Anticonvulsant effects of the BK-channel antagonist paxilline

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SUMMARY

Purpose: Mutations that enhance currents through the Ca\(^{2+}\)- and voltage-gated K\(^+\) channel BK (\(\text{Slo, maxiK, KCNMA1}\)) have been associated with seizure disorders in both rodent models and humans. Previously we have found that seizures themselves induce a gain-of-function in BK channels that is associated with elevated excitability in neocortical neurons. In this study, we sought to examine whether administration of BK-channel antagonists possess anticonvulsant activity in vivo.

Methods: Seizures were induced in animals by intraperitoneal (i.p.) injection of the \(\gamma\)-amino-butyric acid (GABA)\(_{\text{A}}\) antagonists picrotoxin or pentylenetetrazole. Twenty-four hours following induction of the initial seizure episode, animals were reinjected with chemoconvulsant in the presence of the BK-channel antagonist paxilline or saline. The presence and duration of tonic–clonic seizures were evaluated.

Results: Intraperitoneal injection of paxilline was sufficient to eliminate tonic–clonic seizures in picrotoxin-treated animals. Paxilline reduced seizure duration and intensity in pentylenetetrazole-injected animals.

Discussion: The BK-channel antagonist paxilline possesses significant anticonvulsant activity in both picrotoxin and pentylenetetrazole seizure models, an effect that may be related to the seizure-dependent gain-of-function in BK channel previously observed in neocortical neurons in vitro.

KEY WORDS: Anticonvulsant, Abnormal excitability, Sensitization, Channelopathy, BK, MaxiK.
or suppress behavioral seizures in young postnatal animals, using two different seizure models: picrotoxin and pentylenetetrazole-induced seizures. These types of seizure are known to engage both hippocampal and neocortical circuits based upon induction of the immediate-early gene c-fos (Willoughby et al., 1995; Eells et al., 2004). We have previously found that the contribution of BK channels in shaping action-potential waveform and firing rates is minimal in young postnatal animals under control conditions, but is significantly enhanced following chemoconvulsant-induced seizure (Shruti et al., 2008). The timing of this gain-of-function suggested that anticonvulsant-induced seizure (Shruti et al., 2008). The timing of this gain-of-function suggested that anticonvulsant action of BK-channel antagonists might be most effective after an initial seizure event and presented the rationale for this study.

**MATERIALS AND METHODS**

**In vivo injections**

Postnatal days 14–21 (P14-21), C57bl6 mice from our institutional colony or Harlan Sprague Dawley, Inc. (Indianapolis, IN, U.S.A.), were injected with picrotoxin (2 mg/kg body weight at P14) or pentylenetetrazole (80 mg/kg) intraperitoneally (i.p.) and monitored for seizure activity using a modification of the scale developed by Pinel (Pinel & Rovner, 1978), where onset timing, number, and duration (in min) of time spent in tonic–clonic seizure (class 8 on Pinel’s modified scale) were scored by visual inspection and then averaged over all animals within an experimental group. Class 8 seizures were selected for quantitative analysis because of their unambiguous presentation to an observer and because, unlike other more mild manifestations such as brief head shakes, it was reasonable for the observer to assess their duration (tens of seconds to minutes for each tonic–clonic seizure) as an additional measure of seizure intensity. Animals were monitored for 3-h postinjection, and those that died within this observation window or did not have a single class 8 event were not included in the analysis. All mice were P14 on the initial day of the experiments, with age increasing to P21 for the animals studied 7 days after an initial seizure. In total, 82 animals were used for this study.

Except in Fig. 2, animals were never treated with paxilline on the first day of chemoconvulsant injection. However, we found that it was important to compare the same animal’s seizure response on the first day of chemoconvulsant administration to its response on the second day, since there could be significant interanimal variability. Therefore, a group labeled “Day 1 Pax” represents those animals injected with chemoconvulsant that would later (24 h, 48 h, or 7 days later) be injected with paxilline.

Because seizure threshold changed with development, we found that larger doses of picrotoxin were required to elicit tonic–clonic seizures as the animals matured, a finding that held for seizure-naive controls as well as the seizure-primed animals. Therefore, 2.2 mg/kg picrotoxin was injected for animals at P16, and 4.4 mg/kg picrotoxin was injected for animals that were P21.

Paxilline (Sigma, St. Louis, MO, USA), roughly calculated to approximate a final in vivo concentration of 5 nM (the effective concentration for BK-channel antagonist activity in vitro (Knaus et al., 1994) assuming that 1 g of animal was equivalent to 1 ml of solution, was injected at 2.2 µg/kg body weight, i.p. Paxilline was resuspended to a 10 mM concentration in dimethyl sulfoxide (DMSO) and diluted 1:2,000 in phosphate-buffered saline (PBS); therefore, paxilline injections were carried out with a 5-µM solution in 0.05% DMSO. Vehicle solution for control animals was composed of 0.05% DMSO in PBS. In the majority of cases, scoring of seizure onset, number, and cumulative duration was performed blind to vehicle or paxilline injection. All statistical analyses for paxilline versus vehicle-treated animals for seizure onset, number, and duration were carried out using an unpaired t-test.

**Immunohistochemistry**

Because we have extensively analyzed seizure-induced changes in BK-channel dependent excitability of neocortical neurons (Shruti et al., 2008), we elected to examine how in vivo antagonism of BK channels would alter the activity of neurons within this area, specifically primary somatosensory (barrel) cortex. Tissue was dissected and immersion-fixed overnight in 4% paraformaldehyde at 4°C, and then cryoprotected in 30% sucrose and stored at 4°C until sectioning. Animals were in one of the following groups: (1) control animals taken directly from their home cage; (2) animals 24 h after the initial seizure, taken from their home cage where they had recovered; (3) animals sacrificed 2 h after reinjection with picrotoxin 24 h after the initial seizure (to allow for Fos expression to peak); and (4) animals sacrificed 2 h after reinjection with picrotoxin + paxilline 24 h after the initial seizure. Immunohistochemistry was carried out as previously described (Barth et al., 2004). In brief, sections from barrel cortex were taken on a frozen-block microtome at 20-µm thickness and then processed for anti-Fos immunohistochemistry. Primary antibodies against Fos (Ab-5; Calbiochem, San Diego, CA, U.S.A.) were diluted 1:10,000 into PBS with 2% normal donkey serum (PBS-NDS) and applied for 24 h at 4°C. Sections were incubated with Cy-3 conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, U.S.A.) diluted 1:250 into PBS-NDS for 1 h at room temperature and counterstained with 4’,6-diamidino-2-phenylindole (DAPI) before mounting. Barrel cortex was identified by the presence of anatomic barrels in layer 4, which corresponded to a region 0.75–1.75 mm caudal to bregma and from 2.5–3.5 mm lateral to the midline in animals at this developmental age. Photomicrographs of layer 2/3 were taken (40× magnification), and the total number of DAPI-labeled and Fos-IR cells over an
BK Antagonists Block Seizures

Results

Picrotoxin-induced seizures

Seizures were pharmacologically induced in young postnatal animals (P14), and 24 h after the initial event animals were reinjected with picrotoxin + paxilline or picrotoxin + vehicle solution (Fig. 1A). Remarkably, paxilline treatment was sufficient to completely block the induction of tonic–clonic seizures 24 h following the initial sensitizing episode (Fig. 1B–D; picrotoxin + paxilline vs. picrotoxin plus vehicle solution, p < 0.0001 for all comparisons). In contrast, seizure duration and number were not significantly different for control animals between initial and subsequent chemoconvulsant injections (Fig. 1C,D).

The anticonvulsant effect of paxilline was not observed in seizure-naive animals. When coadministered with the first injection of chemoconvulsant, paxilline had no protective effect on the timing of onset (Fig. 2A), number (Fig. 2B), or total duration of tonic–clonic seizures (Fig. 2C), all of which are indicators of chemoconvulsant potency, compared to vehicle-injected controls. This is consistent with our previous findings that under normal conditions, BK channels play a minor role in controlling cellular and network excitability in vitro, at least in neocortical pyramidal neurons under our recording conditions.

Pentylenetetrazole-induced seizures

Pentylenetetrazole, also a γ-aminobutyric acid (GABA)\(_A\) antagonist, is another potent convulsant agent that has been used extensively to study seizure mechanisms in animal models. Seizures induced by pentylenetetrazole injection are qualitatively and quantitatively different from those induced by picrotoxin; mice tend to exhibit a faster seizure onset after injection as well as greater mortality during and after seizures in C57Bl6 mice compared to picrotoxin. Experiments were carried out as described for picrotoxin; specifically, two groups of animals were injected with pentylenetetrazole. Twenty-four hours later, one group was injected with pentylenetetrazole + vehicle solution and the second group with pentylenetetrazole + paxilline.

Typically, we observed that animals would experience a single, brief tonic–clonic seizure immediately following coinjection of paxilline and pentylenetetrazole (although three of eight animals showed no seizures); therefore, paxilline did not significantly alter the mean time of onset for pentylenetetrazole-induced seizures (Fig. 3A). After a single seizure, further tonic–clonic seizures were rare if not absent entirely in the paxilline-treated animals; accordingly, mean number of seizures was significantly reduced in the paxilline-treated group (Fig. 3B; p < 0.05). Consistent with this, we found that paxilline significantly reduced the total duration of pentylenetetrazole-induced seizures (Fig. 3C; p < 0.05).

Duration of paxilline’s anticonvulsant activity

To determine whether the anticonvulsant action of paxilline would be maintained when the interval between the first and second seizures was increased, we evaluated its efficacy at blocking tonic–clonic seizures 48 h and 7 days following an initial picrotoxin-induced seizure (Fig. 4). At 48 h after the initial sensitizing seizure, we found that paxilline continued to show a significant effect at increasing the latency to the first tonic–clonic seizure (Fig. 4A; p < 0.005). Indeed, 7 of 12 animals showed no tonic–clonic seizures after paxilline treatment. The mean number of seizures in paxilline-injected animals was significantly reduced compared to vehicle-injected animals at 48 h (Fig. 4B; p < 0.005). At this time point, the paxilline-injected animals that did seize spent significantly less time in tonic–clonic seizure than vehicle-injected controls (Fig. 4C; p < 0.005).

Seven days after the initial sensitizing event, paxilline injection did not alter the average time to first seizure (Fig. 4D). However, the mean number of seizures in paxilline-injected animals was also significantly reduced compared to vehicle-treated animals at 7 days (Fig. 4E; p < 0.05), and paxilline continued to significantly reduce the total amount of time spent in tonic–clonic seizure (Fig. 4F; p < 0.05), where two of eight paxilline-injected animals showed no tonic–clonic seizures.

Side-effects of paxilline treatment

Although paxilline has been reported to have tremorgenic effects, no such side effects were noted in our...
experiments, possibly because of the low dose of drug applied. After paxilline injection, animal behavior was indistinguishable compared to vehicle-injected controls, that is, they did not appear to be sedated, and mobility in their holding cage was not impaired (data not shown). Indeed, using a simple wire-hang assay (Dabir et al.,

Figure 1.
In vivo administration of BK antagonists eliminates pharmacologically induced seizures. (A) Schematic of experimental procedure. Animals were injected with picrotoxin (Ptx; 2 mg/kg) on day 1 and day 2 of the experiment. On day 1, both groups were treated equivalently; that is, animals received only Ptx. On day 2, animals were either injected with vehicle (control; red bars) in (B–D) or paxilline (Pax; 2.2 μg/kg; yellow bars) in (B–D) intraperitoneally (i.p.) concurrent with Ptx injection. (B) Paxilline treatment eliminates seizures on day 2 compared to picrotoxin- or picrotoxin + vehicle–injected controls. Time to first seizure for individual animals was calculated and averaged (all values are mean ± standard deviation). Statistical comparisons are shown for day 2 values only. Day 2 Ptx + vehicle: 25.6 ± 4.7 min, n = 12 animals; day 2 Ptx + Pax, no seizures, n = 15 animals; **p < 0.0001. (C) Paxilline prevents all tonic–clonic seizures. Day 2 Ptx + vehicle 5.33 ± 1.2 seizures, n = 12 animals; day 2 Ptx + Pax 0 seizures, n = 15 animals; **p < 0.0001. (D) Paxilline treatment abolishes tonic–clonic seizures on day 2. Total time seized was summed for individual animals and group averaged. Day 2 Ptx + vehicle: 49.8 ± 10.1 min, n = 12 animals; day 2 Ptx + Pax, no seizures, n = 15 animals; **p < 0.0001. 

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We found no significant differences in motor function in paxilline-treated animals compared to control (Fig. 5). This was true both for the dose of paxilline used in the chemoconvulsant experiments (2.2 µg/kg) as well as for a 2-fold increase in this dose (4.4 µg/kg).

**Paxilline reduces chemoconvulsant-induced neural firing in vivo**

We next asked whether BK-channel antagonists exerted their anticonvulsant effect via a reduction in the activity of CNS neurons. Although the in vitro data strongly suggest that BK-channel antagonists are eliminating the occurrence of future seizures by reducing neuronal excitability, it was possible that paxilline may reduce the behavioral or motor manifestations of chemoconvulsant-induced seizures without affecting firing activity in the central nervous system (CNS). Although this question could be addressed by electroencephalography (EEG) monitoring of mice following paxilline injection, implantation of chronic EEG electrodes into young postnatal mice before weaning is technically challenging and carries with it the danger of introducing damage at the implantation site that might affect the interpretation of our results. Instead, we selected a less-invasive way to monitor CNS activity, using expression of the immediate-early gene *c-fos* as an indicator of neural activity (Fig. 6).

Under control conditions, a small fraction of neurons in the neocortex show detectable Fos immunoreactivity (Fos-IR). Immediately following picrotoxin-induced seizure, virtually all neurons show robust Fos-IR (data not shown and Willoughby et al., 1995). Consistent with our analysis of elevated spontaneous firing rates in postseizure neurons (Shruti et al., 2008), we observed a slight but not significant elevation in the numbers of Fos-IR cells in layer 2/3 of the neocortex 24 h after the initial chemoconvulsant-induced seizure (Fig. 6A, 6C and Table 1).

Directly after administration of a second chemoconvulsant dose, the percentage of Fos-IR cells was increased nearly 3-fold compared to control (Fig. 6A, 6D and Table 1; *p < 0.005*), indicating that picrotoxin-induced seizures are highly correlated with elevated numbers of Fos-IR cells in the neocortex.

No such picrotoxin-induced increase in Fos-IR cells was observed when animals were cojected with the BK-channel antagonist paxilline at the same time as the second chemoconvulsant dose (Fig. 6A, 6E and Table 1; *p > 0.4* vs. control). This reduction in chemoconvulsant-induced fos-expression was also observed in other brains areas, such as the hippocampus (data not shown). These data indicate that paxilline administration is sufficient to reduce neural activity that can lead to immediate-early gene expression in vivo and suggest that the role of BK channels in mediating network excitability previously characterized in acute slices

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**Figure 2.**
Paxilline does not show an anticonvulsant effect in seizure-naive animals. (A) Time elapsed before first tonic–clonic seizure is not significantly different between vehicle and paxilline-treated seizure-naive animals (first day of treatment only; all values are mean ± standard deviation). Ptx + vehicle (control; red bar): 25.0 ± 5.1 min, *n* = 5 animals; Ptx + Pax (paxilline; yellow bar): 29.0 ± 3.2 min, *n* = 6 animals. (B) Mean number of seizures is not altered in Ptx + vehicle versus Ptx + Pax treated animals. Ptx + vehicle (control; red bar): 3.6 ± 1.1 seizures, *n* = 5 animals; Ptx + Pax (paxilline; yellow bar): 3.2 ± 1.9 seizures, *n* = 6 animals. (C) Total time spent in tonic–clonic seizure is not significantly different in seizure-naive animals (first day of treatment only). Ptx + vehicle (control; red bar): 34.2 ± 22.4 min, *n* = 5 animals; Ptx + Pax (paxilline; yellow bar): 27.8 ± 22.2 min, *n* = 6 animals.

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(Shruti et al., 2008) may also operate to reduce excitability and prevent seizures in vivo.

**DISCUSSION**

Here we describe the anticonvulsant effects of the BK-channel antagonist paxilline for seizures induced by the GABA<sub>A</sub> antagonists picrotoxin and pentylenetetrazole. This effect was most pronounced for picrotoxin-induced seizures 24 h after an initial, sensitizing seizure event, but was also significant for pentylenetetrazole-induced seizures. The anticonvulsant action of paxilline was maintained, although reduced, when longer time intervals separated the initial sensitizing event from the subsequent seizure. Finally, we found that paxilline injection is associated with a decrease in chemoconvulsant-induced Fos-expression in vivo, suggesting that its anticonvulsant action is linked to a reduction in neuronal firing activity.

Previously, we have found that a gain-of-function in BK channels may arise not only from genetic lesion but also as an acquired channelopathy, based upon a history of elevated activity. For example, chemoconvulsant-induced seizures result in enhanced BK-channel function in neocortical pyramidal neurons (Shruti et al., 2008). This gain-of-function is associated with elevated spontaneous firing activity in vitro, an activity that can be normalized to control levels by bath application of BK-channel antagonists. Based upon these data, we hypothesized that systemic administration of BK-channel antagonists may be sufficient to reduce neuronal firing rates and prevent the propagation of epileptic activity in vivo, a hypothesis supported by the data presented in this study.

How do increased BK-channel currents facilitate increased firing activity? It has been hypothesized that because BK channels hasten action potential (AP) repolarization, they may decrease cumulative Ca<sup>2+</sup> entry during repetitive firing, reducing slow K<sup>+</sup> currents (SK) currents that normally suppress firing activity (Brenner et al., 2005). It has also been hypothesized that a larger hyperpolarization caused by BK-channel currents might facilitate Na<sup>+</sup> channel deinactivation and increase firing output (Gu et al., 2007). Alternatively, BK channels may increase firing rates simply because faster AP repolarization...
reduces interspike intervals. Although BK channels are present throughout the cell, they are particularly prominent in the axon and axonal boutons (Hu et al., 2001; Misonou et al., 2006). However, their role in mediating neurotransmitter release remains controversial (Robitaille et al., 1993; Hu et al., 2001; Pattillo et al., 2001; Raffaelli et al., 2004; Xu & Slaughter, 2005). A BK-channel gain-of-function in layer 2/3 pyramidal neurons did not enhance release properties after seizures, at least under our experimental conditions (Shruti et al., 2008).

Our finding that BK-channel antagonists have anticonvulsant activity may be paradoxical in light of previous studies indicating that suppressed K⁺-channel function is linked to enhanced excitability. For example, K⁺-channel antagonists have been linked to epileptic activity (Segal et al., 1984; Fragoso-Veloz et al., 1990; Juhng et al., 1999), and loss-of-function mutations in K⁺ channels have also been linked to seizures (see for example (Rho et al., 1999; Castaldo et al., 2002; Brew et al., 2007). However, other studies have shown that BK channels may be exempt from the assumption that K⁺-channel blockade is proconvulsant. For example, application of BK-channel antagonists to cultured neurons displaying epileptic activity reduced abnormal bursting in these cells (Jin et al., 2000), supporting a possible anticonvulsant effect. In rodents displaying a gain-of-function in BK channels, abnormal excitability in vitro is reduced by application of BK-channel antagonists (Brenner et al., 2005; Shruti et al., 2008), suggesting that these compounds might be effective for some specific forms of inherited epilepsy. Finally, in vivo

Figure 4.
Paxilline treatment reduces picrotoxin-induced seizures 48 h and 7 days after an initial seizure. (A) Paxilline prolongs time to first seizure 48 h after an initial seizure. Data for individual animals was calculated and averaged for all animals, including those that did not exhibit seizures (all values are mean ± standard deviation). Statistical comparisons are shown for 48 h values only. 48 h Ptx + vehicle (control; red bars): 24.7 ± 7.1 min, n = 11 animals; 48 h Ptx + Pax (paxilline; yellow bars): 36.2 ± 5.4 min, n = 12 animals, */p < 0.01. (B) Paxilline significantly reduces the number of tonic–clonic seizures induced 48 h after the initial seizure. 48 h Ptx + vehicle (control; red bars): 4.9 ± 3.5 seizures, n = 11 animals; 48 h Ptx + Pax (paxilline; yellow bars): 1.2 ± 1.5 seizures, n = 12 animals, **/p < 0.005. (C) Paxilline reduces total time seized at 48 h after the initial seizure. 48 h Ptx + vehicle (control; red bars): 11.5 ± 7.8 min, n = 11 animals; Ptx + Pax (paxilline; yellow bars): 1.5 ± 2.4 min, n = 12 animals, **/p < 0.005. (D) As in (A) but for 7 days after the initial seizure. 7d Ptx + vehicle (control; red bars): 27.7 ± 2.9 min, n = 7 animals; 7d Ptx + Pax 32.7 ± 10.1 min, n = 8 animals, p = 0.23. (E) As in (B) but 7 days after an initial seizure. 7d Ptx + vehicle (control; red bars): 4.7 ± 1.6 seizures, n = 7 animals; 7d Ptx + Pax (paxilline; yellow bars): 2.3 ± 1.8 seizures, n = 8 animals, */p < 0.05. (F) As in (C) but 7 days after an initial seizure. 7d Ptx + vehicle (control; red bars): 5.7 ± 1.8 min, n = 7 animals; 7d Ptx + Pax (paxilline; yellow bars): 2.4 ± 1.9 min, n = 8 animals, **/p < 0.005.

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administration of the BK-channel antagonist paxilline does not lead to seizures in seizure-naive rodents (Juhng et al., 1999). This finding is consistent with the results presented in Fig. 2, where paxilline administration did not influence seizures at the initial time of picrotoxin injection.

Because we did not carry out EEG analysis from chemoconvulsant-injected animals, we were unable to determine whether paxilline administration merely blocked the manifestation of tonic–clonic seizures or whether it blocked all abnormal firing activity in the CNS. One limitation of the present study is that only the presence and duration of tonic–clonic seizures were quantified. Other, more mild, behavioral manifestations of abnormal brain activity, such as “wet dog” shakes, were not quantitated in this analysis because of the difficulty in unambiguously detecting and timing these events. Therefore, it remains possible that the BK-channel antagonist paxilline as administered in this study does not fully normalize pathologic brain activity. Further experiments to examine the dose–response range of this anticonvulsant, as well as its half-life in vivo are clearly warranted.

Although our analysis of BK-channel antagonist anticonvulsant action was confined to superficial layers of the neocortex, this compound appears to reduce chemoconvulsant-induced activity in neurons within other brain areas. Therefore, the conclusions drawn here should not be taken to indicate that the activity of layer 2/3 neurons are uniquely affected by antagonist administration, but rather to show that paxilline can prevent seizure-dependent gene expression in this brain area. It is likely that an activity-dependent BK-channel gain-of-function is induced by tonic–clonic seizures in other brain areas, and that paxilline administration will similarly reduce chemoconvulsant-induced firing of neurons in these regions.

Paxilline still conferred a significant anticonvulsant effect 48 h and 7 days after the initial seizure, suggesting that a BK-channel gain-of-function may still be influencing excitability in at least some brain areas at these time points. It was notable that vehicle-injected control animals also showed a reduction in the total time spent in tonic–clonic seizure seizures at 48 h and 7 days after the initial sensitizing event (Fig. 4C, 4F), although the total number of seizures experienced appeared the same or moderately increased. These differences may be caused by an age-dependent increase in seizure threshold (indeed, we were required to increase the chemoconvulsant dose from 2.0 to 2.2 mg/kg at P16 or 4.4 mg/kg at P21), or possibly to some effect of the initial seizure episode. Although we have not investigated whether chemoconvulsant activity leads to a BK-channel gain-of-function in older animals, we expect that this is likely to occur. In such a case, treatment with a BK-channel antagonist may protect against further seizures in adults.

Will BK-channel antagonists have broad anticonvulsant activity, for example, in seizure syndromes linked to dysfunction of another channel type? BK-channel antagonists can reduce abnormal bursting in cultured neurons from the epileptic El strain of mice (Jin et al., 2000). Because the genetic lesion in these mice is not related to the BK-channel gene (Frankel et al., 1995a, 1995b), it is tempting to speculate that augmented BK-channel function can occur whether seizures arise from a genetic or environmental cause, and that BK-channel antagonists might have

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**Figure 5.**

Paxilline injection does not affect motor coordination as assessed by a bar hang task. (A) P14 mouse shown clinging to an inverted wire cage lid. (B) Data summary of total time spent hanging (mean ± standard deviation). Control: 17.6 ± 11.1 s, n = 8 trials in two animals; 2.2 µg/kg paxilline: 17.8 ± 9.5 s, n = 8 trials in two animals; 4.4 µg/kg paxilline: 21.1 ± 15.9 s, n = 8 trials in two animals.

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broad anticonvulsant action for a number of different seizure disorders. Future experiments will examine whether BK-channel antagonists exhibit anticonvulsant activity in vivo for strains of mice that are susceptible to seizures because of genetic lesions in non–BK-channel genes. Taken together, the experimental findings presented here suggest that BK-channel antagonists hold therapeutic promise in the treatment of seizure disorders.

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We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Disclosures: A.L. Barth has applied for a patent for the use of BK channels in the treatment of seizure disorders. None of the other authors has any conflict of interest.

References

Figure 6.
Paxilline reduces chemoconvulsant-induced c-fos expression in the central nervous system (CNS). (A) Summary of the percentage Fos-immunoreactivity (Fos-IR) cells under four conditions: control (11.7 ± 6.0%, n = 4 animals), 24-h post-seizure (19.9 ± 9.1%, n = 2 animals), after the second Ptx injection (second seizure; 33.8 ± 3.8%, n = 4 animals), and after the second Ptx + Pax injection (second seizure + paxilline; 4.78 ± 1.8%, n = 3 animals). Paxilline and picrotoxin coinjection on the second day results in a significant decrease in the percentage of Fos-IR cells compared to Ptx injection alone, indicating that paxilline treatment reduced activity-dependent gene expression in the CNS. p < 0.05 for second seizure versus control and second seizure + paxilline by analysis of variance (ANOVA) with post hoc comparison of means using a Tukey test. (B) Representative Fos-IR in layer 2/3 of a control, untreated animal. (C) Fos-IR in layer 2/3, 24 h after the initial chemoconvulsant-induced seizure. (D) Fos-IR is increased following the second chemoconvulsant-induced seizure. (E) Paxilline coinjection with Ptx 24 h after the initial seizure reduces the fraction of Fos-IR–labeled cells. Bar = 100 μm.

Table 1. Fos expression in somatosensory cortex (layer 2/3)
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