In Vivo Profile of ICA-27243 [N-(6-Chloro-pyridin-3-yl)-3,4-difluoro-benzamide], a Potent and Selective KCNQ2/Q3 (Kv7.2/ Kv7.3) Activator in Rodent Anticonvulsant Models

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ABSTRACT

Openers or activators of neuronal KCNQ2/Q3 potassium channels decrease neuronal excitability and may provide benefit in the treatment of disorders of neuronal excitability such as epilepsy. In the present study, we evaluate the effects of ICA-27243 [N-(6-chloro-pyridin-3-yl)-3,4-difluoro-benzamide], an orally bioavailable, potent, and selective KCNQ2/Q3 opener, in a broad range of rodent seizure models. ICA-27243 was effective against maximal electroshock (MES) and pentylenetetrazole (PTZ)-induced seizures in both rats (MES, ED50 = 1.5 mg/kg p.o.; PTZ, ED50 = 2.2 mg/kg p.o.) and mice (MES, ED50 = 8.6 mg/kg p.o.; PTZ, ED50 = 3.9 mg/kg p.o.) in the rat amygdala kindling model of partial seizures (full protection from seizure at 9 mg/kg p.o.) and in the 6-Hz model of psychomotor seizures in mice (active at 10 mg/kg i.p.). Antiseizure efficacy in all models was observed at doses significantly less than those shown to effect open-field locomotor activity (rat ED50 = 40 mg/kg p.o.) or ability to remain on a Rotorod (no effect in rat at doses up to 100 mg/kg p.o.). There was no evidence of cognition impairment as measured in the Morris water maze in the rat (10 and 30 mg/kg p.o.), nor was there evidence of the development of tolerance after multiple doses of ICA-27243. Our findings suggest that selective KCNQ2/Q3 opening activity in the absence of effects on KCNQ3/Q5 or GABA-activated channels may be sufficient for broad-spectrum antiepileptic activity in rodents.

KCNQ2–5 (Kv7.2–7.5) voltage-dependent potassium channels are potentially attractive targets for novel antiepileptic drugs. These channels are expressed at high levels in the brain, including regions linked to seizure disorders, such as cortex, hippocampus, and thalamus. They represent the molecular correlate of the neuronal M current (IM), a noninactivating, slowly deactivating subthreshold K+ current that opposes depolarizing current and serves to stabilize membrane potential and control neuronal excitability (Brown and Adams, 1980; Wang et al., 1998). KCNQ2/Q3-based M currents may play an important role in the control of neuronal excitability and epileptiform activity because mutations in KCNQ2/Q3 channels lead to benign familial neonatal convulsions, a rare form of neonatal epilepsy in humans (Biervert et al., 1998; Charlier et al., 1998; Singh et al., 1998; Lerche et al., 1999). Benign familial neonatal convulsions are characterized by generalized seizures in early life, which disappear within weeks or months of birth but may return later in life. Moreover, disruption of the KCNQ2 gene in mice results in hypersensitivity to the chemoconvulsant pentylenetetrazole (Watanabe et al., 2000), decreased seizure threshold to electric shock-induced convulsions (Yang et al., 2003), and spontaneous seizures.
taneous seizures (Peters et al., 2005). As this evidence suggests, openers or activators of KCNQ channels should decrease neuronal excitability, making them attractive drug targets for the treatment of epilepsy and related disorders of neuronal excitability (Wickenden et al., 2004).

The first reported KCNQ2/Q3 opener was D-23129 (retigabine). This agent potently enhances KCNQ2/Q3 currents by inducing leftward shifts in the voltage dependence of channel activation (Main et al., 2000; Rundfeldt and Netzer, 2000; Wickenden et al., 2000). Consistent with the widespread distribution of KCNQ2/Q3 channels and the important role played by these channels in neuronal activity, D-23129 exerts anticonvulsant activity in a broad range of seizure models (Rostock et al., 1996; Tober et al., 1996). Furthermore, the efficacy of D-23129 as adjunctive therapy in the treatment of partial seizures has been evaluated in three phase II clinical trials, which have demonstrated that doses at and below the maximum tolerated dose of 1200 mg/day produced statistically significant reductions in monthly total partial seizure rates (Bialer et al., 2007; Porter et al., 2007). Although it seems probable that KCNQ2/Q3 opening plays a significant role in the anticonvulsant actions of D-23129, a potential contribution from other mechanisms, including enhancement of GABAergic transmission in the central nervous system (Kapetanovic et al., 1995), cannot be discounted. Furthermore, D-23129 is a nonselective opener of all neuronal KCNQ channels (KCNQ2–5) (Tatulian et al., 2001; Wickenden et al., 2001), and the relative contribution of each to the anticonvulsant activity is unclear. Final validation of KCNQ2/Q3 channels as antiepileptic drug targets therefore requires identification of highly selective KCNQ2/Q3 openers.

ICA-27243 (Fig. 1) (McNaughton-Smith et al., 2001) is a potent and selective opener of KCNQ2/Q3 channels (ICA-27243 activates KCNQ currents in Chinese hamster ovary cells expressing recombinant KCNQ2/Q3 heteromultimeric channels with an EC₅₀ of 0.4 μM) (Wickenden et al., 2008). It is noteworthy that unlike D-23129, ICA-27243 exhibits selectivity for KCNQ2/Q3 over KCNQ4 (20-fold) and KCNQ3/Q5 (>100-fold) and has no effect on GABA-activated chloride channels, neuronal sodium channels, or voltage-gated calcium channels. In addition, it produced minimal displacement of radioligand binding to a range of central nervous system receptors and transporters (Wickenden et al., 2008). ICA-27243 is rapidly absorbed, bioavailable, and brain penetrant in rodents (Wickenden et al., 2008) and thus represents a valuable tool to assess the validity of KCNQ2/Q3 channels as an anticonvulsant drug target. In the present study, we show that ICA-27243 exhibits anticonvulsant activity in a broad spectrum of rodent seizure models. Tolerance to the anticonvulsant effects did not develop upon repeated dosing and antiepileptic effects could be observed at doses that were without effect on motor coordination or cognition. Our findings suggest that selective KCNQ2/Q3 opening activity, in the absence of effects on KCNQ3/Q5 or GABA-activated channels, may be sufficient for broad-spectrum antiepileptic activity in rodents.

**Materials and Methods**

**Animals**

Male Sprague-Dawley and Wistar rats and male CD-1 mice were obtained from Charles River Laboratories (Raleigh, NC). Male albino CF 1 mice were obtained from Charles River Laboratories (Wilmington, MA). Fisher 344 rats were obtained from Taconic Farms (Germantown, NY). All animal procedures were performed under protocols approved by the governing Institutional Animal Care and Use Committee and according to the Institute of Laboratory Animal Resources (1996).

**Drugs**

ICA-27243 is a proprietary compound synthesized at Icagen, Inc. (Durham, NC). ICA-27243 was formulated in 0.5% methylcellulose for rat maximal electroshock (MES), rat pentylenetetrazole (PTZ), and rat locomotor activity assays, in olive oil for rat kindling, in 5% dimethyl sulfoxide/95% olive oil for mouse MES, in 5% dimethyl sulfoxide/95% (2% Tween 80/0.5% hydroxypropyl methylcellulose) for mouse PTZ and was administered p.o. in a volume of 1 ml/kg in rats and 10 ml/kg in mice. The 6-Hz assay was conducted using ICA-27243 suspended in 0.5% methylcellulose and administered intraperitoneally.

D-23129 was synthesized at Icagen, Inc., formulated in 0.5% methylcellulose, and administered orally in a volume of 1 ml/kg. Pentylenetetrazole was purchased from Sigma-Aldrich (St. Louis, MO), formulated in saline, and administered subcutaneously in a volume of 1 ml/kg in the rat and 10 ml/kg in the mouse. Carbamazepine (purchased from Sigma-Aldrich) was formulated in 2% Tween 80/70% propylene glycol and administered intraperitoneally in a volume of 1 ml/kg. Scopolamine (purchased from Sigma-Aldrich) was formulated in 0.5% methylcellulose and administered intraperitoneally in a volume of 1 ml/kg.

**Seizure Assays**

**MES-Induced Seizure.** Male Wistar rats and male CD-1 mice were tested in the MES assay using the electroshock seizure apparatus designed by Walhquist Instrument Co. (Salt Lake City, UT). The shock level was set at 150 mA (rat) or 50 mA (mouse), and the duration was set at 0.2 s. A drop of 1% proparacaine solution was placed in each eye, the electrodes were placed over the eyes, and the shock was administered. Latency to hind limb extension was measured to the nearest 0.1 s. If extension did not occur within 6 s, the animal was scored as protected, and a value of 6 s was recorded (Swinyard et al., 1989). ICA-27243 was administered orally 30 min before electroshock application in the rat and 10 min before electroshock application in the mouse. D-23129 was tested in a parallel study in rats at doses of 0.3 to 17 mg/kg p.o. administered orally 30 min before shock application.

**PTZ-Induced Seizure.** Male Sprague-Dawley rats and male CD-1 mice were administered ICA-27243 orally before administration of 85 mg/kg PTZ s.c. In rats, ICA-27243 was administered 30 min before PTZ administration, and in mice, the pretreatment time was 45 min. Pretreatment times were determined in preliminary time to peak activity studies. The latency to seizure in minutes was recorded, using administration of PTZ as time 0. If no tonic-clonic seizure was observed within 15 (rat) or 30 (mouse) min post-PTZ, the animal was considered protected, and a latency score of 15 (rat) or 30 (mouse) min was recorded (Swinyard et al., 1989).

After the PTZ-induced seizure dose response studies, we conducted a second series of experiments to explore the duration of action of ICA-27243 in the PTZ model. In this study, latency to PTZ-induced seizure was examined in rats 5 min, 15 min, 30 min, 60 min, and 120 min.
1 h, 2 h, and 4 h after oral administration of a submaximal dose (12 mg/kg) (calculated ED$_{50}$) of ICA-27243. Using a satellite group of adult rats, the oral pharmacokinetics of ICA-27243 was determined to examine the potential correlation between ICA-27243 plasma concentrations and anticonvulsant activity. Bioanalytical experimental methods are described below.

The potential for tolerance to the anticonvulsant activity of ICA-27243 against PTZ-induced seizures was studied in three groups of mice (16 mice per group) that were administered 12 mg/kg ICA-27243 or 20% GST (whichever was less) (ICA-27243 was administered 1 h before test stimulation, carbamazepine was administered 30 min before test stimulation). If the animal did not seize, the current intensity was increased every minute by increments of 40 $\mu$A over the previous stimulation until an afterdischarge of at least 20 s was evoked. Afterdischarge threshold and afterdischarge duration (ADD) were also measured. The effect of drugs was measured on a group of 16 animals that received random doses of ICA-27243, carbamazepine, or the appropriate vehicle. No animal received the same dose of the same drug twice. A drug-free interval of at least 5 days was allowed to reestablish GST.

Impaired motor function and toxicity of ICA-27243 and carbamazepine were examined by the Rotorod test (diameter of the rod, 7 cm; 8 rpm). Kindled rats were trained before drug experiments to remain on the rod for at least 3 min on 3 consecutive days. After drug treatment, the animals were given three trials to maintain their balance on the rod for 1 min. Animals that were not able to remain on the rod for 1 min were considered to exhibit a neurologic deficit.

6-Hz “Psychomotor” Seizure. The 6-Hz psychomotor seizure test was conducted as a part of the National Institutes of Health Anticonvulsant Screening Program (Stables and Kupferberg, 1997). Twenty CF No. 1 mice were pretreated with 10 or 25 mg/kg ICA-27243 i.p. At varying times (0.25, 0.5, 1, 2, and 4 h) after treatment, individual mice (four at each time point) were challenged with sufficient current (32 mA at 6 Hz for 3 s) delivered through corneal electrodes to elicit a psychomotor seizure. In general, this seizure is characterized by a minimal clonic phase, followed by stereotypy and automatistic behaviors described originally as being similar to the aura of human patients with partial seizures. Animals not displaying this behavior are considered protected.

Motor Assessment

To assess potential motor impairment, an open-field locomotor activity assessment was conducted in male Wistar rats. Twenty computer-controlled photocell activity cages (San Diego Instruments, San Diego, CA) were set to accumulate the number of breaks in photocell beams in 10-min bins for 1 h. Treatment conditions (vehicle and compound at 3, 10, 30, 50, 100, and 150 mg/kg for ICA-27243 and vehicle, 10, 25, and 50 mg/kg for D-23129) were distributed equally over the 20 cages. Oral administration occurred 30 min before the rat was placed in the cage. This protocol was designed to produce a high degree of spontaneous exploratory locomotor activity early in the session, so that any reduction by the compound could be easily registered.

In addition to the open-field locomotor activity assessment, motor coordination was assessed in Sprague-Dawley rats using the Rotorod assay. Rats were trained on the day before the drug experiment to remain on a rotating rod (7.6-cm diameter, 12 rpm) for at least 1 min. On the day of drug testing, rats were administered oral doses of 3, 10, 17, 30, 56, or 100 mg/kg ICA-27243 or vehicle. Thirty minutes after drug treatment, each animal was given three attempts to remain on the rotating rod for 1 min. The longest latency time to fall from the rod was recorded in seconds.

Morris Water Maze

To assess the potential for cognitive impairment, a Morris water maze study was conducted in Fisher 344 rats treated with ICA-27243 (10 and 30 mg/kg), vehicle, or scopolamine (1 mg/kg) as an active control. Trials were conducted in a tank 5 feet in diameter containing 2 feet of water with a 4-inch square platform just under the surface of the water. A video camera tracked the rat from release point to platform, and a computer recorded path length and elapsed time. ICA-27243, vehicle, or scopolamine were administered 30 min before each daily test session. A daily test session for a rat consisted of two trials. For the first trial, the rat was released from a designated point around the edge of the tank and allowed up to 2 min to find the platform. If it did so, it was left on the platform for 15 s; if it did not reach the platform within 2 min, it was placed on the platform and left for 15 s. It was then removed from the platform, and 30 s later, it was placed in the second trial, identical to the first except that it was released from a different point around the edge of the tank. The process was done each day for 5 days, with release points varied from day to day. Values for the two trials on each day were summed to yield one value for each rat for each of the 5 days. Evidence of learning is measured...
as a trend of decreasing path length and latency over successive trials.

**Pharmacokinetics**

After completion of the PTZ-induced seizure duration of action study, the oral pharmacokinetics of ICA-27243 in adult rats was determined to examine the potential correlation of ICA-27243 plasma concentrations with latency to PTZ-induced seizure. ICA-27243 was administered orally at 12 mg/kg to jugular vein cannulated adult male Sprague-Dawley rats (n = 2). Blood samples (approximately 0.25 ml) were withdrawn from each rat at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 6 h after compound administration. Plasma was separated from heparin-treated whole blood by centrifugation. Plasma proteins were precipitated and removed with acetonitrile followed by centrifugation. The supernates from this centrifugation were dried under vacuum with a Savant evaporator. The dried extracts were reconstituted with 100 µl of 60% acetonitrile/40% water. ICA-27243 concentrations in the reconstituted extracts were determined by high-performance liquid chromatography-mass spectrometry.

To characterize the plasma and brain exposure after oral administration of ICA-27243, a separate pharmacokinetic study was performed in Sprague-Dawley rats. Animals were dosed orally by gavage with 30 mg/kg ICA-27243 and returned to their cages. Just before the sampling time for an individual rat, the animal was removed from the cage, and blood samples (>0.2 ml) were obtained via cardiac puncture under deep CO2 anesthesia. After obtaining the blood sample, animals were sacrificed by CO2 exposure, and the brains were removed. Plasma and tissue samples were obtained (n = 3 per time point) at the following times postdosing: 0.25, 0.5, 0.75, 1.0, 1.5, and 2 h. Extraction from plasma was performed on-line with a turbulent flow chromatography system. Analysis of extract was by high-performance liquid chromatography-mass spectrometry/mass spectrometry with a lower limit of quantitation of 8 ng/ml. Brain tissue was homogenized in an 80:20 mixture of acetonitrile/water with further extraction performed on-line with a turbulent flow chromatography system. Analysis of ICA-27243 in brain tissue extracts was by high-performance liquid chromatography-mass spectrometry/mass spectrometry, with a lower limit of quantification of 22 ng/g.

**Data Analysis**

MCS- and PTZ-induced seizure latencies, changes in stimulus intensities required to produce ADD and clonic motor seizures in kindled rats, and locomotor activity data were submitted to analysis of variance followed by comparison of dose groups to vehicle using Dunnett’s method (JMP version 5.1; SAS Institute, Cary, NC). Fisher’s exact probability test was used for specific comparisons between each dose treatment and vehicle control group in percentage of animals protected from seizure in each of the dose groups.

**Results**

**MES-Induced Seizure.** The results of the MES-induced seizure studies in mouse and rat are presented in Table 1 and summarized in Fig. 2, A and B. Oral administration of ICA-27243 was protective against MES-induced seizures in both mouse and rat. As we have previously shown, ICA-27243 significantly increased latency to MES-induced seizures in mice (Wickenden et al., 2008). Significant effects were observed at doses of 10 mg/kg and higher (ANOVA, p ≤ 0.05; Dunnett’s test, p ≤ 0.05), and the ED50 value determined from the dose-response curve shown in Fig. 2A was 8.6 mg/kg (95% CI, 6.7–11.1). Protection from MES-induced seizures was achieved in 7, 0, 14, 17, 30, 40, 56 mg/kg ICA-27243, respectively, with statistically significant differences from vehicle at doses of 10 mg/kg and higher (Fisher’s exact probability test, p ≤ 0.05). In rats, there was a significant effect of treatment on seizure latencies (ANOVA, p ≤ 0.05), with doses of 1 mg/kg and higher and an ED50 of 1.5 mg/kg (95% CI, 0.9–2.4) (Fig. 2B). Protection from MES-induced seizures was achieved in 0, 23, 31, 57, 86, 100, and 86% of rats treated with vehicle, 0.3, 1, 3, 5.6, 10, 17, and 30 mg/kg ICA-27243, respectively. The number of rats protected from seizure differed significantly from vehicle at doses of 5.6 mg/kg and greater (Fisher’s exact probability test, p ≤ 0.05). In a parallel experiment, D-23129 was fully protective in the

<table>
<thead>
<tr>
<th><strong>Table 1</strong> Results of the MES- and PTZ-induced seizure studies in mouse and rat following administration of ICA-27243.</th>
<th><strong>CD-1 mouse</strong></th>
<th><strong>Wistar rat</strong></th>
<th><strong>Sprague Dawley rat</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IC50 (mg/kg)</strong></td>
<td>0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Latency to seizure (s)</strong></td>
<td>7.1 ± 0.6</td>
<td>7.1 ± 1.4</td>
<td>7.1 ± 1.4</td>
</tr>
<tr>
<td><strong>Protected from seizure (%)</strong></td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

*Significantly greater than vehicle (ANOVA followed by Dunnett’s test; p ≤ 0.05).†Significantly different from vehicle (Fisher’s exact probability test; p ≤ 0.05).
rat MES assay, with an ED$_{50}$ value of 0.55 mg/kg after oral administration (95% CI, 0.2–1.9) (Fig. 2E).

**PTZ-Induced Seizure.** The effects of ICA-27243 on PTZ-induced seizure studies in CD-1 mouse and Sprague-Dawley rats are presented in Table 1 and shown graphically in Fig. 2, C and D, respectively. In the mouse, ICA-27243 significantly increased PTZ-induced seizure latencies at doses of 4.5 mg/kg and higher (ANOVA, $p \leq 0.05$; Dunnett’s test, $p \leq 0.05$). The ED$_{50}$ value determined from the fitted dose-response curve shown in Fig. 2C was 3.9 mg/kg (95% CI, 3.1–5.0). In addition to increasing latency, ICA-27243 also produced protection from PTZ-induced seizures in mice. However, the dose response relationship appeared bell-shaped, with 0, 14, 14, 43, 86, 42, and 43% of animals fully protected after administration of vehicle, 0.3, 1, 3, 4.5, 10, 17, and 30 mg/kg ICA-27243, respectively, and only the 10 mg/kg dose produced statistically significant protection from seizure compared with vehicle (Fisher’s exact probability test, $p \leq 0.05$). Given the lack of a full response at the two higher doses, these data were not included in the dose-response curve.
Because tolerance is known to develop to the action of some anticonvulsant agents (e.g., phenobarbital, benzodiazepines) and is a theoretical concern for agonist drugs, we explored the effects of repeat, once daily dosing of 12 mg/kg ICA-27243 in the mouse PTZ model. The 12 mg/kg dose was chosen because it represents a dose that would be expected to be effective in 100% of the animals. Mice were administered 12 mg/kg ICA-27243 or vehicle p.o. once daily for 1, 3, or 5 days. The percentages of mice protected from seizure were 75% on day 1, 71% on day 3, and 71% on day 5, and these percentages were not statistically different ($\chi^2$). Group mean seizure latencies are presented for days 1, 3, and 5 in Fig. 3. On each day, there was a statistically significant difference between vehicle and ICA-27243-treated groups in either latency to seizure (days 1 and 3) (Mann-Whitney test, $p \leq 0.05$) or in percentage of mice protected from seizure (days 3 and 5) (Fisher’s exact probability test, $p \leq 0.05$). There was no difference in the percentage of mice protected from seizure across days, suggesting that tolerance does not develop to ICA-27243 after subchronic treatment over 5 days.

ICA-27243 also delayed the onset of PTZ-induced seizures in rats. Effects were significant at doses of 1 mg/kg and higher (ANOVA, $p \leq 0.05$; Dunnett’s test, $p \leq 0.05$), and the $ED_{so}$ value was 2.2 mg/kg (95% CI, 1.1–4.5) (Fig. 2D). ICA-27243 fully protected 7, 14, 33, 53, 73, 90, and 100% of the animals after administration of vehicle, 0.1, 0.3, 1, 3, 10, 30, and 100 mg/kg ICA-27243, respectively, with doses of 3 mg/kg and higher producing statistically significant protection from seizure (Fisher’s exact probability test, $p \leq 0.05$). Having established that ICA-27243 was efficacious in the rat PTZ model and that there was no evidence of tolerance, we employed the rat PTZ model to further explore the duration of anticonvulsant activity of ICA-27243 and the relationship between pharmacodynamic activity and plasma concentration. After administration of a calculated $ED_{so}$ (12 mg/kg ICA-27243), anticonvulsant activity peaked 30 to 60 min after dosing and gradually declined thereafter. Significant anticonvulsant activity could still be observed 4 h postdose (Fig. 4A). In parallel pharmacokinetic studies, plasma levels peaked (maximum drug concentration in plasma = 2.87 $\mu$M) at 1 h after administration of 12 mg/kg, which corresponds closely with the time of peak protection. After 1 h, plasma concentrations gradually declined (Fig. 4A). Because plasma concentrations are not always predictive of concentrations in other compartments, we also measured brain levels of ICA-27243 after a 30 mg/kg (p.o.) dose. Brain concentrations increased with a similar time course to plasma concentrations but were typically 2 to 3-fold higher than plasma concentrations at all time points during the first 2 h after administration (Fig. 4B). These findings suggest that the time of peak anticonvulsant activity in the PTZ assay corresponds to the time of highest brain and plasma exposure.

**Kindling.** ICA-27243 produced clear antiseizure effects in the rat amygdala kindling model (Table 2). ICA-27243 produced a dose-related increase in seizure threshold as evidenced by the increase in current intensity required to evoke an AD. At the highest dose, 9 mg/kg, none of the eight rats tested exhibited an AD at the original test stimulus (statistically significant effect; Fisher’s exact probability test, $p \leq 0.05$), and six of the eight required an average increase stimulus intensity of 121% to evoke an AD. Two of the eight rats at the high dose did not exhibit an AD despite being subjected to the most intense stimulus (statistically significant effect; Fisher’s exact probability test, $p \leq 0.05$). At lower doses, ICA-27243 also raised seizure threshold, with the 1 mg/kg dose increasing the current intensity required to elicit an AD by 11% and the 3 mg/kg group increasing the current intensity required for an AD by 21%.

In addition to raising threshold for induction of local seizures, ICA-27243 also inhibited the propagation of the focal amygdala seizures, as evidenced by the suppression of generalized motor seizures in the ICA-27243-treated rats (Table 2). Of the six rats in the 9 mg/kg dose group that displayed an AD, no generalized tonic-clonic seizure (class 4 or 5) could be evoked in four of them, despite the most powerful stimulus used (1000 $\mu$A with train duration of 2 s) (statistically significant effect; Fisher’s exact probability test, $p \leq 0.05$). In the two high-dose rats in which a class 4 or 5 seizure could be evoked, the current intensity required to evoke the seizure was increased above baseline by at least 5-fold (statistically significant effect; ANOVA, $p \leq 0.05$; Dunnett’s test, $p \leq 0.05$). As illustrated in Fig. 5, lower doses of ICA-27243 also inhibited propagation of focal amygdala seizures. No motor impairment as measured by Rotorod was evident in kindled rats after administration of any of the doses of ICA-27243 (1, 3, 9 mg/kg) (Table 2).

The effect of carbamazepine on amygdala-kindled seizures was tested in a parallel series of studies (Table 2). The lowest dose of carbamazepine selected for study (20 mg/kg) was approximately the $ED_{so}$ described by Albright and Burnham (1980). The current intensity required to elicit a localized AD was increased to a similar extent by all three doses of carbamazepine (78–85%), and the effect was statistically significant (ANOVA, $p \leq 0.05$; Dunnett’s test, $p \leq 0.05$). Carbamazepine also inhibited the propagation of focal amygdala seizures and the development of generalized motor seizures (Fig. 5). Although generalized clonic-tonic seizure
(class 4 or 5) could be evoked in all carbamazepine-treated animals, the current intensity required to elicit these events was increased by up to approximately 2-fold after administration of 20, 30, and 40 mg/kg carbamazepine. Rotorod impairment was marked in the carbamazepine-treated rats (Table 2). At the lowest dose, 40% of the rats displayed impairment. At the two highest doses, all the rats displayed significant motor impairment (Fisher's exact probability test, $p < 0.05$).

**TABLE 2**

Effect of ICA-27243 and carbamazepine on amygdala-kindled rats

Data are submitted as mean ± S.E.M. or as the number of animals displaying a response compared with the number of animals tested.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Animals Responding to Initial Test Stimulus</th>
<th>ADD at Initial Test Stimulus (s)</th>
<th>No. of Animals That Had an ADD at Some Stimulus Intensity</th>
<th>% Increase in Current Intensity for First ADD Evoked</th>
<th>No. of Animals That Had Clonic Motor Seizure at Some Stimulus Intensity</th>
<th>Increase in Stimulus Intensity to Reach Clonic Motor Seizure (%)</th>
<th>Current Intensity Required to Elicit Clonic Motor Seizure &gt; 20 s (μA)</th>
<th>No. of Animals Displaying Rotorod Motor Impairment</th>
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<tr>
<td>ICA-27243</td>
<td>5/5</td>
<td>96 ± 19</td>
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<td>5/5</td>
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<tr>
<td>1 mg/kg</td>
<td>2/6</td>
<td>71 ± 34</td>
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<td>147 ± 10</td>
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<td>9 mg/kg</td>
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<td>121 ± 57</td>
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<td>674 ± 60**‡</td>
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<td>Carbamazepine</td>
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<td>78 ± 26</td>
<td>5/5</td>
<td>78 ± 26</td>
<td>5/5</td>
<td>166 ± 25</td>
<td>450 ± 130</td>
<td>5/5‡</td>
</tr>
</tbody>
</table>

† Significantly different from vehicle (Fisher's exact probability test; $p < 0.05$).

‡ Significantly greater than vehicle (ANOVA followed by Dunnett's test; $p < 0.05$).

* These data reflect the subset of animals that responded within each group; nonresponders are excluded.

** These numbers represent data for the two of eight animals within the group that had a clonic motor seizure at the maximum stimulation of 1000 μA.

Fig. 4. Time course of efficacy and pharmacokinetics of ICA-27243 in rat PTZ-induced seizure assay. A, time course of efficacy after administration of 12 mg/kg (p.o.) ICA-27243 in PTZ-induced seizure assay in Sprague-Dawley rats. ○, latency (mean ± S.E.M.) to seizure ($n = 6–13$); ●, plasma concentrations after administration of 12 mg/kg (p.o.) ICA-27243 to jugular cannulated Sprague-Dawley rats ($n = 2$). B, brain and plasma concentrations at various time points after administration of 30 mg/kg (p.o.) ICA-27243 to Sprague-Dawley rats. Columns, average concentrations (mean ± S.E.M.; $n = 3$ rats per time point).

Fig. 5. ICA-27243 dose-dependently increased current intensity required to elicit generalized seizures in fully kindled rats. Current intensity required to elicit >20-s motor seizure in amygdala-kindled rats 1 h after administration of ICA-27243 p.o. and 30 min after administration of carbamazepine i.p. Symbols represent current intensity in microamperes (mean ± S.E.M.): ▲, 1, 3, and 10 mg/kg p.o. ICA-27243; ●, 20, 30, and 40 mg/kg i.p. carbamazepine; and ■, vehicle. $n = 5$ to 8 per dose group. **, statistically significant differences from vehicle (ANOVA; $p < 0.05$).
6 Hz. In the mouse 6-Hz screening assay, both doses of ICA-27243 tested, 10 and 25 mg/kg i.p., were protective. Although 10 mg/kg protected two of the four mice when tested 15 min after administration, the 25 mg/kg dose was fully protective (n = 4) through three time points up to 1-h postadministration.

**Locomotor Activity.** To evaluate the potential for unwanted effects on motor behavior, we studied the effects of ICA-27243 on Rotorod performance and spontaneous locomotor activity in rats. In the rat Rotorod assay, ICA-27243 had no effect on the ability to remain on the Rotorod at doses up to 100 mg/kg p.o. (data not shown). The results of the spontaneous locomotor activity studies are summarized in Fig. 6A, in which the mean ± S.E.M. total activity counts determined over a 1-h period are plotted against ICA-27243 dose. ICA-27243 reduced spontaneous locomotor activity in a dose-related manner, with statistically significant reductions observed after administration of 50, 100, and 150 mg/kg ICA-27243 (ANOVA, p < 0.05; Dunnett’s test, p < 0.05). The ED₅₀ value was 40 mg/kg (95% CI, 28–58). In a parallel study, D-23129 caused a decrease in locomotor activity, with an ED₅₀ value of 11 mg/kg after oral administration (95% CI, 5.8–21) (Fig. 6B).

**Morris Water Maze.** ICA-27243 was evaluated in the Morris water maze to assess the effects of this compound on cognitive function. These data are presented in Fig. 7. Performance in the water maze was measured as either the time taken to reach the platform (Fig. 7A) or the path length (Fig. 7B) and was measured over 5 consecutive days.

The data show that performance improved in all groups in the water maze over days as the subjects became more familiar with the task and learning occurred. There was no significant effect of ICA-27243 (10 or 30 mg/kg) on either latency or path length in the water maze. The active control, scopolamine, significantly impaired performance in both measures of water maze performance, confirming the ability of the assay to demonstrate cognition impairment. Latency in the scopolamine group was greater than latency in the vehicle group and reached statistical significance (ANOVA, p ≤ 0.05) on days 1 and 4. Path length was longer in the scopolamine-treated group than in vehicle-treated group and reached statistical significance on days 1 to 4 (ANOVA, p ≤ 0.05).

**Discussion**

**Anticonvulsant Assays and Results.** ICA-27243 is a potent and selective KCNQ2/Q3 activator, with broad-spectrum anticonvulsant activity in rodent seizure models commonly used for assessment of potential antiseizure drugs. The compound was active at very low doses and produced full protection in two of the most widely used anticonvulsant models, the MES- and PTZ-induced seizure assays. ICA-27243 was also very active in the 6-Hz assay, which produces psychomotor seizures reminiscent of partial or limbic seizures in humans (Barton et al., 2001).

In the rat PTZ assay, the compound is active within 5 min of oral administration, with significant activity evident as long as 4 h after administration and with levels of protection that correlate well with plasma levels. Studies of tissue and plasma concentrations provide evidence that ICA-27243 penetrates the brain very well, with brain levels generally 2 to 3-fold higher than plasma levels. ICA-27243 has been determined to be 56% bound to rat serum plasma (Cerep, Poitiers, France). At the time of peak activity after administration of an ED₉₀ dose, plasma concentrations were 2.87 μM, and brain levels are assumed to be 2 to 3-fold higher. Based on these estimates, peak anticonvulsant activity was produced by brain concentrations of ~6 to 9 μM. These concentrations agree nicely with in vitro estimates of potency (~4 μM for half-maximal shift in the V₅₀ for KCNQ2/Q3 activation, 1–10 μM for suppression of seizure-like activity in hippocampal slices; see Wickenden et al., 2008). The close agreement between in vitro and in vivo estimates of potency and the high degree of selectivity exhibited by this compound (Wickenden et al., 2008) strongly suggests that KCNQ2/Q3 opening is the primary mechanism responsible for the anticonvulsant activity of ICA-27243 in the rat PTZ model.

ICA-27243 is also effective in mouse seizure assays, although the efficacious doses are slightly higher than in the rat. This difference may be the result of a lower brain penetration ratio in the mouse (Wickenden et al., 2008). The
bell-shaped dose response relationship seen in the mouse PTZ model was not observed in the rat PTZ model or any of the other behavioral assays reported here. The apparent decrease in protection against PTZ-induced seizure observed only in the mouse at higher doses of ICA-27243 may be related to gross behavioral effects observed at high doses, such as tremor. These are observed more often in the mouse than in the rat and may interfere with the assessment of PTZ-induced convulsive behavior.

The MES, PTZ, and 6-Hz assays are similar in that they evoke seizures in a normal or naive nervous system and suffer from the limitation that they do not model the epileptic nervous system of humans treated for seizures. The rat kindling model addresses this limitation, at least in part. Induction of kindling in a rat by periodic electrical stimulation of a nucleus such as the amygdala results in a lifelong lowered threshold to intense partial and generalized tonic-clonic seizure. This process of induction of kindling may be somewhat similar to that of the human epileptic patient, in that once seizures occur, patients may have a lifelong lowered threshold to experience generalized seizures. In addition, because the seizures are evoked by focal stimulation of the amygdala, this is a model of partial epilepsy. ICA-27243 produced dose-dependent elevations of the threshold of focal seizures, suppressed the propagation of focal seizures, and demonstrated these powerful antiseizure effects in the absence of detectable toxicity as measured by motor effects. By contrast, carbamazepine was far less effective in the kindling assay and produced ataxia at the same doses that produced antiseizure effects.

The potential of ICA-27243 to induce tolerance was investigated in the PTZ assay in mice. Subchronic administration of a high dose (corresponding to a maximally effective dose) did not result in any change in efficacy of ICA-27243. Giar- dina et al. (2005) report that in a similar paradigm employing an i.v. PTZ-induced seizure model, diazepam was significantly less effective after 4 days of repeated treatment than after a single acute treatment, whereas valproate and the experimental test compound ABT-769 were equally effective after repeat and single treatments. Studies have also shown that anticonvulsants such as phenobarbital and benzodiazepines are less effective against MES-induced seizures after 4 days of repeat dosing (Fariello et al., 1998; Villetti et al., 2001). Based on these limited data, there is no indication of the development of tolerance for ICA-27243. However, further studies with longer periods of administration and using additional seizure models are necessary to confirm this observation.

ICA-27243 displayed a very good therapeutic index between anticonvulsant activity and motor impairment (Table 3). When comparing locomotor activity and anticonvulsant activity in the rat, the therapeutic index for ICA-27243 ranged from 27 to 67 in the MES model, from 18 to 45 in the PTZ model, and from 4 to 33 in the amygdala-kindling model, depending on the motor parameter used. In contrast, carbamazepine had a therapeutic index of 1 when kindling activity was compared with Rotorod motor impairment.

**Assessment of the Potential for Cognition Impairment.** The effects of KCNQ channels on neuronal excitability make KCNQ blockers potential targets for cognition enhancers, and a number of the earliest KCNQ blockers were developed as cognition-enhancing drugs (Gribkoff, 2003). The KCNQ blocker linopirdine has been shown to enhance cognition in a variety of animal models (Cook et al., 1990; Fontana et al., 2000). ICA-27243 was administered in the Morris water maze (Fig. 7). No evidence of cognition impairment was observed after administration of ICA-27243 as measured in the Morris water maze. A, latency to reach the platform (seconds); B, length of the path taken to reach the platform (arbitrary units) as the sum of each day's two trials for 5 days for ICA-27243 (10 and 30 mg/kg p.o.), vehicle (p.o.), and 1 mg/kg i.p. scopolamine ($n = 12$ per drug treatment). **,** statistically significant differences from vehicle (ANOVA; $p < 0.05$).

<table>
<thead>
<tr>
<th>Response</th>
<th>Therapeutic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat MES</td>
<td>1.5 (0.9–2.4)</td>
</tr>
<tr>
<td>Mouse MES</td>
<td>8.6 (6.7–11.1)</td>
</tr>
<tr>
<td>Rat PTZ</td>
<td>2.2 (1.1–4.5)</td>
</tr>
<tr>
<td>Mouse 6 Hz (i.p.)</td>
<td>10–25†</td>
</tr>
<tr>
<td>Rat amygdala kindling</td>
<td>3–9†</td>
</tr>
<tr>
<td>Rat locomotor activity</td>
<td>40 (28–58)</td>
</tr>
<tr>
<td>Rat Rotorod</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

LMA, rat locomotor activity; RR, rat Rotorod.
† ED$_{50}$ not determined; numbers indicate active doses.
et al., 1994), although the compound produced equivocal results in human clinical trials (Rockwood et al., 1997; Borjeson et al., 1999). XE911, a potent blocker of KCNQ channels has also shown in vivo activity suggestive of cognition-enhancing ability (Zaczek et al., 1998). In contrast, it could be speculated that enhancing the M current via the KCNQ channel activation may impair cognition. Flupirtine, a KCNQ opener currently marketed in Europe as an analgesic, has been shown to diminish the degree of LTP and enhance LTD in mouse hippocampal slices, activity consistent with cognition impairment (Azad et al., 2004). In the present study, we show that ICA-27243 had no detrimental effect on learning and memory at doses far above those needed for anticonvulsant efficacy, suggesting that cognition impairment may not be a side effect of anticonvulsant treatment with ICA-27243.

Conclusions

In conclusion, the present data indicate that ICA-27243, an orally bioavailable, potent, and selective KCNQ2/3 opener, is active in multiple seizure models and therefore may be effective against generalized tonic-clonic, absence, and partial seizures. Efficacy was observed in all rodent assays at doses as low as 1 mg/kg p.o. Motor impairment was not observed at anticonvulsant doses but rather at doses 4 to 28 times higher, suggesting that the therapeutic index of ICA-27243 compares favorably with other anticonvulsants. There was no evidence of tolerance development after multiple doses of ICA-27243; importantly, there is no indication of an impairment of cognition after ICA-27243 administration. ICA-27243’s potent anticonvulsant activity and the lack of evidence for tolerance development and cognition impairment suggest that this compound represents a novel class of selective KCNQ activators, thus validating KCNQ2/3 as an antiepileptic drug target.

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References


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KCNQ2/3 activator in multiple seizure models and therefore may be


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