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In vivo pharmacological effects of JZP-4, a novel anticonvulsant, in models for anticonvulsant, antimania and antidepressant activity

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Abstract

JZP-4 is a potent calcium and sodium channel blocker, which is currently being evaluated in patients as an anticonvulsant and mood stabilizer. In the current studies, JZP-4 was evaluated in a variety of animal models for anticonvulsant, antimania and antidepressant activity. In the mouse and rat maximal electroshock models, JZP-4 was slightly more potent than LTG. In the mouse pentylenetetrazole induced seizures model, JZP-4 was approximately twice as potent as lamotrigine in prolonging the time to clonus. In the mouse 6-Hz model for drug resistant or refractory epilepsy, JZP-4 had potent anticonvulsant activity at all current intensities, whereas LTG was active at only the lowest current intensity. In the mouse amphetamine-chlordiazepoxide model for antimanic effects, JZP-4, but not LTG, produced dose-related and significant effects at 3 and 10 mg/kg i.p. In the rat forced swim model of antidepressant activity, JZP-4 (30 mg/kg i.p.) produced a significant reduction in immobility and an increase in climbing behavior. LTG (30 mg/kg i.p.) produced similar effects but these effects did not achieve statistical significance. The specificity of this antidepressant response was confirmed in the rat locomotor test. In this test, JZP-4 produced dose-related and significant reductions in locomotor activity, indicating that it was not a CNS stimulant. LTG produced no significant effects in the rat locomotor test. The studies have demonstrated that JZP-4 has greater potency and efficacy than LTG in models of refractory epilepsy, antidepressant activity and antimania activity. The variance between the effects of LTG and JZP-4 may be related to the greater potency at sodium channels or the additional pharmacological actions of JZP-4 on calcium channels.

Keywords: JZP-4; Lamotrigine; Anticonvulsant; Mood stabilizer; Antidepressant; Mania; Epilepsy; Bipolar disorder

1. Introduction

Anticonvulsants are among the most widely utilized pharmacological agents for CNS disorders with applications for neurological disorders such as epilepsy, essential tremor and neuropathic pain and for psychiatric disorders such as mood stabilizers in bipolar disorder, schizophrenia and drug withdrawal (Goldsmith et al., 2003; French et al., 2004; Morley-Forster, 2006; Weisler et al., 2006). Although these compounds are used in a broad range of patients, many of these drugs have dose limiting side-effects, such as sedation, cognitive impairment, weight gain or birth defects. A few of these compounds may even have rare, but life-threatening side-effects such as serious rash, blood dyscrasias, hepatic failure or cardiac arrhythmias (Stefan and Feuerstein, 2007). To avoid some of these adverse events, many anticonvulsants have to be titrated slowly to a therapeutic level, which also implies that the patients will have minimal benefit during the initial subtherapeutic phase (Ketter et al., 2005). There remains a

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need for drugs that have broad spectrum efficacy and the tolerability and pharmacokinetic profiles that allow them to be dosed rapidly to achieve steady state pharmacokinetics at therapeutic doses.

Lamotrigine (LTG), one of the better tolerated anticonvulsants, is clinically useful as an anticonvulsant for the treatment of refractory partial epilepsy, generalized seizures, absence seizures and Lennox-Gastaut syndrome (Leach et al., 2002) and as a mood stabilizer in bipolar II disorder (Bowden, 1998; Shelton, 2002; Goldsmith et al., 2003). Although LTG is generally well tolerated, it has to be titrated to therapeutic doses because it can induce an immunologic hypersensitivity reaction (Ketter et al., 2005). The cause of this hypersensitivity has been linked to an arene oxide metabolite, which has been proposed to be the immunoreactive antigen (Maggs et al., 2000; Anderson, 2002; Bavdekar et al., 2004, Fig. 1). Further complicating the titration of LTG is its long (14–60 h) half-life which requires a prolonged titration period to achieve steady state serum concentrations.

JZP-4 (3-(2,3,5-trichloro-phenyl)-pyrazine-2,6-diamine) is a potent and selective sodium and calcium channel blocker (Foreman et al., submitted for publication) that is currently in clinical trials as an anticonvulsant and mood stabilizer. It has structural similarities related to LTG but has a trichlorophenyl ring rather than a dichlorophenyl ring and a pyrazine rather than a triazine ring (Fig. 1). These modifications may be responsible for its greater potency for both sodium and calcium channels and a greater selectivity for the central compared to peripheral ion channels (Foreman et al., submitted for publication). The structural characteristics of JZP-4 also change in pathways involved with the metabolism from predominately metabolism by UGT1A4 for lamotrigine to oxidation by CYP1A2 followed by glucuronidation by UGT2B15 for JZP-4. The additional chlorine on the phenyl ring also reduces the possibility of the formation of the potentially immunogenic arene oxide (Fig. 1), which may lower its risk of serious dermatological side-effects. JZP-4 also has a lower half-life compared to LTG (manuscript in preparation), making it pharmacokinetically possible to achieve therapeutic doses at steady state with a shorter titration period (Eller et al., in preparation).

The current studies focus on the comparative effects of JZP-4 in a variety of animal models for different forms of epilepsy, bipolar mania and depression. The animal models for anticonvulsant activity included are those that are considered predictive for anticonvulsant activity in generalized myoclonic seizures, generalized tonic–clonic seizures, partial seizures with secondary generalization and treatment-resistant epilepsy. The animal models for psychiatric disorders included the amphetamine-chlordiazepoxide induced hyperactivity model for antimania activity, the forced swim test for antidepressant activity and the locomotor activity evaluation that discriminates generalized stimulatory or sedative effects that can lead to false positive responses in the other behavioral models. Each of these psychopharmacological models are considered to be predictive and have been extensively validated.

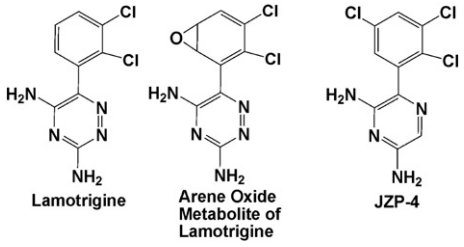

Fig. 1. Structures of lamotrigine, the arene oxide metabolite of lamotrigine and JZP-4.

2. Methods

2.1. Animals and testing vehicle

Male albino CF1 mice (18–25 g, Charles River, Portage, MI) and male albino Sprague Dawley rats (100–150 g; Simonsen, Gliroy, CA and Charles River, Raleigh, NC) were used as experimental animals in the MES, and 6 Hz tests conducted at the NINDS-Anticonvulsant Testing Center. Male C57Bl/6J mice (8 weeks of age, Jackson Laboratories, Bar Harbor, Maine) were used in the mouse amphetamine-chlordiazepoxide induced locomotor model of antimania activity conducted at Psychogenics, Inc. Young adult, male Sprague Dawley rats (approximately 150 g) from Harlan Laboratories (Indianapolis, IN) were used in the rat forced swim test for antidepressant activity and the locomotor activity tests conducted at Psychogenics, Inc. Male Lister hooded rats (280–350 g at the time of surgery) used for the kindling studies; young adult (5–8 weeks of age) EL mice used for the EL mouse studies, and male CD1 mice (25 g) used for the intravenous PTZ tests conducted at GlaxoSmithKline were obtained from the GlaxoSmithKline Rodent Breeding Unit.

All rats and mice used in the studies at Psychogenics and the NINDS-Anticonvulsant Screening Center were housed, fed and handled in a manner consistent with the recommendations in the National Research Council Publication, “Guide for the Care and Use of Laboratory Animals” and used only once. No insecticides capable of altering hepatic drug metabolism enzymes were used in the animal facilities. All animals were euthanized in accordance with the Institute of Laboratory Resources policies on the humane care of laboratory animals. The animal care and use procedures for the studies conducted at GlaxoSmithKline were in accordance with UK Home Office regulations.

For the mouse MES, PTZ and 6 Hz anticonvulsant studies and the mania studies, all test suspensions were administered either i.p. or p.o. at volumes of 0.01 ml/g body weight in a 0.5% methylcellulose suspension. For the rat MES, all test suspensions were administered at 4 ml/kg body weight in a 0.5% methylcellulose suspension. In the rat forced swim and locomotor activity studies, all suspensions were administered in volumes of 1 ml/kg. For the kindled rat studies, JZP-4 was administered p.o. in a 0.25% methylcellulose suspension at a volume of 1 ml/kg p.o.

2.2. MES test and 6 Hz test

For the MES and 6 Hz tests, a drop of anesthetic/electrolyte solution (0.5% tetracaine hydrochloride in 0.9% saline) was applied to the eyes of each animal prior to placement of the corneal electrodes. Eight animals per treatment group were used in both the mouse and rat MES studies. The doses used for the

MES included 5, 6.25, 7.5, 10 and 20 mg/kg i.p. The electrical stimulus in the MES tests was 50 mA, 60 Hz, for mice and 150 mA, 60 Hz for rates delivered over a 0.2 s period using an apparatus that was similar to that originally described by Woodbury and Davenport (1952). Abolition of the hind leg tonic extensor component of the seizure was used as the endpoint.

The ability of JZP-4 to prevent seizures induced by 3 s duration 6 Hz corneal stimulation was evaluated at currents of 22, 32 and 44 mA. Eight mice were used per dose in the 6 Hz model, 4 doses were used to determine the ED_{50} values at each of the 3 current strengths. At 22 mA the doses used included 3, 6, 9 and 15 mg/kg i.p., at 32 mA the dosed used included 3, 6, 12 and 25 mg/kg i.p. and at 44 mA the doses used included 15, 18, 23, and 35 mg/kg i.p. Four to sixteen mice were used at each dose for the determination of the ED_{50} with fewer animals used at doses that had either low or very high anticonvulsant activity and higher numbers of mice were used at doses near the ED_{50}. These 6 Hz seizures are characterized by a minimal clonic phase that is followed by stereotyped, automatistic behaviors described as being similar to the aura of human patients with partial seizures (Barton et al., 2001; Toman et al 1952). Animals not displaying this behavior were considered to be protected.

2.3. Seizures induced by pentylenetetrazole (PTZ)-intravenous infusion

Male CD1 mice (approximately 25 g) were brought into the procedure room at least 2 h prior to testing and food was withdrawn. One hour prior to treatment with PTZ, mice were dosed with vehicle (0.25% methyl cellulose) or JZP-4 (4–40 mg/kg i.p.) using a random dosing schedule. PTZ was infused into the lateral tail vein at a rate of 50 μl/min and a concentration of 10 mg/ml. During the infusion time the mice were freely mobile within a small observation cage. The latencies in seconds to the first twitch, and clonus with loss of righting reflex were recorded. The first twitch was noted when a myoclonic jerk that rippled through the body occurred. This usually occurs approximately 100 s after the beginning of the infusion. These myoclonic jerks become more frequent until there is a clonus with a loss of righting reflex. This clonic state is noted when the mouse lies on his side, and shows repetitive movements with his front paws and jaws. The animal is euthanized at this second endpoint or at 300 s, whichever occurs first. If the second endpoint is not reached a range value of >300 is recorded. Each dose was evaluated in 4–12 mice with the lowest number of mice used at near 0% and 100% protection and progressively higher doses used near the ED_{50}.

2.4. Motor impairment tests

Motor impairment in mice was identified by the rotorod procedure (Durham and Miya, 1957; Loscher and Nolting, 1991c). One hour prior to testing, the mice or rats were dosed with vehicle (0.25% methyl cellulose) or JZP-4 (10–40 mg/kg i.p. in mice and 30–90 mg/kg p.o. in rats) using a random dosing schedule. Eight animals per treatment group were used in both the mouse and rat motor impairment studies. In this test a mouse is placed on a 1-inch knurled rod that rotates at a speed of 6 r.p.m. Usually, mice will maintain balance on the bar in control testing consisting of three 1 min test periods. Minimal motor impairment was considered to be induced if the treated mice fell off the rotating rod three times during a 1-min period. In rats, motor impairment was determined by overt evidence of ataxia, abnormal gait and stance. Motor impairment for these studies is considered to be a minimal toxicological effect and therapeutic range or protective index (PI) is calculated as the ratio of the median toxic dose (TD_{50}) and the median of the effective anticonvulsant dose (ED_{50}). The ED_{50} or TD_{50} were determined by testing groups of at least 8 animals with various doses of JZP-4 until at least two doses were established between the limits of 100% of the test group with seizure protection or motor impairment and 0% of the test group with seizure protection or motor impairment. The ED_{50} and TD_{50} were calculated by a computer program based upon the method described by Finney (1971).

2.5. Amygdala kindling rat model

The rat kindling tests were conducted as described by Southam et al., 2002; and Stratton et al., 2003. JZP-4 (5, 10, 20 or 40 mg/kg i.p.) was evaluated for its ability to block the kindled motor seizure (seizure scores of 4 and 5) and limbic behavioral seizure (seizure score between 1 and 3) and to affect changes in after discharge duration (ADD). Rats were implanted with bipolar electrodes into the right basolateral amygdala according to previously published procedures and stereotaxic coordinates (Southam et al., 2002; Stratton et al., 2003). The chronic bipolar stimulating and recording electrodes used in these studies consisted of 15 mm stainless steel insulated with polymide (Plastics One Inc.).

For kindling to occur, the stimulation must be delivered at a current that initially produces an after-discharge in the amygdala. An after-discharge was defined as a continuous train of two or more spikes occurring at a rate of at least 1/s after the stimulation had ceased. Therefore, prior to kindling, the after-discharge threshold (ADT) was determined for each rat by applying a stimulation starting at 80 μA and increasing 10 μA steps on consecutive days until an after-discharge was observed, up to a maximum of 400 μA. If no ADT could be determined within these limits the animal was not included in the study.

Rats were kindled within an enclosed unit containing a connector cable for attachment to the electrode pedestal. The cable was connected to a filter (1–30 Hz) and an amplifier (X500) via a swivel attachment that allowed animals to move freely within the chamber. A stimulator (Cambridge Neurotechnology, Cambridgeshire, UK) was set to deliver a 1 s train of biphasic square wave pulses (1 ms pulse width) with a variable current (10–500 mA in 10 mA steps) at 60 Hz (Stratton et al., 2003). The recoded waveform was digitized (CED 1401*plus* interface) and processed by Spike 2 data capture and analysis software (Cambridge Electronic Design, Cambridge, UK). Each rat was kindled by application of a once daily (Monday through Friday) stimulation at 125% of the ADT. Baseline EEG was recorded for 10 s followed by the application of a stimulus. The EEG was

recorded until a return to the prestimulus baseline was observed and the waveform was saved to file for future analysis of the ADD. The behavior of the rats was observed throughout the stimulation and the seizures were scored according to the following criteria: Stage 1 – frozen immobility; mouth and facial clonus; Stage 2 – Stage1 plus either rhythmic whisker twitching, head nodding or chewing; Stage 3 – Stage 2 plus unilateral or bilateral forelimb tonus or clonus; Stage 4 – Stage 3 plus rearing; Stage 5 – Stage 4 plus repeated rearing and falling (modified from Racine, 1972). Rats were considered to be fully kindled when three consecutive Stage 5 seizures had been observed. Having reached this state, rats continued to receive a weekly stimulation (maintenance stimulation) and tested with potential anticonvulsant agents.

A drug test consisted of 3 stimulations, applied over a period of 4 days, with each rat serving as its own control. Day 1 was the pretreatment control during which the rats received vehicle (1 ml/kg p.o.); Day 2 was an off treatment day; Day 3 was a treatment day when the rats received either 5 (N=6 rats), 10 (N=8 rats), 20 (N=6 rats) or 40 (N=4 rats) mg/kg p.o. of JZP-4 and finally on Day 4 rats received a post-treatment dose of vehicle. For humane reasons, the lowest number of rats used at near 0% and 100% protection and progressively larger number of rats were used near the ED_{50}. All treatments were administered 2 h prior to stimulation. Following each stimulation, the seizure score and ADD were measured. The mean seizure stage and ADD values were calculated for each dose group for pre- and post-treatment controls and for drug treatment. The data were statistically analyzed using a paired Student's t test.

2.6. Epilepsy-like mouse model

Epilepsy-like (EL) mouse is an inbred mutant strain derived from DDY mice, which exhibit seizures with rhythmic vestibular stimulation (Imazumi et al., 1959; Imazumi, 1964; King and LaMotte, 1989; Southam et al., 2002). These mice have been accepted as a murine model of human hereditary, sensory-precipitated, temporal lobe epilepsy. EL mice of both sexes were sensitized to vestibular stimulation as described previously (King and LaMotte, 1989; Southam et al., 2002). Briefly, these animals were sensitized to vestibular stimulation by gently being tossed 10–15 cm into the air for a maximum of 30 repetitions or until seizure initiation. The sensitization period was conducted at twice weekly intervals for 2–4 weeks and was considered complete when the mice exhibited 3 consecutive ictal seizures (tonic–clonic convulsions with a loss of postural equilibrium, Straub tail, and salivation). The mean number of tosses plus 10 was determined to be the cut-off point for each mouse. The EL mouse seizures have prodromal, ictal and postictal phases defined as: prodromal – squeaking and transient immobility followed by "running fits"; ictal – convulsions starting with the hind limbs but rapidly becoming tonic–clonic seizures, with loss of postural equilibrium, Straub tail, salivation, defecation and urination; and postictal lethargy, sitting on hind limbs with forelimbs elevated, grooming behavior, moving head side to side and occasional hyperirritability. The severity of the seizure was assessed by applying the following scores: squeaking and running (prodromal seizures) 1 point each; clonus, Straub tail, hindlimb extension and loss of righting reflex, 2 points each; with a maximum score of 10 points for a full ictal seizure and a minimum of 0 points for no seizure. The percent protection was calculated as the mean seizure score for the control group minus the mean seizure score of the drug group multiplied by 100. JZP-4 was given in doses ranging from 2.5–20 mg/kg i.p. and lamotrigine 20 mg/kg i.p. was used as a positive control. Each treatment group had 6–12 mice. As in the other anticonvulsant tests, the lowest number of mice used at near 0% and 100% protection and progressively larger number of animals were used near the ED_{50}. Five mice were used at 2.5, 10 and 20 mg/kg and 10 mice were used at 5 mg/kg.

2.7. Mania model (amphetamine-chlordiazepoxide induced increase in open field activity)

The d-amphetamine(AMPH)-chlordiazepoxide(CDP) induced increase in open field activity model for evaluating antimania drugs has been demonstrated to be responsive to lithium, VPA, LTG, carbamazepine and levetiracetam (Rushton and Steinberg, 1966; Vale and Radcliffe, 1987; Aylmer et al., 1987; Cao and Peng, 1993; Lamberty et al., 2001; Arban et al 2005). This test includes the evaluation of open field activity under three conditions including open field activity following treatment with the vehicles alone for baseline activity, treatment with vehicle (sterile water; VEH-2) and AMPH to identify responses to AMPH amplified activity or treatment with vehicle and a combination of AMPH and CDP to differentiate agents that are antimanic at doses that are not sedative under the previous conditions.

In the present studies, the primary endpoint, distance traveled in an open field, was accessed in plexiglas square chambers (27.3×27.3×20.3 cm; Med Associates Inc., St Albans, VT) surrounded by infrared photobeam sources (16×16×16) by determining the total distance covered between consecutive beam breaks. The test compounds, LTG, JZP-4 and VPA (400 mg/kg i.p.), were dissolved in a vehicle (VEH-1) containing 0.5% (w/w) solution of hydroxypropylmethylcellulose (HPMC) in sterile water and given in doses of 1, 3, 10, and 30 mg/kg i.p. Valproate, the internal control, was dissolved in sterile water and given at a dose of 400 mg/kg. LTG, JZP-4, VPA or VEH-1 were administered 30 min prior to a second injection with VEH-2, AMPH (4 mg/kg, Sigma) or a mixture of AMPH and CDP (2.5 mg/kg; Sigma). All solutions were administered i.p. at a dose volume of 10 ml/kg. Following the administration of VEH-2, AMPH or AMPH and CDP the mice were placed in the chambers for a 60 min test session. Twelve mice were used in each treatment group, but statistical outliers with a total distance traveled that fell above or below 2 standard deviations from the mean were removed from the final analysis. Data from the groups of mice treated with either vehicle (VEH-2), amphetamine (AMPH), or amphetamine and chlordiazepoxide (AMPH and CDP) were analyzed separately by analysis of variance (ANOVA) followed by Dunnett's test. An effect was considered significant if $p<0.05$.

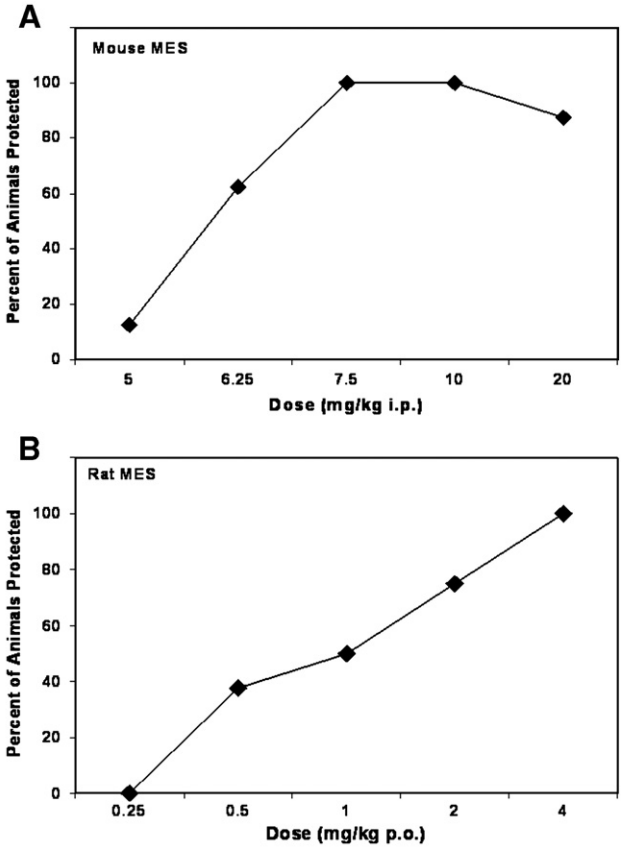

Fig. 2. A and B: MES studies in mouse (A) and rat (B) comparing the percentage of animals protected from the maximal electroshock seizure by different doses of JZP-4.

2.8. Rat forced swim test (FST) for antidepressant-like activity

The force swim chamber was constructed of clear acrylic (height=40 cm; diameter=20.3 cm) and filled with water (23±1 °C) at 16 cm deep during habituation (Day 1) and 30 cm deep during test (Day 2). The rats were exposed on the first day to a 15-min pre-swim session. After completion of the habituation session, rats were injected with vehicle, desipramine (20 mg/kg i.p.), LTG (3, 10 or 30 mg/kg i.p.) or JZP-4 (3, 10 or 30 mg/kg i.p.). Twenty-four hours following the pre-swim, animals were placed back in the test chamber for a 5-minute test session. Pretreatment time for all compounds was 30 min. Each test session was recorded and the tapes were scored using the program Observer v3.0.

Table 1
Comparative effects of anticonvulsants in the mouse MES model

Compound	MES ED_{50} (mg/kg i.p.)	TD_{50} (mg/kg i.p.)	PI
JZP-4	5.9	21.8	3.7
Lamotrigine [a]	7.8	30.0	4.0
Carbamazepine [a]	7.8	45.4	5.8
Valproic acid [a]	263	398	1.5
Levetiracetam [a]	Not active	–	–
Topiramate [a]	33.0	401	12
Felbamate [a]	35.5	220	6.2

[a] White et al., 2002.

Table 2
Comparative effects of anticonvulsants in the rat MES model

Compound	ED_{50} MES (mg/kg p.o.)	TD_{50} (mg/kg p.o.)	PI
JZP-4	0.9	52.3	56
Lamotrigine [a]	1.3	411	316
Carbamazepine [a]	5.4	364	67
Valproic acid [a]	485	784	1.6
Levetiracetam [a]	Not active	>500	–
Topiramate [a]	3.3	>500	>153
Felbamate [a]	25.3	>500	–

[a] White et al., 2002.

Immobility, climbing, and swimming behaviors were recorded as a frequency of total behavior over the 5 min trial. The analysis of the videotapes was performed by raters, who were blinded to the treatments. Eight animals were tested at one time and ten rats were used in each treatment group. Data were analyzed by analysis of variance (ANOVA) followed by a Dunnett's test post-hoc analysis. An effect was considered significant if $p<0.05$. Data are represented as the mean and standard error of the mean.

2.9. Rat locomotor activity study

Locomotor activity was assessed using photocell monitored cages (Hamilton Kinder, San Diego). Each cage consists of a standard plastic rodent cage surrounded by a stainless steel frame

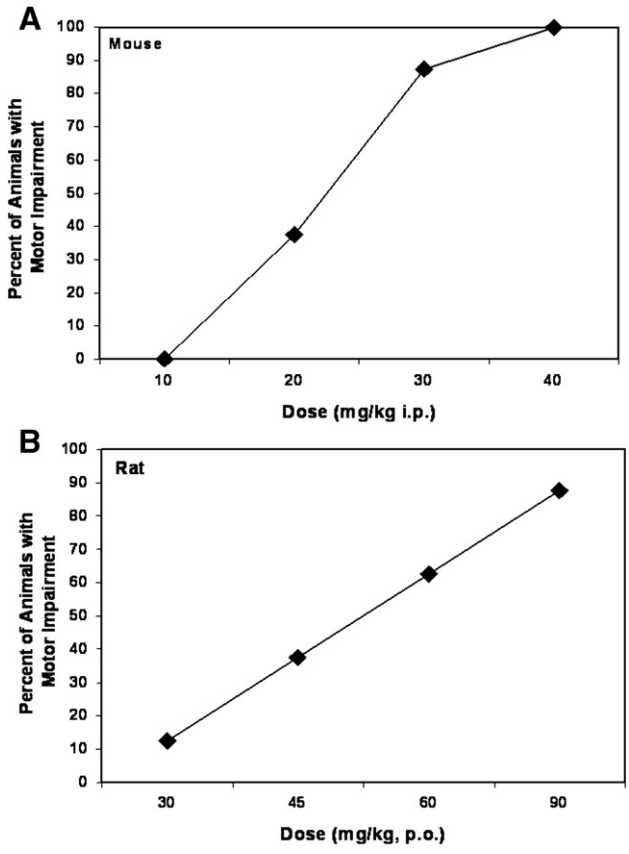

Fig. 3. A and B: Mouse (A) and rat (B) motor impairment tests comparing the level of impairment induced by different doses of JZP-4.

Table 3
Comparative effects of anticonvulsants in the mouse 6 Hz model

Compound	ED_{50} (mg/kg i.p.)		
	22 mA	32 mA	44 mA
JZP-4	5.3	10.6	18.3
Phenytoin [a]	9.4	>60	>60
Ethosuximide [a]	86.9	167	>600
Lamotrigine [a]	18.8	>60	>60
Levetiracetam [a]	4.6	19.4	1089
Valproic acid [a]	41.5	126	310

[a] White et al., 2002.

containing photocell beams. Two sets of infrared photobeam cells are located along the long axis of the frame to detect ambulatory distance traveled and rearing activity. Rats were injected with vehicle, LTG or JZP-4 at 3, 10 or 30 mg/kg i.p. and placed in holding cages for 30 min following which they were placed individually in the activity chambers. Ten rats were used per treatment group. Locomotor and rearing activities during the 60-min test were measured by consecutive photobeam breaks as the animal moved. Data were analyzed by analysis of variance (ANOVA) followed by post-hoc comparisons with Dunnett's test when appropriate. An effect was considered significant if $p<0.05$. Data are represented as the mean and standard error of the mean.

3. Results

3.1. Rat and mouse MES models

JZP-4 potently suppressed the tonic extension seizures induced by maximal electroshock in both mice and rats with ED_{50} values of 5.9 mg/kg i.p. and 0.9 mg/kg p.o., respectively (Fig. 2). This potency placed JZP-4 among the most potent of the current anticonvulsant agents (White et al. 2002; Tables 1 and 2). The TD_{50} values or dose at which minimal motor impairment was observed in half of the animals for JZP-4 in mice and rats were 21.8 mg/kg i.p. and 52.3 mg/kg p.o., respectively (Fig. 3), which yielded protective indices of 3.7 and 58.1 for mice and rats, respectively. JZP-4 was more potent than other currently available anticonvulsants and has a high therapeutic margin (White et al., 2002).

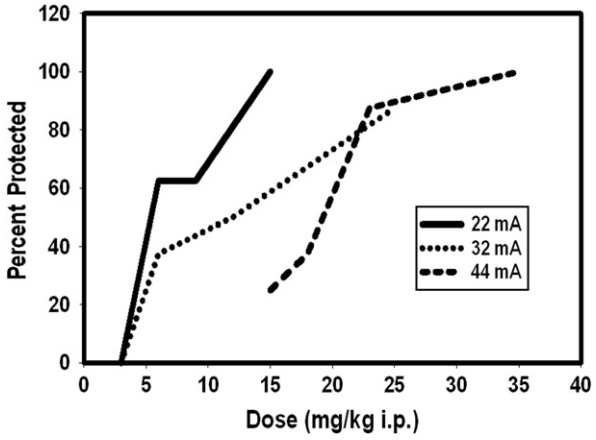

Fig. 4. Dose–response curves for the anticonvulsant effects of JZP-4 at current strengths of 22, 32 and 44 mA in the 6 Hz mouse of treatment-resistant epilepsy.

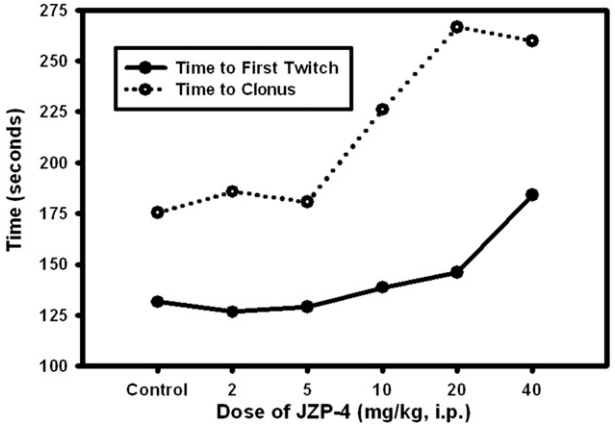

Fig. 5. Dose–response curve for the effects of JZP-4 on time to first twitch (solid line) and time to clonus with loss of righting reflex (dashed line) in the mouse PTZ induced seizure model.

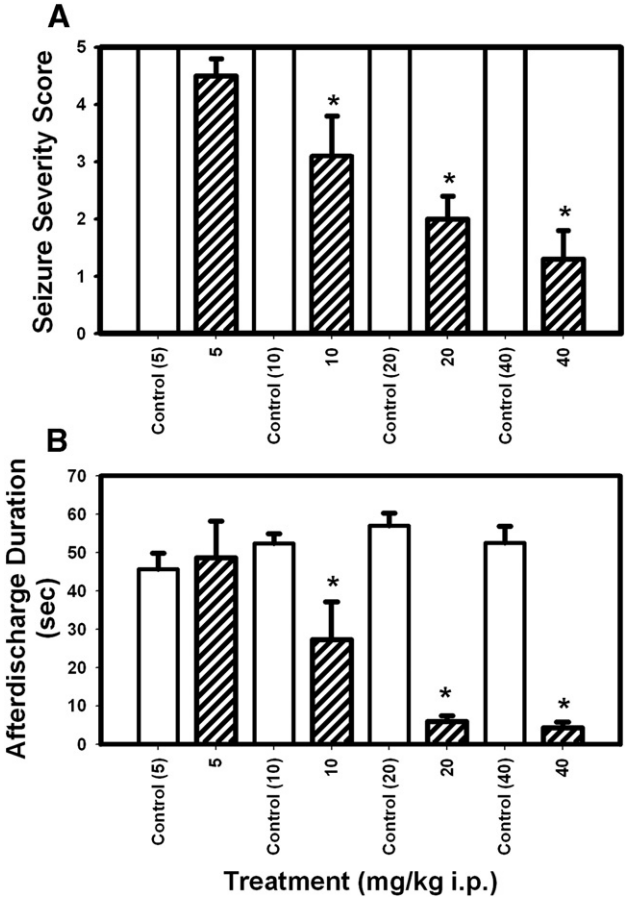

Fig. 6. A and B: Dose-related effects of JZP-4 on seizure severity (A) and after discharge duration in hippocampal (B) kindled rats. Data represent the mean ± SEM. Asterisk denotes statistically significant changes. The number of rats used in the 5, 10, 20 and 40 mg/kg groups were 6, 8, 6, and 4 respectively.

3.2. Mouse 6 Hz model

In the mouse 6 Hz model, JZP-4 showed potent anticonvulsant effects at all of the currents (22, 32 and 44 mA) tested (Table 3 and Fig. 4). JZP-4 showed a greater efficacy at the higher current levels than LTG which was active at only the 22 mA. JZP-4 was 60 and 17 times more potent than levetiracetam and VPA, respectively, in previous 44 mA studies.

3.3. Mouse pentylenetetrazole models

In the mouse model using seizures induced by intravenous infusion of pentylenetetrazole, JZP-4 (Fig. 5) and LTG prolonged the time to clonus with loss of righting reflex with ED_{50} values of 5.6 and 10 mg/kg i.p., respectively. No significant effects were noticed for either compound on the latency to first twitch, therefore, comparison to other AEDs tested in this seizure model was not possible.

3.4. Effect of JZP-4 on behavioral seizure scores and ADD in amygdala kindled rats

Pretreatment with JZP-4 (5–40 mg/kg p.o.) 2 h prior to electrical stimulation of the basolateral amygdala reduced the seizure severity and ADD in kindled rats in a dose-dependent manner. The findings on the seizure severity score and the ADD are summarized in Fig. 6A/B and a representative EEG with notations of behavior in a rat pretreated with 20 mg/kg p.o. is shown in Fig. 7A/B. The number of rats used in the 5, 10, 20 and 40 mg/kg groups were 6, 8, 6 and 4, respectively. The minimal effective dose to produce a statistically significant change for both parameters was 10 mg/kg p.o. In the paired *t* test comparisons between seizure severity score obtained during the pretest vehicle and JZP-4 treatment, the effect were not significant at 5 mg/kg (*t*-ratio = −1.464; *df* = 5) but was significant at 10 mg/kg (*t*-ratio = −2.707; *df* = 7; $p = 0.0303$), 20 mg/kg (*t*-ratio = −8.216; *df* = 5, $p < 0.001$), and 40 mg/kg (*t*-ratio = −7.383; *df* = 3; $p < 0.001$). For the after discharge duration, the comparisons between pretest and drug treatment with JZP-4 in the paired *t* test were not significant at 5 mg/kg (*t*-ratio = 0.2744; *df* = 5) but were significant at 10 mg/kg (*t*-ratio = 2.394; *df* = 7; $p = 0.0479$), 20 mg/kg (*t*-ratio = −12.581; *df* = 5; $p < 0.001$), and 40 mg/kg (*t*-ratio = 17.089; *df* = 3;

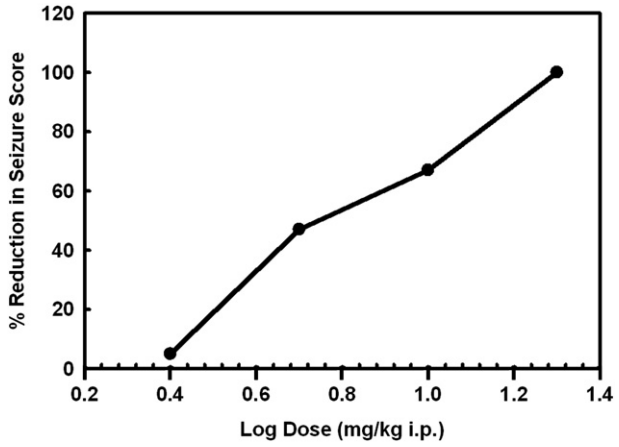

Fig. 8. Dose–response of percentage seizure suppression with various doses of JZP-4 in the EL mutant mouse.

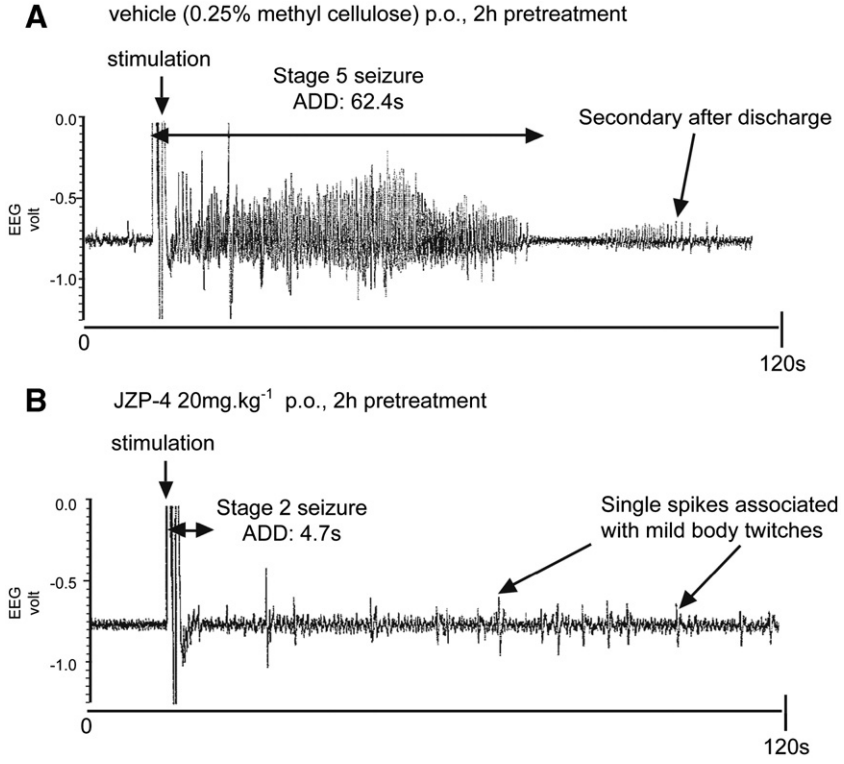

Fig. 7. Representative EEG tracings of kindled rats with and without pretreatment with JZP-4.

$p<0.001$). The estimated ED_{50} for reducing ADD was 10.3 mg/kg p.o. compared to LTG and levetiracetam which were previously reported to have ED_{50} values in reducing the ADD of 14 mg/kg p.o. and 32 mg/kg p.o., respectively, for this same testing procedure (Stratton et al., 2003).

3.5. EL mouse model

JZP-4 and LTG produced significant reductions in seizure scores in EL mice following vestibular stimulation with ED_{50} values of 5.9 and 4.5 mg/kg i.p., respectively. The dose–response curve for the response to JZP-4 is shown in Fig. 8.

3.6. Mouse mania model

The total distance traveled summed over the 60 min period following pretreatment with VPA (400 mg/kg i.p.), LTG (1, 3, and 10 mg/kg i.p.) and JZP-4 (1, 3 and 10 mg/kg) followed by treatment with vehicle, AMPH or AMPH plus CDP is shown in Fig. 9A/B. JZP-4 (1–10 mg/kg i.p.) had no significant effect on

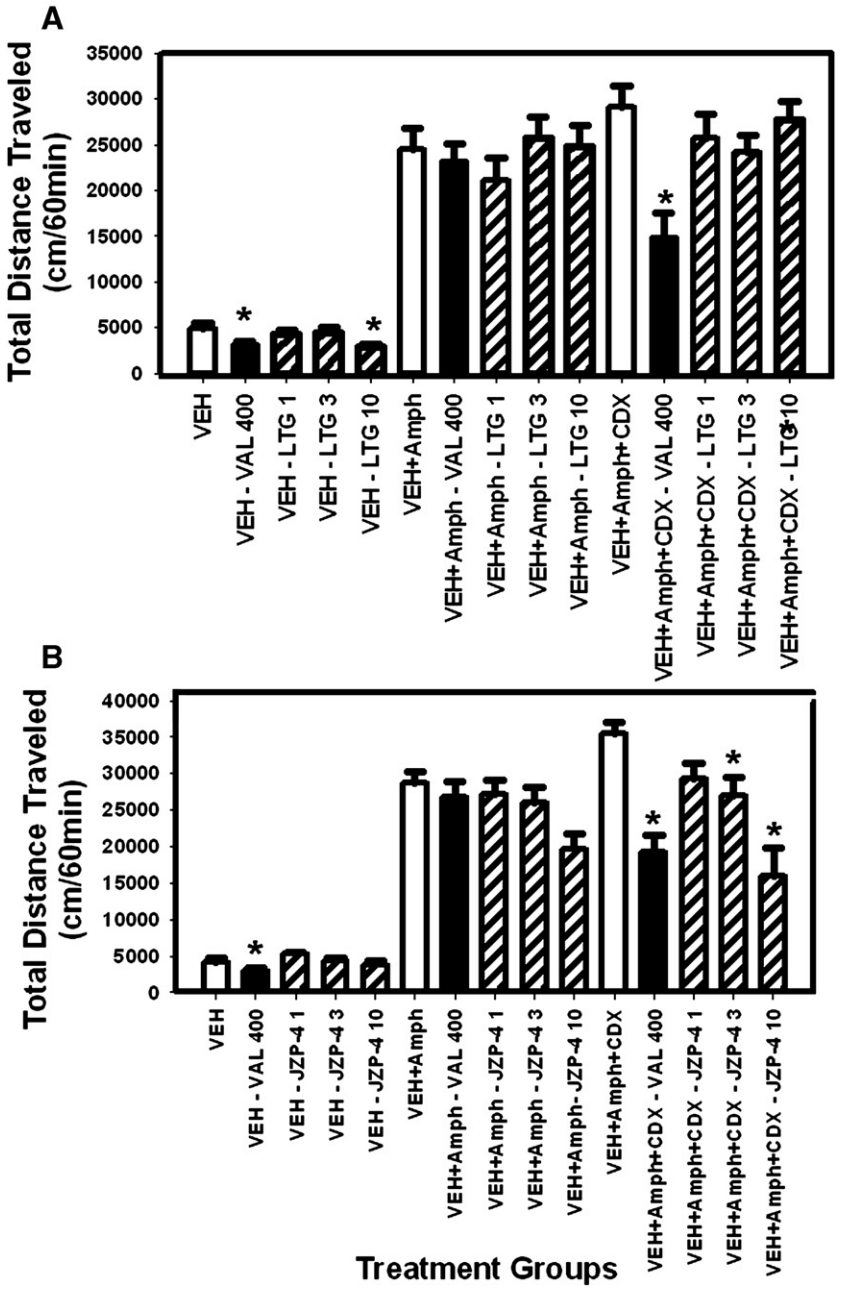

Fig. 9. A and B: Total distance traveled summed over the 60-min test period in the mouse amphetamine plus chlorodiazepoxide model of mania for lamotrigine (A) and JZP-4 (B) treatments. The white bars represent the effects of vehicle treatments, the black bars represent the effects of treatment with VPA and the hatched bars represent the effects of treatments with LTG or JZP-4. Data represent mean±SEM. Asterisk represents a significant difference from the vehicle response of at least $p<0.05$.

basal activity. However, statistically significant decreases in basal locomotor activity were observed with valproate [400 mg/kg i.p.; $F(4, 53)=6.1041$; $P=0.004$; $df=57$, Dunnett's test: $p=0.0296$] in the study with JZP-4, and LTG [10 mg/kg i.p.; $F(4, 52)=3.2853$, $df=56$, $p=0.0178$, Dunnett's test: $p=0.0179$]. In the AMPH-stimulated locomotor activity studies, the administration of 10 mg/kg of JZP-4 produced a reduction of locomotor activity which did not reach statistical significance in the ANOVA [$F(4, 59)=2.3883$, $p=0.061$; $df=63$]. In the AMPH+CDP stimulated locomotor activity studies, all of the test substances, VPA (400 mg/kg i.p.), LTG (1, 3 and 10 mg/kg i.p.) and JZP-4 (1, 3 and 10 mg/kg i.p.), produced some reduction in the mean total distance traveled compared to the VEH–AMPH+CDP control. However, statistically significant decreases in locomotor activity were achieved only with VPA [400 mg/kg i.p., $F(4, 55)-7.3762$; $p<0.001$, $df=59$; Dunnett's test $p<0.001$ in studies with LTG; $F(4, 68)=11.8504$, $df=72$, $p<0.001$; Dunnett's test: $p<0.001$ in studies with JZP-4], and JZP-4 [3 and 10 mg/kg i.p.; $F(4, 68)=11.8504$; $df=72$, $p<0.001$, Dunnett's test: $p=0.0069$ for 3 mg/kg and $p<0.0001$ for 10 mg/kg].

3.7. Rat forced swim model

The effects of LTG and JZP-4 on immobility and climbing behaviors in the rat forced swim test are shown in Fig. 10. Desipramine (20 mg/kg i.p.), LTG (30 mg/kg i.p.), and JZP-4 (30 mg/kg i.p.) decreased the frequency of immobility behavior and increased frequency of climbing behavior without affecting swimming behavior. The changes in immobility reached significance for only the desipramine (20 mg/kg i.p.; $t=5.1367$, $df=18$, $p<0.001$ for the LTG study; $t=4.8197$, $p<0.001$ for the JZP-4 study) and the JZP-4 [30 mg/kg i.p.; $F(3, 36)=3.9178$, $df=39$, $df=19$, $p<0.0161$; Dunnett's test: $p=0.0426$] for comparisons with their matched vehicles. The changes in climbing behavior reached significance for only the desipramine (20 mg/kg i.p.; $t=-4.9865$, $df=18$, $p<0.001$ for the LTG study; $t=4.9865$, $p<0.001$ for the JZP-4 study) and the JZP-4 [30 mg/kg i.p.; $F(3, 36)=3.9178$, $df=39$, $df=19$, $p<0.0121$; Dunnett's test: $p=0.0286$] for comparisons with their matched vehicles. The response to the vehicle for desipramine and the vehicle for LTG and JZP-4 was not significantly different. Notably, the fact that animals treated with desipramine, LTG, and JZP-4 did not display swimming behavior suggests that there may be some common feature in the underlying pharmacological mechanism for the induction of this effect.

3.8. Rat spontaneous locomotor activity

JZP-4 ($F(3, 35)=0.1448$, $df=38$, $p=0.9323$), but not LTG produced dose-related reductions in both total distance traveled

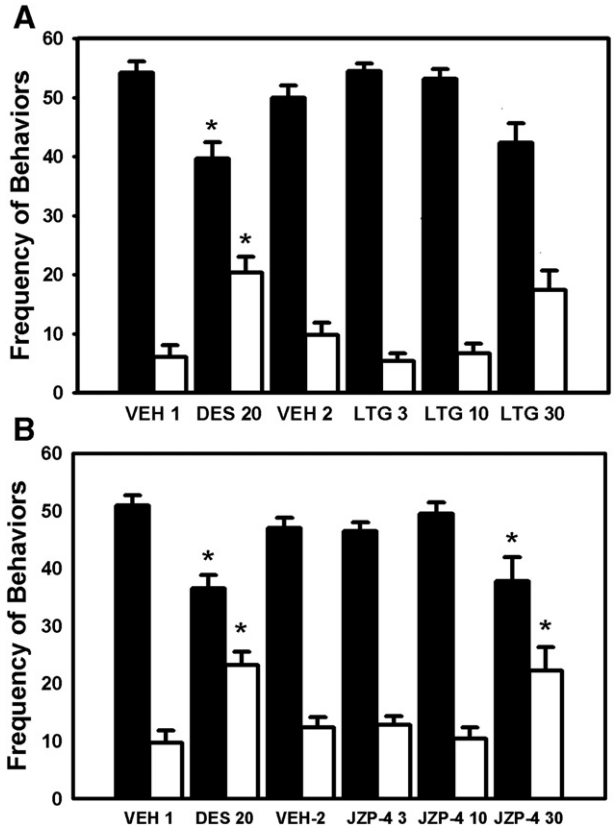

Fig. 10. A and B: Effects of desipramine, lamotrigine (A) and JZP-4 (B) in the rat forced swim test. The black bars represent the frequency of immobility behavior and the white bars represent the frequency of climbing behavior. VEH 1=water vehicle used for desipramine; VEH 2=5% methylcellulose vehicle used for LTG and JZP-4; DES 20=Desipramine 20 mg/kg i.p.; LTG 3=lamotrigine 3 mg/kg i.p.; LTG 10=lamotrigine 10 mg/kg i.p.; LTG 30=lamotrigine 30 mg/kg i.p.; JZP-4 3=JZP-4 3 mg/kg i.p.; JZP-4 10=JZP-4 10 mg/kg i.p.; JZP-4 30=JZP-4 30 mg/kg i.p.; Asterisk represents a significant difference from the vehicle response of at least $p<0.05$. None of the treatments affected the frequency of swimming behavior (data not shown).

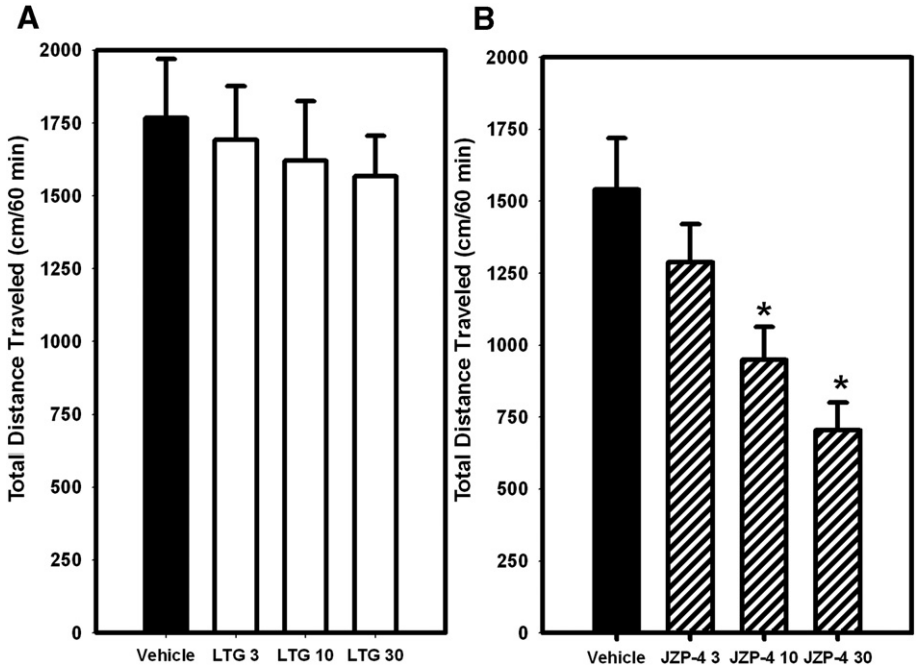

Fig. 11. A and B: Effects of lamotrigine (A) and JZP-4 (B) on total distance traveled in 60 min in the rat locomotor activity test. The black bars represent the effects of vehicle, the white bars represent the effects of LTG and the hatched bars represent the effects of JZP-4. Asterisk represents a significant difference from the vehicle response of at least $p<0.05$.

during the 60 min test (Fig. 11). The mean values for total distance traveled reached statistical significance at 10 and 30 mg/kg i.p. for JZP-4 [$F(3, 36)=7.4937$, $df=39$, $p<0.0005$; Dunnett's test for 10 mg/kg: $p=0.0113$; for 30 mg/kg: $p<0.0003$]. These findings indicate that the effects of LTG and JZP-4 in forced swim model were not due to nonspecific activational effects.

4. Discussion

The primary objective of the present series of experiments was to determine the potency and efficacy of JZP-4 in animal models that are considered to be predictive of anticonvulsant, antimania and antidepressant activity in humans in order to provide a preclinical justification for extensive clinical evaluation for efficacy against different forms of epilepsy and diverse psychiatric disorders.

Anticonvulsant activity was established by chemical and electrical evoked seizure models, and a genetic mutant model that has epilepsy-like characteristics. The electrical tests used included the maximal electrical shock (MES) seizure test, the 6 Hz psychomotor seizure test, and the amygdala kindled rat test. The maximal electroshock model in mice and rats is considered to be a model for generalized tonic–clonic seizures in humans. In this model, JZP-4 ($ED_{50}=5.9$ mg/kg i.p. mouse; 0.9 mg/kg p.o. rats) was more potent than LTG ($ED_{50}=7.8$ mg/kg i.p. mouse; 1.3 mg/kg p.o. rats) and the other anticonvulsants reported by the NINDS-Anticonvulsant Screening Program (Tables 1 and 2; White et al., 2002). JZP-4 also had a protective index of 3.7 in mice and 56 in the rats demonstrating a safety margin comparable to other new AEDs. In the mouse 6 Hz model (Table 3), JZP-4 was more potent and/or more efficacious than the other AEDs that have been reported, including LTG (White et al., 2002). Since the 6 Hz model is viewed as a model of refractory epilepsy (Barton et al., 2001), this study suggests that JZP-4 may be a new more potent alternative to LTG, VPA, felbamate and levetiracetam in treating these treatment-resistant patients. The efficacy of JZP-4 in patients with different forms of epilepsy is currently being evaluated.

The chemical induced seizure model was the mouse intravenous PTZ model, which has been proposed as a model of generalized myoclonic seizures (Loscher, 1988; Loscher et al., 1991b) and absence seizures (White et al 1995; 2002). In this model, JZP-4, like LTG, prolonged the time to clonus with loss of righting reflex with ED_{50} of 5.6 compared to 10 mg/kg i.p. for LTG. However, neither JZP-4 nor LTG abolished convulsions as commonly observed with VPA (White et al., 2002).

In the amygdala kindled rat and EL mutant mouse models (Figs. 6–8), which have been proposed to be models of partial seizures with secondary generalization (King and LaMotte, 1989; Southam et al., 2002), JZP-4 showed dose-related and significant anticonvulsant effects with a potency equal to or greater than LTG.

In addition to the predictive validity for anticonvulsant activity, some of the models used in this study have been thought to have predictive validity for specific psychiatric disorders. Efficacy in the kindling rat model is also considered to be predictive of efficacy in bipolar disorder since the repetitive subthreshold traumas are viewed as parallelisms to the development of psychiatric disorders like bipolar disorder (Goddard et al., 1969; Goldberg and Harrow, 1994; Post et al., 1995; Post et al., 1996). Efficacy in the PTZ model has also been

thought to be reflective of anxiolytic effects (Emmett-Oglesby et al., 1983). In support of this latter possibility, LTG has also been found to have an anxiolytic profile in the rat conditioned emotional response test (Mirza et al., 2005). The efficacy of JZP-4 in both the kindling and PTZ models is suggestive that it could have beneficial effects in bipolar disorder and anxiety states, respectively.

In the mouse AMPH+CDP model for effects on mania, only VPA (400 mg/kg i.p.) and JZP-4 (3 and 10 mg/kg i.p.) produced significant suppression of AMPH+CDP induced increases in locomotor activity. In the current study, LTG did not produce significant changes in locomotor activity at doses below 30 mg/kg i.p. In a previous study, LTG was found to have significant effects in this model but only at a dose of 20 mg/kg, which also induced a lower response to AMPH (Arban et al., 1995). In bipolar patients, LTG has not had reproducible benefit for patients with acute bipolar mania. The data from the current study are in agreement with the clinical findings of inconsistent effects of LTG on acute bipolar mania and are suggestive that JZP-4 may also be of benefit during the mania phase of the disorder. However, clinical studies to test the effects of JZP-4 will be completed in order to confirm these effects.

Although there is no animal model specifically for bipolar depression, there are models that have high pharmacological validity for compounds with antidepressant-like activity. The most common model is the forced swim model in rats, which is responsive to a variety of known antidepressants such as tricyclic antidepressants, serotonin, dopamine and norepinephrine reuptake inhibitors, monoamine oxidase inhibitors and 5-HT_{1A} receptor agonists (Porsolt et al., 1978; Foreman et al., 1994). Many of the tricyclic antidepressants, 5-HT reuptake inhibitors and dopamine reuptake inhibitors are also known to have sodium channel blocking activity (Aronstam, 1981; Lenkey et al., 2006; Ogata et al., 1989). In addition to these agents, anticonvulsants such as carbamazepine, oxcarbazepine and others that have Na^+ and Ca^{2+} channel antagonist activity are also active in this model (Maj et al., 1985; Beijamini et al., 1998; Joca et al., 2000; Vamvakides et al., 2002; Hudgens et al., 2006). In the current studies, JZP-4 (30 mg/kg i.p.) but not LTG (30 mg/kg i.p.) was found to have statistically significant antidepressant effects that were comparable to the magnitude of effects observed with desipramine (20 mg/kg i.p.). These antidepressant effects observed for JZP-4 occurred at doses which produced no effects or significant decreases in locomotor activity indicating that the antidepressant effects are not due to nonspecific activational effects. These data are suggestive that JZP-4 may have clinical utility for unipolar or bipolar depression, but additional preclinical and clinical studies will be needed to be performed to confirm these findings. Although the effects in the forced swim test are at higher doses than the minimum effective doses in the various anticonvulsant tests, the forced swim test is known to show effects that are well above the clinically effective doses.

In the *in vitro* pharmacology studies on $Na_v1.2A$ and $Na_v1.3$, JZP-4 appeared to be between 10 and 20 times more potent than LTG depending on whether the evaluations were conducted at either 50% or full inactivation V_h (Foreman et al., submitted for publication). In the studies on high-voltage activated (HVA) calcium channels on dorsal root ganglia neurons, JZP-4 had an IC_{50} of 74 μM where as LTG was without effect at 1000 μM. In studies with human L, and P/Q channels on xenopus oocyte cells, JZP-4 but not lamotrigine showed both voltage and use dependent inhibition. The increased potency of JZP-4 compared to LTG at type IIA ($Na_v1.2A$) and types III ($Na_v1.3$) as well as its novel effects on L and P/Q Ca^{2+} channels may help explain why JZP-4 had an increase in potency and efficacy in the mouse 6 Hz model at all current levels and the mania model compared to LTG. JZP-4 also has significant affinity for the dopamine and norepinephrine reuptake sites within the effective concentration ranges for Na^+ and Ca^{2+} channel (Foreman et al., submitted for publication).

Collectively, the results from the anticonvulsant tests and the antimania and antidepressant tests provide evidence for a diverse profile of JZP-4 as a novel, potentially high potency anticonvulsant and mood stabilizer. In addition to these pharmacological effects, one major attribute of JZP-4 is that it may produce a lower incidence of hypersensitivity reactions in patients because of its structural modifications that may prevent it from forming the reactive arene oxide metabolite that is hypothesized to trigger this hypersensitivity response. However, extensive clinical studies will be required to determine the risk of side-effects with JZP-4 administration. Considering the fact that the use of many anticonvulsants is limited by their serious side-effects, JZP-4 has the potential to have a wider variety of clinical applications than many of the current agents of this class. This possible reduced side-effect potential combined with a shorter half-life provides a means to potentially rapidly titrate JZP-4 to steady state at therapeutic levels. This potential change in the clinical treatment paradigm means a potentially shorter treatment duration at which patients will be at subtherapeutic levels and opens the possibility of a broader range of clinical trials in psychiatric and neurologic disorders which require faster titration to effective doses in order to discriminate clinical benefits.

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