizures was determined to be five-fold more potent than the standard anticonvulsant phenytoin. Since the clinical need is for novel medicines with efficacy against a broad spectrum of seizure types, we have now tested BW534U87 in a battery of rat and mouse seizure models with components thought to represent a range of partial and generalised seizure (King and LaMotte, 1989; Loscher, 1988; Loscher et al., 1991a,b) in order to gain an insight into its likely breadth of activity in human epilepsies.

An early clue to a possible mechanism of action of BW534U87 was the observation that it blocked binding of radiolabelled 2′deoxycoformycin to adenosine deaminase, one of the enzymes responsible for adenosine metabolism (Jones, Cox and Morgan, unpublished observations). More recently, in vitro electrophysiology provided evidence to suggest that BW534U87 limited hyperexcitability in rat hippocampal slices partly through an adenosine-dependent mechanism (Dupere et al., 1999). The significance of the inhibitory tone provided by endogenous adenosine, primarily via adenosine A1 receptor mechanisms (Brundege and Dunwiddie, 1997), in limiting seizure activity in epilepsy is well recognised (Dragunow, 1988; Stevens and Haas, 1997;

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Young and Dragunow, 1994). Consequently, we have performed a series of behavioural and biochemical experiments designed to investigate further the possible contribution of an adenosinergic component to the mechanism of action of BW534U87. These included testing the effect of adenosine A1 receptor blockade on the antiseizure activity of the drug by predosing genetically seizure-prone epilepsy-like (EL) mice with the selective A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (Haleen et al., 1987), examining the effect of the drug on rat brain adenosine deaminase activity in vitro, and testing the anticonvulsant activity of a potent inhibitor of adenosine deaminase (*erythro*-9-(2-hydroxy-3-nonyl)adenine, EHNA) (Schaeffer and Schwender, 1974) in EL mice and mice infused with the convulsant compound pentylenetetrazole (PTZ).

2. Methods

2.1. Animal models

All animal studies were performed in strict accordance with the UK Animals Scientific Procedures Act 1986.

2.2. EL mouse

Young adult (5–8 weeks) EL mice of both sexes (experience suggests that gender does not influence seizure susceptibility, GlaxoSmithKline Rodent Breeding Unit) were sensitized to vestibular stimulation as described previously (King and LaMotte, 1989). Briefly, animals were gently tossed 10–15 cm into the air up to a maximum of 30 times, or until seizure initiation, at twice weekly intervals. The sensitization process was considered complete (2–4 weeks) when animals exhibited ictal seizures (tonic–clonic convulsions with loss of postural equilibrium, straub tail and salivation) on three consecutive occasions. After the administration of test substances or vehicle, animals were stimulated to seizure or up to a maximum of 10 tosses more than that required to induce seizures in the absence of drug. Seizure severity was scored according to the features described in Table 1. On the basis of preliminary experiments, mice (groups of 6–12) were dosed intraperitoneally with BW534U87 60 min prior to stimulation and EHNA subcutaneously 20 min before stimulation. DPCPX was administered intraperitoneally 20 min prior to BW534U87. Statistical comparisons were made using the Kruskal–Wallis multiple comparisons test or nonparametric Mann–Whitney *U* test as appropriate.

Table 1
Scoring table for assessing seizure severity in EL mice

Signs of seizure activity	Score
Squeaking	1
Running	1
Clonus	2
Straub tail	2
Loss of righting reflex	2
Hindlimb extension	2
Cumulative score	10

Total seizure scores comprise the sum of scores assigned for each of the observed signs of seizure activity.

2.3. Mouse PTZ infusion

Details of the mouse PTZ infusion model have been described previously (Loscher et al., 1991b). Briefly, male CD-1 mice (25–30 g, Charles River, UK) were infused via the lateral tail vein with 10 mg ml^{-1} PTZ at a rate of 50 μl min^{-1} and observed for signs of seizure, initially a generalised myoclonic jerk (first twitch) followed by clonus with loss of righting reflex. Latency to seizure (up to a maximum of 300 s) was noted. Groups of 6–12 mice were dosed intraperitoneally with BW534U87 or subcutaneously with EHNA, 60 and 20 min, respectively, before PTZ. Statistical comparisons were made using the Kruskal–Wallis test.

2.4. Mouse threshold maximal electroshock seizure

Male CD-1 mice (20–30 g, Charles River) were subjected to electric current, initially 10 mA for 300 ms, via transauricular electrodes, and observed for tonic hindlimb extension. When an animal exhibited tonic hindlimb extension, the current passed to the next animal was reduced by an increment of 1 mA. Conversely, when no hindlimb extension was observed, current applied to subsequent animals was increased in 1 mA increments. The current required to produce tonic hindlimb extension in 50% of mice (calculated $current_{50}$, CC_{50}) was derived from the numbers of positive and negative responses within a group of 20 animals administered vehicle or BW534U87 (subcutaneously, 30 min before testing) and the standard error of the samples calculated according to Kimball et al. (1957).

2.5. Rat supramaximal electroshock seizure

Electric current (a 300-ms pulse of 200 mA, approximately 10-fold the threshold required to elicit tonic hindlimb extension) was delivered to male Han Wistar rats (120–180 g, Charles River) via transauricular electrodes and the presence or absence of tonic hindlimb extension noted. Results are expressed as the percentage of animals protected from hindlimb extension. Animals (five to seven per group) were dosed orally via gavage needle with BW534U87 60 min before testing. Minimum effective dose was calculated using the two-sided Fisher's exact test.

2.6. Amygdala kindled rats

Male Lister Hooded rats (250–300 g, GlaxoSmithKline Rodent Breeding Unit) were anaesthetised by the subcutaneous administration of 1.1 ml kg^{-1} Hypnovel (midazolam,

Table 2
Modified Racine Scale of seizure severity in amygdala kindled rats (modified from Racine, 1972)

Seizure class	Description
1	Frozen immobility/facial twitches
2	Rhythmic whisker twitching or rhythmic head nodding or rhythmic chewing
3	Unilateral or bilateral forelimb tonus and/or clonus
4	Bilateral forelimb clonus and raising of torso into rearing position
5	Four with falling or bilateral hindlimb clonus

2 mg ml^{-1}) followed by 1.1 ml kg^{-1} Hypnorm (fentanyl, 0.315 mg ml^{-1} and fluanisone, 10 mg ml^{-1}) and bipolar stimulating/recording electrodes were chronically implanted into the right basolateral amygdala according to the following stereotaxic coordinates with the incisor bar set at −3.3 mm: 2.8 mm posterior to bregma, 4.8 mm lateral and 7.2 mm below skull surface (Paxinos and Watson, 1982). At least 2 weeks after surgery, the afterdischarge threshold (minimum current required to elicit a train of spikes in the amygdala at a rate of at least one per second) was determined by stimulating initially at 80 μA (1 s train of biphasic square wave pulses, 1 ms pulse width, 60 Hz) and increasing in 10 μA increments until an afterdischarge was observed. Animals were then kindled by stimulating once daily (Monday to Friday) at a current 25% greater than that determined for afterdischarge threshold until seizures of class 5 severity according to a modified Racine Scale (see Table 2) were elicited on 3 consecutive days. For testing, animals ($n=5$) were stimulated at the same current on alternate days over a 5-day period with vehicle administered on the first and fifth days, and BW534U87 (100 mg kg^{-1}, orally, 2-h prestimulation) on the third day. Seizure severity and afterdischarge duration (determined from electroencephalogram recordings) were compared before (Day 1) and after (Day 3) the administration of BW534U87 using a paired Student's t-test.

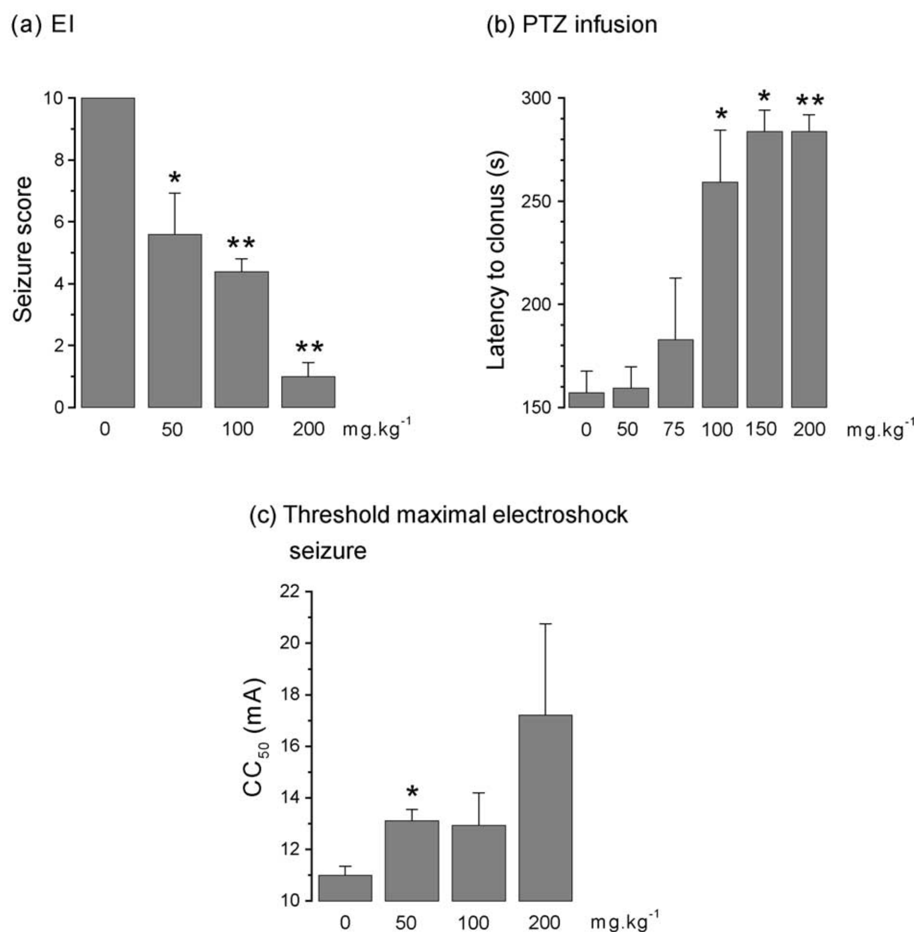

Fig. 1. Anticonvulsant effects of BW534U87 in mouse seizure models. (a) Dose-dependent reduction of seizure severity in EL mice stimulated by spatial disorientation. (b) Dose-dependent increase in latency to clonus with loss of righting reflex in mice infused with PTZ. (c) Increase in current (CC_{50}) required to elicit tonic hind limb extension in threshold maximal electroshock test. Each column represents the mean (±S.E.) of groups of 6–12 animals (a, b) or CC_{50} (±S.E.) of 20 animals (c). *, ** Significantly different from vehicle, $P<.05$ and .01, respectively (Kruskal–Wallis test).

2.7. Rat brain homogenate adenosine deaminase activity studies

2.7.1. Preparation of rat brain homogenate

Forebrain was quickly removed from adult male Han Wistar rats (Charles River), immersed in ice-cold 50 mM phosphate buffer pH 7.0 containing 1 mM $MgCl_2$ and 1 mM ethylenediaminetetraacetic acid (EDTA), and homogenized using an Ystral mechanical homogenizer. The soluble fraction was isolated by centrifugation and frozen (−70 °C) until use.

2.7.2. Adenosine deaminase activity assay

Adenosine deaminase activity was determined using a colourimetric method (Guisti and Galanti, 1984) in which the stoichiometric conversion of adenosine to inosine and ammonia was monitored by inducing the latter to react with an alkaline hypochlorite/phenol solution to generate a blue indophenol dye. One milliliter of rat brain homogenate (protein concentration ~0.5 mg ml^{-1}, as determined according to Bradford, 1976) was mixed with 50 μl adenosine solution (final concentrations in the range 50–500 μM) and incubated at 37 °C for 15 min. Enzyme activity was terminated by the addition of 3 ml 0.17 mM sodium nitroprusside in 106 mM phenol plus 3 ml 11 mM NaOCl in 125 mM NaOH. Colour was allowed to develop for 15 min at 37 °C before the absorbence at 620 nm was determined using a spectrophotometer (Cecil Instruments, Cambridge, UK). Sample blank was prepared by adding the adenosine solution after the phenol/hypochlorite solutions. A standard curve was constructed by replacing the 1 ml rat brain homogenate with $(NH_4)_2SO_4$ solutions in the range 20–

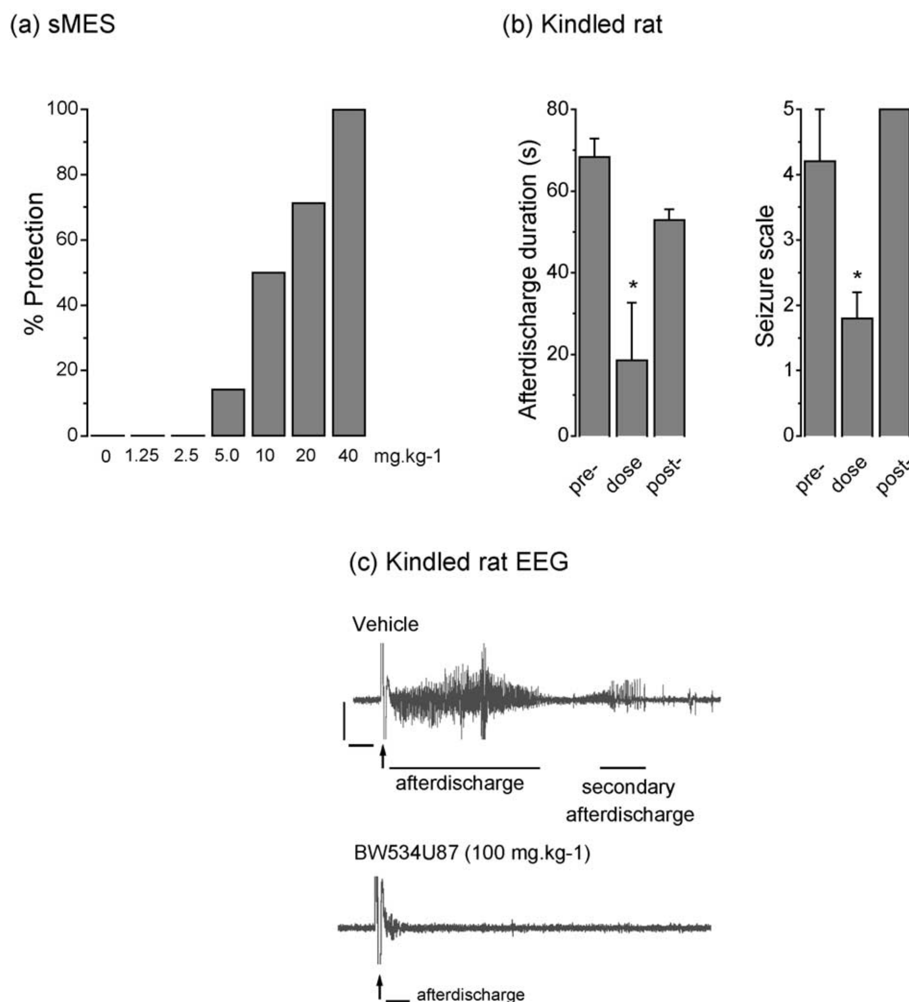

Fig. 2. Anticonvulsant effects of BW534U87 in rat seizure models. (a) Dose-dependent protection against hindlimb extension induced by supramaximal electroshock. Minimum effective dose of BW534U87 was 20 mg kg^{-1} ($P<.05$, two-sided Fisher's exact test). Columns represent mean % protection in groups of five to seven animals. (b) BW534U87 (100 mg kg^{-1}) reduced afterdischarge duration and seizure severity in kindled rats. Each column represents mean (±S.E.) of afterdischarge duration or modified Racine Scale estimation of seizure severity in groups of five animals. (c) Representative electroencephalograms (EEG) demonstrating the marked reduction in afterdischarge duration in fully kindled rats administered 100 mg kg^{-1} BW534U87. Scale bars: vertical 1 μV; horizontal 10 s. During the afterdischarge, the vehicle- and BW534U87-treated animals from which the EEGs were recorded exhibited seizures of Modified Racine Scales 5 and 2, respectively. * Significantly different from predose values, $P<.05$, respectively (Kruskal–Wallis test).

200 μM. Each data point represents the mean of three separate experiments.

3. Materials

BW534U87 and lamotrigine isothionate were synthesized by GlaxoSmithKline. Protein assay reagents were purchased from Biorad, UK. DPCPX, EHNA and all other chemicals were obtained from Sigma-Aldrich, UK. For in vivo administration, BW534U87 was prepared as a suspension in 0.25% methyl cellulose and EHNA was dissolved in saline. DPCPX was initially dissolved in 1/10th volume dimethyl sulfoxide (DMSO)/1 M sodium hydroxide before making up the volume with 0.25% methyl cellulose. Maximum drug volume administered to mice was 10 ml kg^{-1} and to rats 1 ml kg^{-1}. For in vitro investigations, BW534U87, EHNA and lamotrigine were dissolved in 45% 2-hydroxypropyl β-cyclodextrin. Data plotting and curve fitting was conducted using Origin 5.0.

4. Results

4.1. Profile of BW534U87 in animal seizure models

Figs. 1 and 2 demonstrate that BW534U87 exerted antiseizure activity in a range of both mouse and rat models. In the EL mouse, there was a dose-dependent reduction in seizure score, which was statistically significant at doses of 50 mg kg^{-1} and above (Fig. 1a). In mice continuously infused with PTZ, there was a dose-dependent increase in latency to clonus with loss of righting reflex, which was significant at 100 mg kg^{-1} (Fig. 1b), although there was no corresponding increase in the latency to the appearance of the first twitch (not shown). In the mouse threshold maximal electroshock test, a dose-related trend was observed for BW534U87 to increase CC_{50} values, although, due to greater variation at higher doses, this effect only reached statistical significance at 50 mg kg^{-1} (Fig. 1c).

In rats, BW534U87 dose-dependently protected against supramaximal electroshock-induced tonic hindlimb extension (Fig. 2a) with a minimum effective dose of 20 mg kg^{-1}. In kindled rats, both afterdischarge duration (Fig. 2b,c) and seizure severity (Fig. 2b) were significantly reduced by 100 mg kg^{-1} BW534U87 but appeared to have no effect at 20 mg kg^{-1} (n = 3, not shown).

Signs of central side-effects were minimal. Rats subjected to supramaximal electroshock were visually assessed for ataxia prior to stimulation. Mild (normal gait but with occasional unsteadiness) to moderate (abnormal gait) ataxia was observed in half of the animals administered doses of 20 mg kg^{-1} or greater. No evidence of sedation was observed in rats or mice in any of these studies.

4.2. Effect of adenosine A1 receptor blockade on antiseizure efficacy of BW534U87

The contribution of adenosine A1 receptor activation on the efficacy of BW534U87 in the EL mouse model was tested by prior dosing with the selective A1 receptor antagonist DPCPX (Fig. 3). In the absence of the antagonist, 150 mg kg^{-1} BW534U87 reduced seizure severity score by about 70%. In the presence of 3 and 10 mg kg^{-1} DPCPX, the antiseizure activity of BW534U87 was attenuated as demonstrated by a significant increase in seizure score (P < .05 and .01, respectively). The mean effect of 10 mg kg^{-1} DPCPX was no greater than that of the lower dose indicating that the observed effect of adenosine A1 receptor blockade on seizure protection by BW534U87 was likely to be maximal. This data suggests that adenosine A1 receptor activation partly but not entirely mediates the antiseizure activity of BW534U87 in this model.

4.3. Effect of BW534U87 on adenosine deaminase activity

Rat brain homogenate adenosine deaminase activity was inhibited by BW534U87 in a concentration-dependent manner (Fig. 4a). With a substrate concentration of 250 μM, 50% inhibition was achieved by 66 μM BW534U87. As a positive control, EHNA (Schaeffer and Schwender, 1974) was tested and also found to exert concentration-dependent inhibition of rat brain homogenate adenosine deaminase activity (IC_{50} = 433 nM). In contrast, the unrelated anticonvulsant lamotrigine failed to inhibit rat brain adenosine deaminase activity in vitro at concentrations up to 1 mM.

A double reciprocal plot (Fig. 4b) describing rat brain homogenate adenosine deaminase activity as a function of substrate concentration revealed a k_m for the enzyme (intercept on the abscissa) of 45 μM. In the presence of 30 μM

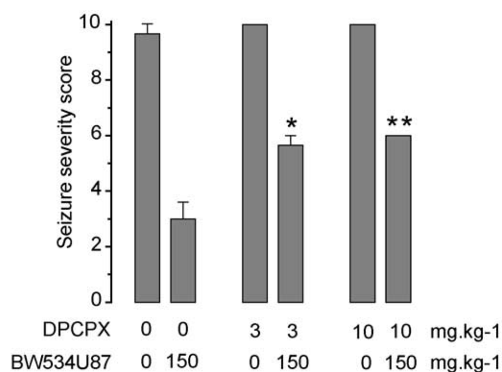

Fig. 3. Partial reversal of the anticonvulsant action of BW534U87 in EL mice by the selective adenosine A1 receptor antagonist DPCPX. Each column represents the mean (± S.E.) of groups of 6–12 animals. Absence of error bars indicates that all animals within the group received identical seizure severity ratings. *, ** Significantly different from seizure severity score in animals administered BW534U87 alone, P < .05 and .01, respectively (Mann–Whitney U test).

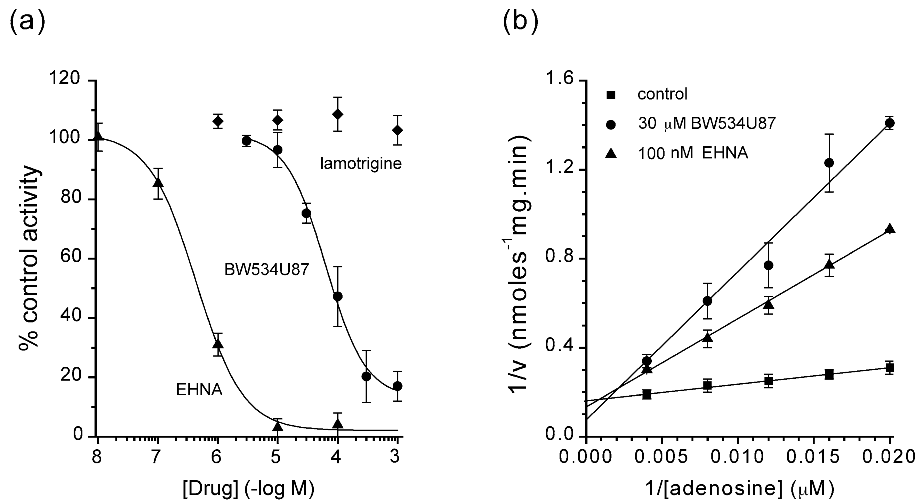

Fig. 4. BW534U87 inhibits rat brain homogenate adenosine deaminase activity. (a) Concentration inhibition curves demonstrating the effects BW534U87 and the selective adenosine deaminase inhibitor EHNA on the adenosine deaminase activity of rat brain homogenate incubated in the presence of 250 μM adenosine. The unrelated anticonvulsant lamotrigine (< 1 mM) did not modulate enzyme activity. (b) Double reciprocal plots of adenosine deaminase activity (1/v) as a function of substrate concentration (1/[adenosine]) in the absence and presence of 30 μM BW534U87 or 100 nM EHNA indicate competitive inhibition by both compounds (K_i = 7 μM and 12 nM, respectively). Each data point represents the mean (± S.E.) of three separate determinations.

BW534U87, the slope of the plot was markedly increased, whereas the v_{max} (intercept on the ordinate) was unchanged indicating competitive inhibition. The k_i for BW534U87 was calculated as 7 μM. EHNA also inhibited the adenosine deaminase activity of rat brain homogenate in a competitive fashion with its k_i determined as 12 nM.

4.4. Effect of EHNA in mouse seizure models

In EL mice, EHNA, administered 20 min before the commencement of vestibular stimulation, dose-dependently reduced seizure score with seizures completely eliminated by 100 mg kg^{-1} EHNA (P < .05, Fig. 5a). In mice infused with PTZ, EHNA significantly increased the latency to the appearance of clonus at 150 mg kg^{-1} (Fig. 5b, P < .01).

5. Discussion

A key objective of the present series of investigations was to discover whether BW534U87 was an effective anticonvulsant in a battery of animal models thought to represent many of the human epilepsies and thus to gain an insight into whether this compound might address the pressing clinical need for novel drugs with improved efficacy against a broad spectrum of epileptic seizure types. Initially, we confirmed that BW534U87 provided seizure protection to rats subjected to supramaximal electroshock (Kelley et al., 1995) and also demonstrated efficacy against seizures induced in mice by threshold maximal electroshock. Both maximal electroshock models are thought to represent generalised tonic clonic seizures (Loscher et al.,

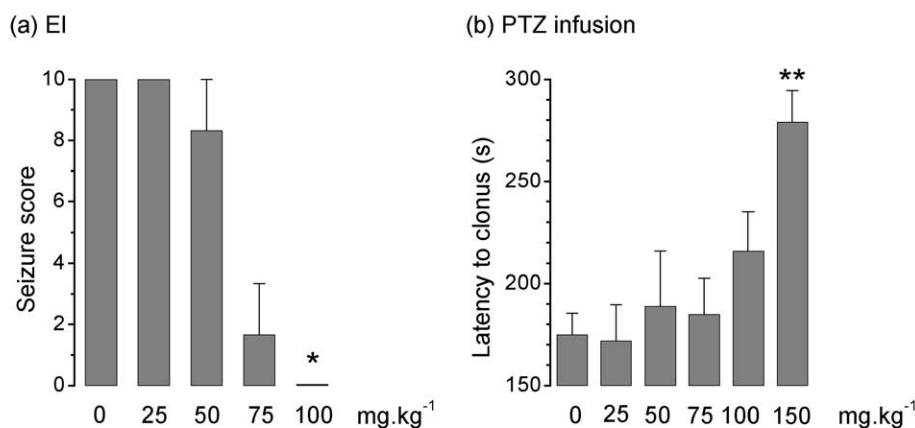

Fig. 5. Anticonvulsant effects of the selective adenosine deaminase inhibitor EHNA in mouse seizure models. (a) Dose-dependent reduction of seizure severity in EL mice stimulated by spatial disorientation with complete blockade of seizures at 100 mg kg^{-1}. (b) Dose-dependent increase in latency to clonus and loss of righting reflex in mice infused with PTZ. Each column represents the mean (± S.E.) of groups of 6–12 animals. *, ** Significantly different from vehicle, P < .05 and .01, respectively (Kruskal–Wallis test).

1991a). In addition, BW534U87 was effective against seizures in EL mice and electrically kindled rats, models which have features in common with partial seizures with secondary generalisation (Goddard et al., 1969; King and LaMotte, 1989). Finally, BW534U87 reduced seizure activity induced in mice by PTZ infusion, a model of generalised myoclonic seizures (Loscher, 1988). Although a model thought to represent absence seizures was not included in the battery of tests performed in this study, the range of seizure models in which BW534U87 was shown to be effective is consistent with the profile of a broad-spectrum anticonvulsant.

Having established that BW534U87 was effective in combating a wide range of experimental seizure types, we considered the mechanism by which this compound might exert its anticonvulsant activity. Recently published in vitro electrophysiological evidence suggested that BW534U87 limited hyperexcitability in rat hippocampal slices partly through an adenosine-dependent mechanism (Dupere et al., 1999). Activation of adenosine A1 receptors has been widely reported to reduce seizure activity in a variety of animal seizure models (Abdul-Ghani et al., 1997; Concas et al., 1993; MacGregor et al., 1993; Malhotra and Gupta, 1997; Pourgholami et al., 1997), an effect reversed by the potent and selective A1 receptor antagonist DPCPX (Malhotra and Gupta, 1997). The observed reduction in seizure protection provided by BW534U87 in EL mice administered DPCPX (at doses not noticeably pro-convulsant) does suggest that A1 receptor activation is a factor in the antiseizure activity of BW534U87. However, the incomplete reversal of the anticonvulsant effects of BW534U87 by DPCPX suggests that adenosine A1 receptor activation is only partially responsible for the efficacy of the drug, at least in this particular model. It is possible that other adenosine receptors may also contribute since adenosine $A2_A$ receptor activation has been reported to depress seizure activity (Adami et al., 1995). If so, it remains possible that adenosinergic mechanisms may entirely account for the anticonvulsant activity of BW534U87. Alternatively, a non-adenosinergic mechanism may contribute to the action of BW534U87. In limiting hyperexcitability in rat hippocampal slices, BW534U87 appeared to act by a dual mechanism, one adenosinergic, the second the voltage- and frequency-dependent inhibition of voltage gated sodium channels (Dupere et al., 1999), observations which are entirely consistent with the current behavioural data.

The importance of endogenous adenosine in restricting seizure activity is illustrated by the convulsant or proconvulsant effect of A1 receptor antagonism by methylxanthines (Ault et al., 1987; Eldridge et al., 1989; Murray et al., 1985; Zwillich et al., 1975), the striking elevations in extracellular adenosine concentrations in the hippocampus of sufferers of intractable temporal lobe epilepsy (During and Spencer, 1992) and in the brains of experimental animals subjected to convulsive procedures (Berman et al., 2000; Schultz and Lownstein, 1978; Winn et al., 1980), and by data identifying adenosine as a potential mediator of seizure arrest and postictal refractoriness in human epilepsy (During and Spencer, 1992). These observations led to the idea that adenosine may be an endogenous anticonvulsant (Dragunow, 1988; Stevens and Haas, 1997) and thereby suggest that boosting concentrations of endogenous adenosine by inhibiting its metabolism may be an effective antiseizure strategy.

Metabolism of adenosine occurs via two routes: phosphorylation and deamination. k_m values for adenosine kinase in rat brain extract are reported to be about 2 μM (Phillips and Newsholme, 1979), close to resting extracellular adenosine concentrations in the brain (Van Wylen et al., 1986), which suggests that, under normal conditions, adenosine is likely to be phosphorylated. However, during seizure activity, when extracellular adenosine concentrations rise by up to 30-fold in affected brain regions (During and Spencer, 1992; Schultz and Lownstein, 1978; Winn et al., 1980), adenosine kinase will rapidly become saturated. Since adenosine deaminase has both a higher k_m and capacity than adenosine kinase (present investigation, Geiger and Nagy, 1986; Phillips and Newsholme, 1979), it is likely that deamination will be the more significant metabolic route during seizure activity. The present demonstration that an in vitro preparation of rat brain adenosine deaminase was inhibited by BW534U87 raises the possibility that inhibition of adenosine deaminase may contribute to the activity of BW534U87 by promoting adenosinergic inhibitory tone. Enzyme kinetic studies revealed the inhibition to be competitive with a k_i of 7 μM. The significance of this figure is illustrated by unpublished pharmacodynamic data suggesting that the ED_{50} for BW534U87 in the rat supramaximal electroshock model results in peak brain concentrations of the drug in the range 1–10 μM.

Evidence supporting the approach of combating seizure activity in the brain by inhibiting adenosine deaminase is limited (Zhang et al., 1993). Our observations that EHNA, a well-characterised inhibitor of adenosine deaminase (Schaeffer and Schwender, 1974), is an effective anticonvulsant in both EL and PTZ-infused mice suggest that blocking adenosine metabolism by this route is an effective means of protecting against seizure activity in these models. Furthermore, in contrast to the previously reported 30% maximum efficacy in inhibiting bicuculline-induced seizures in the rat prepiriform cortex with the adenosine deaminase inhibitor 2′deoxycoformycin (Zhang et al., 1993), we saw no such ceiling of efficacy in blocking seizures in EL mice with EHNA.

In conclusion, we provide evidence to suggest that: (1) the putative anticonvulsant BW534U87 inhibits seizures in a wide range of animal models thereby raising hopes that it may be effective against a broad spectrum of human seizures; (2) based on the partial reversal of the antiseizure activity of BW534U87 in EL mice by the blockade of adenosine A1 receptors, adenosine may contribute to its mechanism of action; (3) BW534U87 is an inhibitor of

adenosine deaminase in vitro; and (4) inhibition of adenosine metabolism by adenosine deaminase is an effective strategy for limiting seizures in animal models.

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