

Topiramate Reduces Excitability in the Basolateral Amygdala by Selectively Inhibiting GluK1 (GluR5) Kainate Receptors on Interneurons and Positively Modulating GABA_A Receptors on Principal Neurons

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ABSTRACT

Topiramate [2,3,4,5-bis-*O*-(1-methylethylidene)- β -D-fructopyranose sulfamate] is a structurally novel antiepileptic drug that has broad efficacy in epilepsy, but the mechanisms underlying its therapeutic activity are not fully understood. We have found that topiramate selectively inhibits GluK1 (GluR5) kainate receptor-mediated excitatory postsynaptic responses in rat basolateral amygdala (BLA) principal neurons and protects against seizures induced by the GluK1 kainate receptor agonist (*R,S*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid (ATPA). Here, we demonstrate that topiramate also modulates inhibitory function in the BLA. Using whole-cell recordings in rat amygdala slices, we found that 0.3 to 10 μ M topiramate 1) inhibited ATPA-evoked postsynaptic currents recorded from BLA interneurons; 2) suppressed ATPA-induced enhancement of spontaneous inhibitory postsynaptic currents (IPSCs) recorded from BLA pyramidal cells; and 3) blocked ATPA-induced suppression of evoked IPSCs, which is mediated by presynaptic GluK1 kainate receptors present on BLA

interneurons. Topiramate (10 μ M) had no effect on the AMPA [(*R,S*)- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid]-induced enhancement of spontaneous activity of BLA neurons. Thus, although topiramate inhibits GluK1 kainate receptor-mediated enhancement of interneuron firing, it promotes evoked GABA release, leading to a net inhibition of circuit excitability. In addition, we found that topiramate (0.3–10 μ M) increased the amplitude of evoked, spontaneous, and miniature IPSCs in BLA pyramidal neurons, indicating an enhancement of postsynaptic GABA_A receptor responses. Taken together with our previous findings, we conclude that topiramate protects against hyperexcitability in the BLA by suppressing the GluK1 kainate receptor-mediated excitation of principal neurons by glutamatergic afferents, blocking the suppression of GABA release from interneurons mediated by presynaptic GluK1 kainate receptors and directly enhancing GABA_A receptor-mediated inhibitory currents.

The antiepileptic drug topiramate [2,3,4,5-bis-*O*-(1-methylethylidene)- β -D-fructopyranose sulfamate] is an *O*-alkyl sulfamate derivative of the naturally occurring monosaccharide D-fructose (Shank et al., 2000). Topiramate has broad utility in the treatment of partial and generalized seizures, as well as seizures associated with the Lennox-Gastaut syn-

drome. Antiepileptic drugs exert their therapeutic effects largely through direct or indirect actions on voltage-gated or neurotransmitter-gated ion channels (Rogawski and Löscher, 2004). Topiramate has actions on diverse ion channel targets. The drug has been reported to suppress the activity of voltage-gated sodium channels and calcium channels (Zona et al., 1997; Taverna et al., 1999; Zhang et al., 2000), enhance GABA_A receptor-mediated currents (White et al., 1997, 2000), and inhibit non-NMDA (AMPA/kainate) receptor-mediated synaptic transmission (Gibbs et al., 2000; Sk-

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; AMPA, (*R,S*)- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BLA, basolateral amygdala; artificial cerebrospinal fluid; QX-314, *N*-(2,6-dimethylphenylcarbonylmethyl)triethylammonium; IPSC, inhibitory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; ATPA, (*R,S*)-2-amino-3-(3-hydroxy-5-*tert*-butylisoxazol-4-yl)propanoic acid; SCH 50911, (2*S*)-(+)-5,5-dimethyl-2-morpholineacetic acid; D-APV, D-2-amino-5-phosphonovaleric acid; GYKI 53655, 1-(4-aminophenyl)-3-methylcarbonyl-4-methyl-7,8-methylenedioxy-3,4-dihydro-5*H*-2,3-benzodiazepine, LY293558, (3*S*,4*aR*,6*R*,8*aR*)-6-[2-(1(2)*H*-tetrazole-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid; sIPSC, spontaneous inhibitory postsynaptic current; CNQX, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline.

radski and White, 2000; Smith et al., 2000; Poulsen et al., 2004). We have demonstrated that among non-NMDA receptors, topiramate is selective for kainate receptors containing the GluK1 subunit (formerly GluR5; Collingridge et al., 2009). Thus, in rat basolateral amygdala (BLA) principal neurons, topiramate inhibited pharmacologically isolated GluK1 kainate receptor-mediated postsynaptic currents, whereas AMPA receptor-mediated currents were only modestly reduced (Gryder and Rogawski, 2003). In addition, topiramate provided protection against seizures induced by intravenous infusion of the selective GluK1 kainate receptor agonist ATPA, but it was less effective against seizures induced by AMPA or NMDA, indicating that the selective interaction of topiramate with GluK1 kainate receptors is relevant to its anticonvulsant properties (Kaminski et al., 2004).

In the BLA, GluK1 kainate receptors mediate a component of the postsynaptic excitation of principal neurons (Li and Rogawski, 1998). In addition, GluK1 kainate receptors play a key role in regulating inhibitory synaptic transmission. Activation of somatodendritic GluK1 kainate receptors on BLA interneurons stimulates GABA release in an action potential-dependent manner. GluK1 kainate receptors are also present on presynaptic terminals of BLA interneurons where they modulate GABA release in a bidirectional, agonist concentration-dependent manner (Braga et al., 2003). When activated by basal, ambient concentrations of endogenous glutamate, or low concentrations of exogenously applied GluK1 kainate receptor-selective agonists, these presynaptic GluK1 kainate receptors facilitate GABA release. In contrast, at higher agonist concentrations that mimic the concentrations of extracellular glutamate that are present during intense neuronal activity and seizures, presynaptic GluK1 kainate receptors suppress GABA release, thus contributing to further enhancement of circuit excitation (Aroniadou-Anderjaska et al., 2008). This effect, along with a depolarization of principal neurons, apparently over-rides the enhancement of inhibition associated with the GluK1 kainate receptor mediated-depolarization of GABAergic interneurons, because the net effect of GluK1 kainate receptor activation by sufficiently high agonist concentrations, as revealed by intracellular and field potential recordings, is a dramatic enhancement in the excitability and responsiveness of the BLA neuronal network and frank epileptiform activity (Li et al., 2001; Yonekawa et al., 2002; Rogawski et al., 2003). Topiramate may reduce this network excitability by antagonizing the GluK1 kainate receptors mediating the depolarization of BLA principal neurons (Gryder and Rogawski, 2003). In addition, topiramate could interact with GluK1 kainate receptors on interneurons to modify inhibitory tone in the BLA (Braga et al., 2003), which is a potentially powerful way of influencing the overall excitability of the nucleus. To evaluate this latter possibility, in the present study we investigated whether topiramate can modulate interneuron function and inhibitory synaptic transmission within the BLA. We found that topiramate interacts with GluK1 kainate receptors on BLA interneurons and that, furthermore, it can shape inhibition in the BLA by a direct action on GABA_A receptors.

Materials and Methods

Slice Preparation. Coronal slices containing the amygdala were prepared from 18- to 24-day-old male Sprague-Dawley rats. The rats were anesthetized with halothane and then decapitated. The brain was rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) composed of 125 mM NaCl, 2.5 mM KCl, 2.0 mM CaCl₂, 1.0 mM MgCl₂, 25 mM NaHCO₃, 1.25 mM NaH₂PO₄, and 11 mM glucose, bubbled with 95% O₂, 5% CO₂ to maintain a pH of 7.4. A block containing the amygdala region was prepared by rostral and caudal coronal cuts, and 400- μ m-thick slices were cut using a Vibratome slicer (series 1000; Technical Products International, St. Louis, MO). Slices were kept in a holding chamber containing oxygenated ACSF at room temperature, and recordings were initiated ≥ 1 h after slice preparation.

Electrophysiology. Slices were transferred to a submersion-type recording chamber for whole-cell recordings, in which they were continuously perfused with oxygenated ACSF at a rate of 3 to 4 ml/min. Neurons were visualized with an upright microscope (Eclipse E600fn; Nikon, Tokyo, Japan) using Nomarski-type differential interference optics through a 60 \times water immersion objective. All experiments were carried out at room temperature (24°C). Tight-seal (>1 G Ω) whole-cell recordings were obtained from the cell body of neurons in the BLA region. Patch electrodes were fabricated from borosilicate glass and had a resistance of 2.5 to 6.0 M Ω when filled with a solution containing 120 mM CsCl, 10 mM KCl, 1 mM MgCl₂, 0.025 mM CaCl₂, 0.1 mM EGTA, 10 mM HEPES, 1 mM ATP, 0.2 mM GTP, 5 mM QX-314, and 0.4% Lucifer yellow (pH 7.2; 285–290 mOsm/kg H₂O). Neurons were voltage-clamped using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Inhibitory postsynaptic currents (IPSCs) were pharmacologically isolated and recorded at a holding potential of -60 mV. Synaptic responses were evoked with sharpened tungsten bipolar stimulating electrodes (2 μ m in diameter; WPI, Sarasota, FL) placed in the BLA, approximately 50 μ m from the recording electrode. Minimal stimulation was applied at 0.1 Hz, using a photoelectric stimulus isolation unit having a constant current output (PSIU6; Grass Instruments, West Warwick, RI). Stimulation of a single presynaptic axon was confirmed by the intensity-threshold test (for references, see Braga et al., 2003). The mean IPSC amplitude showed a steep all-or-none threshold as a function of stimulating current intensity. Increasing current intensity by 40 to 60% above the threshold for evoking an IPSC had no effect on the IPSC amplitude, indicating the stimulation of a single presynaptic axon. Access resistance (5–24 M Ω) was regularly monitored during recordings, and cells were rejected if resistance changed by more than 15% during the experiment. The signals were filtered at 2 kHz, digitized (Digidata 1322A; Axon Instruments), and stored on a computer using the pCLAMP8 software (Axon Instruments). The peak amplitude, 10 to 90% rise time, and decay time constant of IPSCs were analyzed offline using pCLAMP8 software (Axon Instruments) and the Mini Analysis Program (Synaptosoft, Leonia, NJ). The visual method was used to identify failures and calculate failure rates. The percentage of failures was calculated from the responses to a cohort of at least 30 stimulus pulses before, during, and after drug application. Miniature IPSCs (mIPSCs) were detected offline using the Mini Analysis Program with the threshold arbitrarily set to optimize mIPSC detection (approximately 1.5 times the baseline noise amplitude). In some experiments, postsynaptic currents were elicited by application of 10 μ M ATPA or 10 μ M muscimol. Fast micropressure application of agonist solutions was performed with a Picospritzer (Parker Hannifin, Pine Brook, NJ) using borosilicate glass micropipette with a tip diameter of ~ 3 μ m. Applications were made for 2 s at 3-min intervals at pressures from 60 to 200 kPa.

The activity of spontaneous synaptic potentials was facilitated by bath application of agonists. Applications were for 40 to 60 s, but only the last 30 s was subjected to analysis. Events were detected when they were >3 times the average baseline noise. The records were

analyzed automatically using the software, but each was inspected visually and events seeming to represent noise were discarded. Interevent histograms were generated using the Mini Analysis Program. The onset of some of the actions of topiramate was slow. Therefore, all agonist applications included in the analyses were made after exposure of the slice to topiramate for >20 min, and ~20 min was allowed to elapse before testing after a concentration change.

All data are presented as mean \pm S.E.M. Results were tested for statistical significance using the Student's paired *t* test.

Drugs. D-2-Amino-5-phosphonovaleric acid (D-APV), an NMDA receptor antagonist; muscimol, a GABA_A receptor agonist; AMPA, an AMPA receptor agonist; and SCH 50911, a GABA_B receptor antagonist, were from Tocris Biosciences (Ellisville, MO). Bicuculline methiodide, a GABA_A receptor antagonist; ATPA; tetrodotoxin, a sodium channel blocker; and acetazolamide, a carbonic anhydrase inhibitor, were from Sigma-Aldrich (St. Louis, MO). GYKI 53655, an AMPA receptor antagonist, and LY293558, a GluK1 kainate receptor antagonist (Alt et al., 2004), were gifts from Lilly Research Laboratories, Eli Lilly & Co. (Indianapolis, IN). (GYKI 53655 may also block some non-GluK1 kainate receptor isoforms; Perrais et al., 2009.) Topiramate was a gift of Johnson & Johnson Pharmaceutical Research and Development (Spring House, PA). Drugs were dissolved immediately before use in ACSF except for ATPA. ATPA was prepared in a stock solution of 10% dimethyl sulfoxide and then diluted in ACSF so that the final dimethyl sulfoxide concentration was <0.01%.

Morphology. Pyramidal-like (principal) neurons and interneurons were visually (microscopically) distinguished based on their size and shape. An initial tentative identification was based on the size and shape of the soma: larger neurons were classified as pyramidal neurons, whereas smaller neurons were classified as interneurons. To confirm this initial classification, during whole-cell recordings, neurons were filled passively with 0.4% Lucifer yellow (Molecular Probes, Carlsbad, CA) for post hoc morphological identification. Slices were fixed for 1 h at 4°C in a phosphate-buffered saline, pH 7.4, containing 4% paraformaldehyde, and subsequently mounted and coverslipped in 10% phosphate-buffered saline in glycerine. The fluorescence image of the dye-filled neurons was captured by a fluorescence microscope (Mikroskop DM RXA; Leica Microsystems, Inc., Deerfield, IL) equipped with a SPOT2 digital camera (Diagnostic Instruments, Sterling Heights, MI) and a laser scanning confocal microscope (MRC-600; Bio-Rad Laboratories, Hercules, CA). Neurons considered "pyramidal-like" had a clearly distinguishable primary dendrite and a high spine density. Neurons considered to be GABAergic interneurons had smaller somata than the pyramidal-like neurons, and a dendritic field with no apparent primary dendrite, and with absent or sparse spines. Following previous classifications (Washburn and Moises, 1992; Rainnie et al., 1993; Sah et al., 2003), we use the term "pyramidal cell" for the larger pyramidal-like neurons and the term "interneurons" for the smaller nonpyramidal neurons.

Results

Topiramate Inhibits Somatodendritic GluK1 Kainate Receptors on GABAergic Interneurons. Whole-cell recordings from BLA interneurons were obtained at a holding potential of -60 mV. GluK1 kainate receptor-mediated postsynaptic currents were evoked by micropressure application of the selective GluK1 agonist ATPA ($10 \mu\text{M}$; Clarke et al., 1997; Jane et al., 2009), in the presence of D-APV ($50 \mu\text{M}$), GYKI 53655 ($50 \mu\text{M}$), bicuculline ($10 \mu\text{M}$), and SCH 50911 ($10 \mu\text{M}$), to block NMDA, AMPA, GABA_A, and GABA_B receptors, respectively. Under control conditions, the mean peak amplitude of ATPA-evoked postsynaptic currents was 692 ± 87 pA (nine interneurons). Bath application of

LY293558 ($30 \mu\text{M}$) eliminated these currents, confirming that they are mediated by activation of GluK1 kainate receptors (data not shown). Inclusion of topiramate (0.3 – $10 \mu\text{M}$) in the perfusion solution caused a concentration-dependent decrease in the mean peak amplitude of the ATPA-evoked postsynaptic currents (to $71.9 \pm 5.4\%$ of control levels by 300 nM topiramate, $54.2 \pm 8.4\%$ by $1 \mu\text{M}$ topiramate, and $12.3 \pm 9.2\%$ by $10 \mu\text{M}$ topiramate; Fig. 1) that persisted throughout the application of topiramate and was completely reversed after removal of the drug (Fig. 1). The blocking effect of topiramate occurred gradually so that full blockade required 20-min exposure to the drug. Therefore, in this and subsequent experiments, test responses in the presence of topiramate were only included in the analyses when the exposure was for at least 20 min. In addition, at least 20 min was allowed to elapse after a change in topiramate concentration before testing.

Activation of GluK1 kainate receptors on somatodendritic regions of GABAergic neurons enhances interneuronal firing and increases the frequency of action potential-dependent sIPSCs recorded from BLA pyramidal neurons (Braga et al., 2003). Because topiramate inhibits these GluK1 kainate receptors, topiramate should also inhibit the enhancement of spontaneous IPSCs induced by GluK1 kainate receptor activation. To confirm this prediction, we recorded sIPSCs from BLA pyramidal neurons at a holding potential of -60 mV in the presence of D-APV ($50 \mu\text{M}$), GYKI 53655 ($50 \mu\text{M}$), and SCH 50911 ($10 \mu\text{M}$). Bath application of ATPA ($10 \mu\text{M}$) caused an increase in the frequency of sIPSCs from 2.1 ± 1.3 to 18.7 ± 2.9 Hz ($895 \pm 142\%$ of control levels; eight pyra-

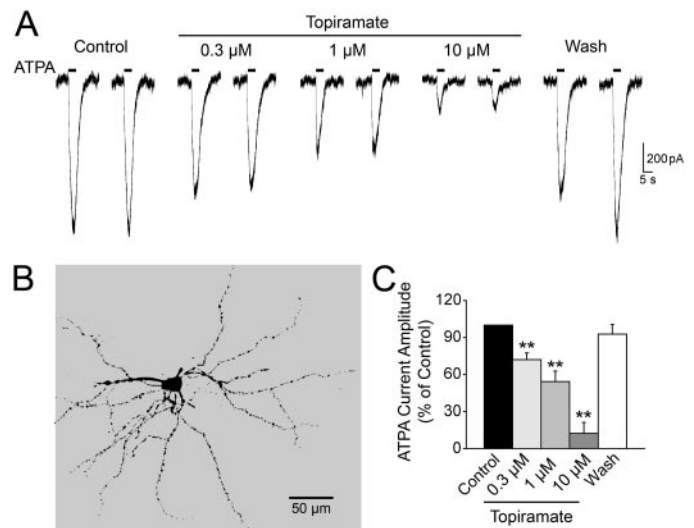


Fig. 1. Topiramate inhibits the activation of GluK1 kainate receptors in BLA interneurons. GluK1 kainate receptor-mediated postsynaptic currents were recorded from BLA interneurons in response to micropressure application of the selective GluK1 agonist ATPA ($10 \mu\text{M}$) in the presence of D-APV ($50 \mu\text{M}$), GYKI 53655 ($50 \mu\text{M}$), bicuculline ($10 \mu\text{M}$), and SCH 50911 ($10 \mu\text{M}$). Holding potential, -60 mV. A, ATPA-evoked currents from an interneuron before, during, and after the perfusion of topiramate at various concentrations. The small horizontal bar above each current trace indicates the duration of the ATPA pressure application pulse. Topiramate applied in the slice perfusion solution reversibly reduced the amplitude of ATPA-evoked currents in a concentration-dependent manner. The traces shown were acquired after at least 20-min exposure to the indicated topiramate concentration. B, Lucifer yellow fill of the interneuron. C, mean \pm S.E.M. peak ATPA current amplitudes with respect to the control current amplitude in nine interneuron recordings similar to that shown in A. **, $p < 0.01$ with respect to control.

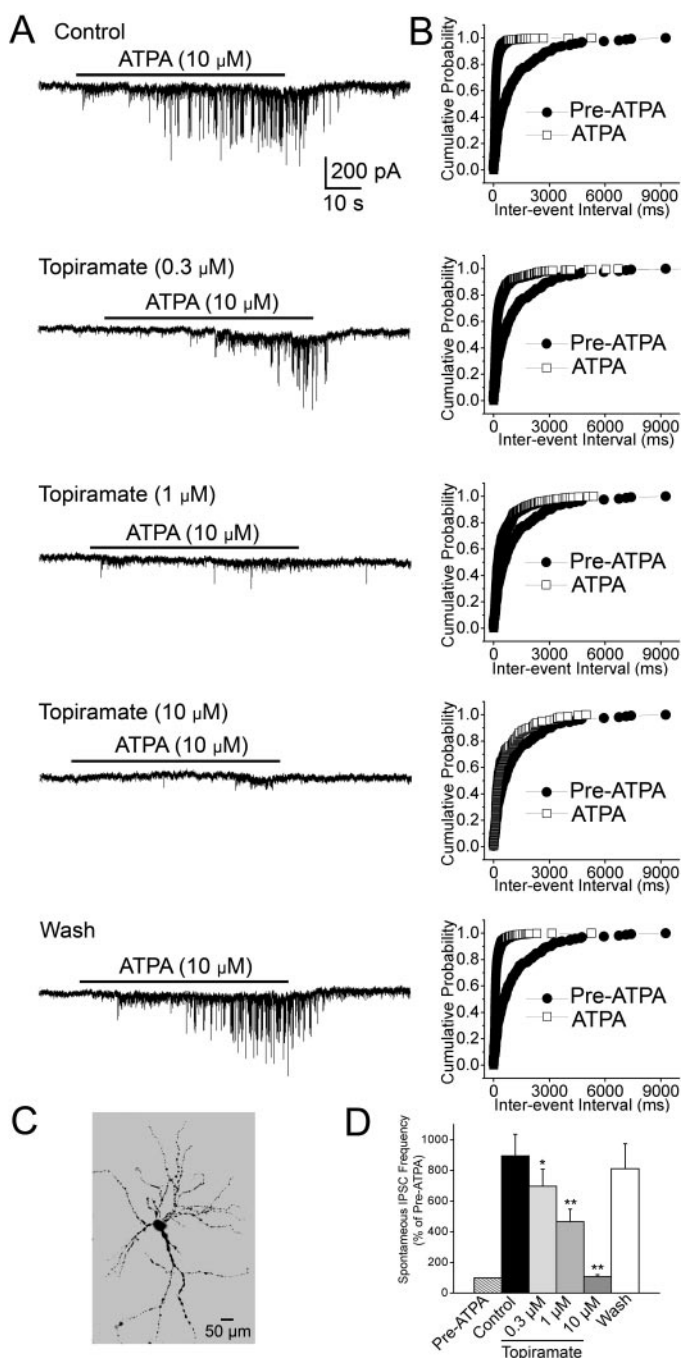


Fig. 2. Topiramate inhibits the GluK1 kainate receptor-mediated enhancement of sIPSCs in BLA pyramidal neurons. Recordings were carried out in the presence of D-APV (50 μ M), GYKI 53655 (50 μ M), and SCH 50911 (10 μ M) at a holding potential of -60 mV. A and B, bath application of the selective GluK1 kainate receptor agonist ATPA (10 μ M) significantly increased the frequency of sIPSCs as evidenced by a reduction in interevent intervals shown in the cumulative probability plot. Inclusion of topiramate (0.3–10 μ M) along with ATPA antagonized the increase in sIPSC frequency in a concentration-dependent manner. For each topiramate concentration change, at least 20 min was allowed to elapse before ATPA application. C, the Lucifer yellow fill of the pyramidal cell is shown. The bar graph presents the mean sIPSC frequencies in similar experiments with eight BLA pyramidal neurons. **, $p < 0.01$ with respect to control. D, the bar graph shows the mean \pm S.E.M. percentage of change in sIPSC frequency in eight similar experiments. *, $p < 0.05$; **, $p < 0.01$ with respect to control.

midal neurons; $p < 0.01$; Fig. 2). Topiramate (0.3–10 μ M), in a concentration-dependent manner, significantly reduced the ATPA-mediated increase in sIPSC frequency (from 895 ± 142 to $697 \pm 111\%$ for 300 nM topiramate, $466 \pm 82\%$ for 1 μ M topiramate, and $108 \pm 17\%$ for 10 μ M topiramate; eight pyramidal neurons; $p < 0.01$; Fig. 2). The reduction in sIPSP frequency persisted as long as topiramate was present in the perfusion solution (the longest duration tested was 40 min) and was fully reversed by perfusion with topiramate-free ACSF.

Topiramate Has No Effect on AMPA Receptor-Mediated BLA Neuron Excitation as Assessed by Recordings of Spontaneous Synaptic Currents. Having demonstrated that topiramate inhibits GluK1 kainate receptor-mediated effects, we sought to determine whether AMPA receptor-mediated responses are affected at the same drug concentrations. Recordings were carried out in the presence of D-APV (50 μ M), LY293558 (30 μ M), and SCH 50911 (10 μ M). Spontaneous synaptic currents in BLA pyramidal neurons were facilitated by depolarization of presynaptic neurons with the selective AMPA receptor agonist AMPA. During perfusion with AMPA (200 μ M), the frequency of spontaneous synaptic currents increased to $1012 \pm 138\%$ of preperfusion control levels (six pyramidal neuron recordings; $p < 0.01$; Fig. 3). Topiramate (10 μ M) failed to affect the AMPA receptor-mediated increase in the frequency of these current (Fig. 3). Thus, topiramate inhibits GluK1 kainate receptor-mediated but not AMPA receptor-mediated depolarization of BLA neurons. In addition, the absence of a significant effect of 10 μ M topiramate on the frequency of AMPA-induced synaptic potentials indicates that any effect of this concentration of topiramate on voltage-gated sodium or calcium channels is not sufficient to influence the excitability of BLA neurons.

Topiramate Blocks the GluK1 Kainate Receptor-Mediated Suppression of Evoked IPSCs. We have demonstrated previously that GluK1 kainate receptors are present on GABAergic terminals in the BLA and that activation of these receptors by high concentrations of glutamate (30–200 μ M) or ATPA (10 μ M) suppresses interneuron-to-

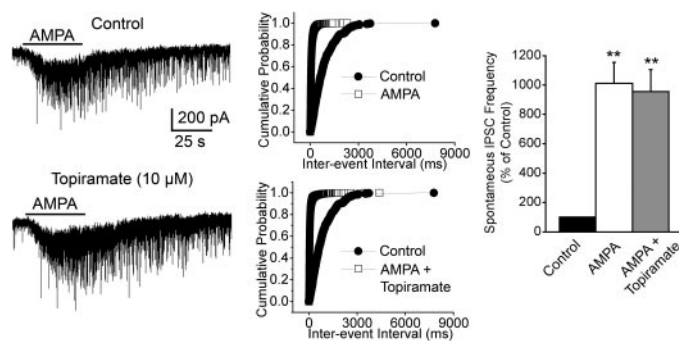


Fig. 3. Topiramate has no effect on AMPA receptor-mediated enhancement of the spontaneous activity of BLA neurons. AMPA receptors were activated by bath application of AMPA (200 μ M), and spontaneous postsynaptic currents were recorded from BLA pyramidal cells in the presence of D-APV (50 μ M), LY293558 (30 μ M), and SCH 50911 (10 μ M). Holding potential, -60 mV. In a pyramidal neuron, the current traces (left) and interevent interval cumulative probability plots (middle) show that AMPA significantly increased the frequency of spontaneous postsynaptic currents with respect to the preperfusion control frequency, and this effect was not prevented by 20-min exposure to 10 μ M topiramate in the perfusion solution. The bar graph shows the mean \pm S.E.M. percentage of change in sIPSC frequency in six similar experiments. **, $p < 0.01$.

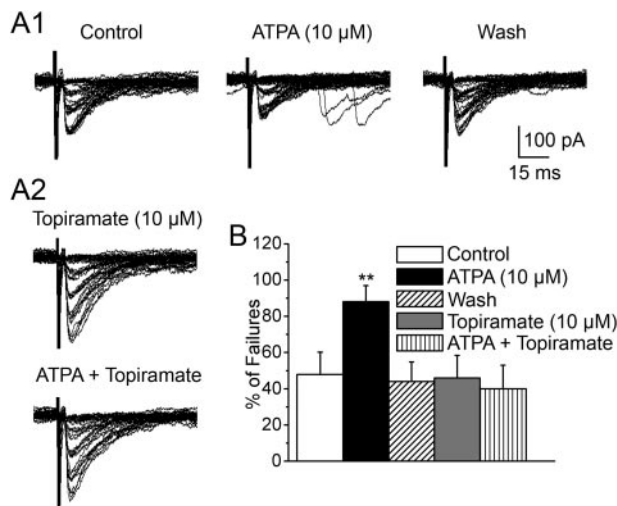


Fig. 4. Topiramate blocks the GluK1 kainate receptor-mediated suppression of evoked IPSCs recorded in BLA pyramidal neurons. A1, superimposed evoked IPSCs recorded in response to minimal electric stimulation of a presynaptic GABAergic axon in the presence of D-APV (50 μ M), GYKI 53655 (50 μ M), and SCH 50911 (10 μ M). Holding potential, -60 mV. Bath application of the GluK1 kainate receptor agonist ATPA (10 μ M) caused a reversible increase in failures and reduced the amplitude of the evoked IPSCs. A2, in the same recording, the effects of ATPA were prevented by coapplication of topiramate (10 μ M). Testing occurred 20 min after inclusion of topiramate in the perfusion solution. Note that topiramate increases the IPSC amplitude but did not increase the failure rate, indicating that the amplitude change does not influence the assessment of failure frequency. B, mean \pm S.E.M. percentage of failures for eight similar experiments. **, $p < 0.01$.

pyramidal neuron synaptic transmission (Braga et al., 2003). Here, we examined whether topiramate prevents the GluK1 kainate receptor-mediated suppression of IPSCs evoked by minimal stimulation of a GABAergic axon, in the presence of D-APV (50 μ M), GYKI 53655 (50 μ M), and SCH 50911 (10 μ M). ATPA (10 μ M in the perfusion solution) produced a marked increase in the rate of failures of synaptic transmission, from 48 ± 10.2 to $88 \pm 9\%$ (eight pyramidal neuron recordings; $p < 0.01$; Fig. 4), along with a reduction in the amplitude of the evoked IPSCs. Subsequent perfusion of the slices with ATPA-free ACSF completely reversed the effects of the agonist. Bath application of topiramate (10 μ M) increased the amplitude of the evoked IPSCs (to $172 \pm 7.6\%$ of control levels), with no significant effect on the failure rate of GABAergic synaptic transmission (Fig. 4). In the presence of topiramate (10 μ M), ATPA (10 μ M) no longer increased the

failure rate or reduced the IPSC amplitude. These results demonstrate that topiramate antagonizes the activation of presynaptic GluK1 kainate receptors that mediate inhibition of GABA release. In addition, the absence of an impact of topiramate on failures is further evidence that 10 μ M topiramate does not interfere with interneuron excitability by effects on voltage-gated sodium or calcium channels or other actions.

Topiramate Enhances GABAergic Transmission in BLA Interneuron-to-Pyramidal Cell Synapses by a Direct Effect on Postsynaptic GABA_A Receptors. Previous studies in cultured cortical and cerebellar neurons have suggested that topiramate enhances GABA_A receptor-mediated currents by a direct effect on GABA_A receptors (White et al., 1997, 2000). In our experiments, application of topiramate alone enhanced the amplitude of evoked IPSCs (Fig. 4). Therefore, we investigated further whether topiramate directly affects GABA_A receptor function.

First, we examined the effects of topiramate on single-fiber-evoked IPSCs recorded from BLA pyramidal neurons. D-APV (50 μ M), CNQX (20 μ M), and SCH 50911 (10 μ M) were present in the perfusion medium to block NMDA, AMPA/kainate, and GABA_B receptors, respectively. In control conditions, the mean peak current amplitude of evoked IPSCs was 464 ± 24 pA, the mean 10 to 90% rise time was 1.2 ± 0.3 ms, and the mean 90 to 10% decay time was 12.3 ± 1.5 ms (eight pyramidal neurons). Bath application of bicuculline (10 μ M) eliminated evoked IPSCs, confirming that they were mediated by GABA_A receptors (data not shown). As shown in Fig. 5, topiramate reversibly augmented the evoked IPSC amplitude in a concentration-dependent manner (to $133 \pm 7\%$ with 300 nM topiramate, $154 \pm 8\%$ with 1 μ M topiramate, and $182 \pm 9.2\%$ with 10 μ M topiramate; eight pyramidal neurons). Topiramate did not cause any significant change in the rise time or decay time constant of evoked IPSCs. In this or other experiments, topiramate did not affect the holding current, indicating that it does not directly activate GABA_A receptors. As shown in the time course of Fig. 5C, the time to achieve maximal augmentation was ~ 3 min. The action of topiramate on GABA responses was therefore more rapid than on GluK1 kainate receptors. Although the rates are based on room temperature recordings and do not necessarily reflect rates at body temperature, the relatively slower time course for blockade of GluK1 responses is compatible with an indirect action.

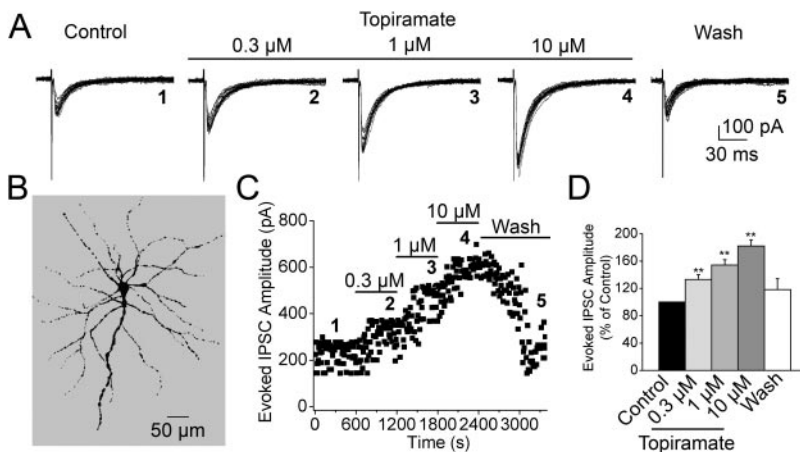


Fig. 5. Topiramate increases the amplitude of evoked IPSCs recorded from BLA pyramidal neurons. IPSCs were evoked by suprathreshold stimulation at a rate of 0.1 Hz in BLA pyramidal neurons in the presence of CNQX (20 μ M), D-APV (50 μ M), and SCH 50911 (10 μ M). Holding potential, -60 mV. A, inclusion of increasing concentrations of topiramate augmented the amplitude of the evoked IPSCs in a concentration-dependent and fully reversible manner. B, Lucifer yellow fill of the pyramidal neuron. C, individual IPSC amplitudes showing time course of the effects of topiramate. D, mean \pm S.E.M. percentage of change in IPSC amplitudes with respect to control in eight similar experiments with separate pyramidal neurons. **, $p < 0.01$.

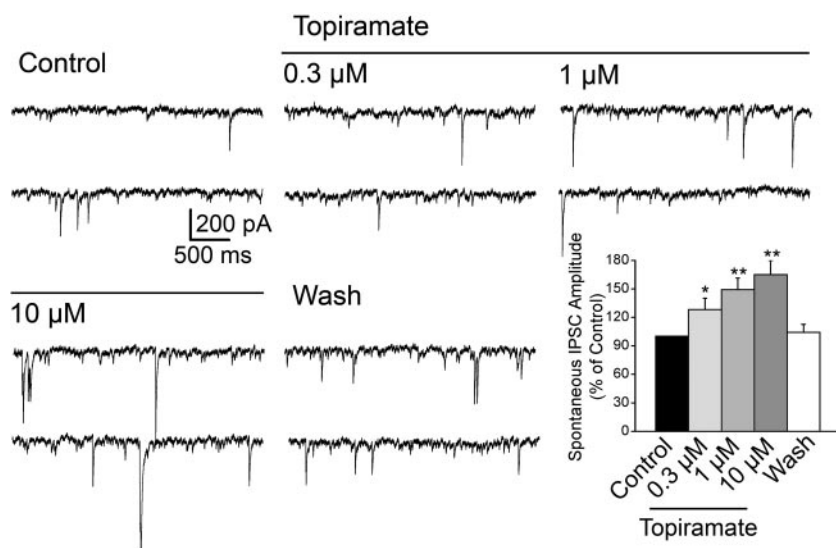


Fig. 6. Topiramate increases the amplitude of action potential-dependent sIPSCs recorded from BLA pyramidal cells. sIPSCs were recorded from BLA pyramidal neurons in the presence of CNQX (20 μ M), D-APV (50 μ M), and SCH 50911 (10 μ M). Holding potential, -60 mV. Topiramate (0.3–10 μ M) reversibly augmented the amplitude of the sIPSCs in a concentration-dependent manner. The traces shown were acquired after at least 20-min exposure to the indicated topiramate concentration. The bar graph shows the mean \pm S.E.M. percentage of change in sIPSC amplitude in nine similar experiments with separate pyramidal neurons. *, $p < 0.05$; **, $p < 0.01$.

Topiramate (0.3–10 μ M) also increased the amplitude of action potential-dependent sIPSCs recorded from BLA pyramidal neurons. In control conditions, the mean frequency of sIPSCs recorded in BLA pyramidal neuron somata was 2.9 ± 1.5 Hz, the mean peak current amplitude was 543 ± 194 pA, the mean 10 to 90% rise time was 3.2 ± 0.6 ms, and the mean 90 to 10% decay time was 28 ± 14.3 ms (nine pyramidal neurons). Topiramate reversibly augmented in a concentration-dependent manner the mean sIPSC amplitude (to $128 \pm 12\%$ by 300 nM topiramate, $149 \pm 12\%$ by 1 μ M topiramate, and $165 \pm 15\%$ by 10 μ M topiramate; nine pyramidal neurons; Fig. 6). The augmentation persisted throughout the application of topiramate and was completely reversed on washout of the drug. There was no significant change in the frequency, rise time, or decay time constant of sIPSCs.

Topiramate weakly inhibits carbonic anhydrase types II and IV (Dodgson et al., 2000), which would be expected to reduce bicarbonate. GABA_A receptors are permeable to bicarbonate, and activation of GABA_A receptors ordinarily causes an inward (depolarizing) bicarbonate current (Kaila, 1994). Therefore, inhibition of extracellular carbonic anhydrase would reduce extracellular bicarbonate, which would increase the bicarbonate gradient and increase the GABA_A receptor inward current and could be one of the mechanisms underlying the topiramate-induced enhancement of the amplitude of GABA_A receptor-mediated IPSCs (Herrero et al., 2002). To examine this possibility, we pretreated amygdala slices with acetazolamide (20 μ M), a carbonic anhydrase inhibitor, and investigated the effects of topiramate on evoked and sIPSCs under the same experimental conditions as described above. Acetazolamide by itself did not significantly affect the amplitude of evoked and sIPSPS. In addition, acetazolamide had no significant effect on the topiramate-induced increase in the amplitude of evoked and sIPSCs (six pyramidal neurons; data not shown).

The topiramate-induced enhancement in evoked and sIPSC amplitude could be due to a direct postsynaptic action of topiramate on GABA_A receptors or could be the result of an increase in GABA release. To distinguish these possibilities, we recorded mIPSCs in a medium containing the same antagonists as described above, with the additional presence of 1 μ M tetrodotoxin. A change in the amplitude of miniature

IPSCs without a change in their frequency would be indicative of a postsynaptic site of action of topiramate at GABA_A receptors. In control conditions, the mean frequency of mIPSCs recorded from the soma of BLA pyramidal neurons was 2.3 ± 0.6 Hz, the mean peak current amplitude was 42.4 ± 3.9 pA, the mean 10 to 90% rise time was 1.1 ± 0.4 ms, and the mean 90 to 10% decay time was 8.5 ± 0.7 ms (seven neurons). Bath application of bicuculline (10 μ M) eliminated mIPSCs, confirming that they are mediated by GABA_A receptors. Topiramate (0.3–10 μ M) augmented the mean mIPSC amplitude in a concentration-dependent manner (to $116 \pm 10\%$ of the control amplitude by 300 nM topiramate, $132 \pm 9\%$ by 1 μ M topiramate, and $142 \pm 12\%$ by 10 μ M topiramate; seven neurons; Fig. 7). The augmentation persisted throughout the application of topiramate and was completely reversed on washout of the drug. There was no significant change in the frequency, rise time, or decay time constant of mIPSCs.

The effects of topiramate on the amplitude of mIPSCs provide strong evidence that topiramate enhances inhibitory synaptic transmission by a direct action on postsynaptic GABA_A receptors. To provide further support for this conclusion, we examined the effects of topiramate (0.3–10 μ M) on the amplitude of muscimol-evoked postsynaptic currents recorded from BLA pyramidal cells. GABA_A receptor-mediated postsynaptic currents were evoked by micropressure application of muscimol (10 μ M) in the presence of CNQX (20 μ M), D-APV (50 μ M), and SCH 50911 (10 μ M). In control conditions, the mean peak amplitude of muscimol-evoked postsynaptic currents was 1702 ± 107 pA (seven neurons). Topiramate (0.3–10 μ M) caused a concentration-dependent increase in the mean peak amplitude of these currents (to $124 \pm 6\%$ of control levels by 300 nM topiramate, $144 \pm 9\%$ by 1 μ M topiramate, and $186 \pm 8\%$ by 10 μ M topiramate; seven neurons; Fig. 8) that persisted throughout the application of topiramate and was completely reversed after removal of the drug.

Discussion

The main observations of this study are as follows: 1) topiramate selectively inhibits the GluK1 kainate receptor-

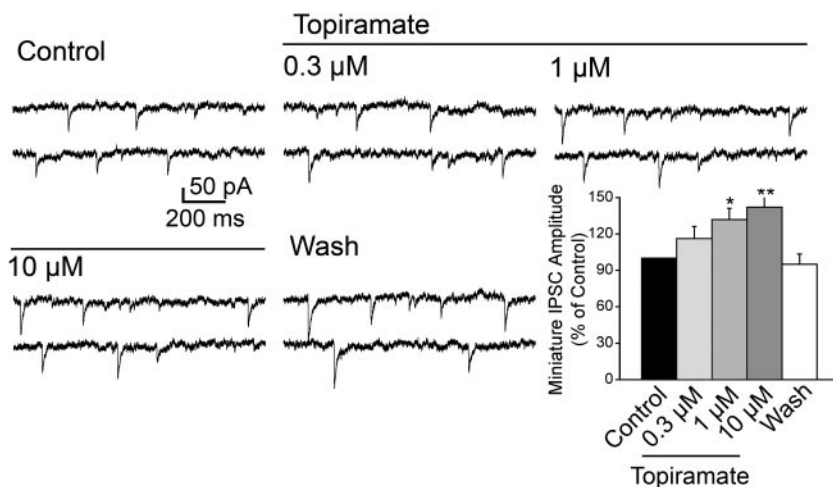


Fig. 7. Topiramate increases the amplitude of mIPSCs recorded from BLA pyramidal cells. mIPSCs were recorded from BLA pyramidal neurons in the presence of CNQX (20 μ M), D-APV (50 μ M), SCH 50911 (10 μ M), and tetrodotoxin (1 μ M). Holding potential, -60 mV. Topiramate (0.3–10 μ M) reversibly augmented the amplitude of mIPSCs in a concentration-dependent manner, indicating that topiramate acts directly on postsynaptic GABA_A receptors. The traces shown were acquired after at least 20-min exposure to the indicated topiramate concentration. The bar graph shows the mean \pm S.E.M. percentage of change in sIPSC amplitude in seven similar experiments with separate pyramidal neurons. *, $p < 0.05$; **, $p < 0.01$.

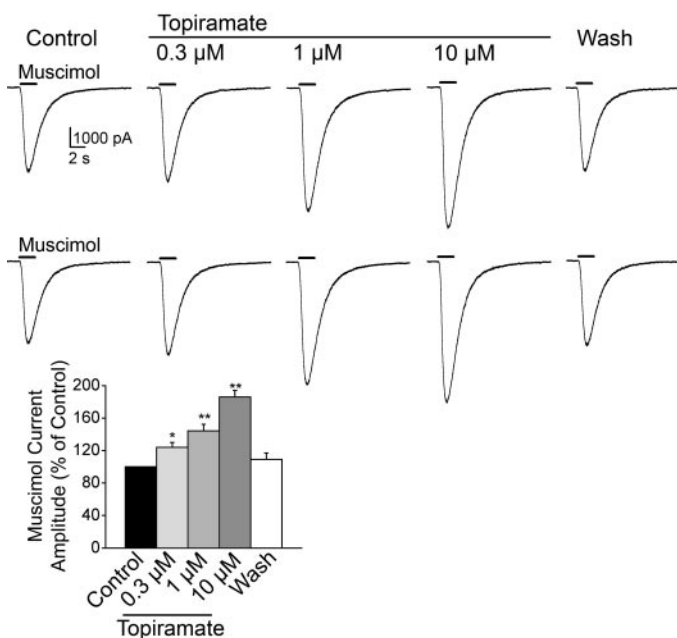


Fig. 8. Topiramate enhances the amplitude of muscimol-evoked postsynaptic currents recorded from BLA pyramidal cells. GABA_A receptor-mediated postsynaptic currents were evoked in BLA pyramidal neurons by microperfusion of muscimol (10 μ M) in the presence of CNQX (20 μ M), D-APV (50 μ M), and SCH 50911 (10 μ M). Holding potential, -60 mV. The short horizontal bars above the current traces denote the duration of the muscimol application. Topiramate (0.3–10 μ M) enhanced the amplitude of the muscimol-evoked currents in a reversible and concentration-dependent manner. The traces shown were acquired after at least 20-min exposure to the indicated topiramate concentration. The bar graph shows the mean \pm S.E.M. percentage of change in peak muscimol current amplitude in seven similar experiments with separate pyramidal neurons. *, $p < 0.05$; **, $p < 0.01$.

mediated depolarization of interneurons in the BLA, thereby reducing action potential-dependent GABA release; 2) topiramate blocks GluK1 kainate receptors present on the presynaptic terminals of BLA interneurons, thereby preventing the GluK1 kainate receptor-mediated suppression of GABA release; and 3) topiramate enhances GABA_A receptor-mediated currents by an effect on postsynaptic GABA_A receptors. All of these actions occur at the same clinically relevant range of concentrations so that they are likely to occur together. [Plasma topiramate concentrations in clinical trials have normally ranged from 2 to 5 μ g/ml (5.9–14.8 μ M),

although in some cases the C_{max} values have been greater (Perucca and Bialer, 1996; Twyman et al., 1999; May et al., 2002).] The net effect of these various actions is complex. However, when extracellular glutamate concentrations are high (as during epileptic seizures), activation of GluK1 kainate receptors on GABAergic terminals inhibits GABA-mediated neurotransmission, further promoting hyperexcitability (Braga et al., 2003). Therefore, the dominant effect of topiramate on interneurons would be to enhance their inhibitory output, because an effect on interneuron terminals would be expected to over-ride any somatodendritic actions. Taken together with the potentiation of postsynaptic GABA_A receptors, the overall effect of topiramate would be to reduce BLA circuit excitability.

Topiramate Selectively Inhibits Interneuron GluK1 Kainate Receptors. Topiramate inhibited inward currents evoked in BLA interneurons by the GluK1 kainate receptor agonist ATPA as well as sIPSCs recorded from pyramidal cells in response to depolarization of interneurons by bath application of ATPA. In addition, topiramate prevented the ATPA-induced suppression of electrical stimulation-evoked IPSCs, which we demonstrated previously is mediated by GluK1 kainate receptors on the presynaptic terminals of interneurons (Braga et al., 2003). Although ATPA is a highly potent and selective agonist at GluK1 kainate receptors, it is also a weak AMPA agonist (Clarke et al., 1997; Stensbøl et al., 1999). However, the selective AMPA receptor antagonist GYKI 53655 was included in the bathing solution so the effects of ATPA cannot be attributed to an interaction with AMPA receptors. Thus, various lines of converging evidence indicate that topiramate inhibits GluK1 kainate receptors on BLA interneurons, whether they are somatodendritic or on presynaptic terminals. It is noteworthy that these actions of topiramate cannot be attributed to effects of the drug on voltage-dependent ion channels, including sodium channels (Zona et al., 1997; Taverna et al., 1999) or high voltage-activated calcium channels (Zhang et al., 2000), or as an activator of potassium channels (Herrero et al., 2002) because topiramate inhibited ATPA-evoked currents, which are not dependent upon synaptic transmission or on the degree of membrane shunting (Fig. 1). In addition, as demonstrated in the experiment of Fig. 4, topiramate does not by itself influence the evoked synaptic release of GABA, although it prevents ATPA-induced inhibition of such release.

There is considerable previous evidence that non-NMDA (AMPA/kainate) receptors represent a target of topiramate. Thus, in cultured neurons, topiramate blocks currents evoked by kainate, an agonist of kainate and AMPA receptors (Gibbs et al., 2000; Skradski and White, 2000; Angehagen et al., 2004; Poulsen et al., 2004) or by AMPA alone (Poulsen et al., 2004). These studies indicated that topiramate can inhibit AMPA- and kainate receptor-mediated responses, but they did not address the relative selectivity of topiramate for the two non-NMDA receptor subfamilies. However, in BLA pyramidal neurons, we found that topiramate is a more potent and effective inhibitor of GluK1 kainate receptor-mediated responses than AMPA receptor responses (Gryder and Rogawski, 2003). Likewise, in the present study, at the concentrations tested, we failed to observe an effect of topiramate on AMPA responses (Fig. 3), indicating that topiramate selectively inhibits GluK1 kainate receptors. ATPA selectively activates homomeric and heteromeric GluK1-containing kainate receptors (Alt et al., 2004; Jane et al., 2009). However, it has no activity on homomeric GluK2 (GluR6) kainate receptors and is extremely weak on heteromeric GluK2K5 (GluR6/KA-2) kainate receptors. Although GluK2 subunits are expressed in the BLA (Li et al., 2001), the extent to which they form active receptors and participate in synaptic transmission is unknown because of the unavailability of appropriate pharmacological tools. For the same reason, we were not able to determine whether topiramate influences non-GluK1-containing kainate receptors.

The mechanism by which topiramate antagonizes the activation of GluK1 kainate receptors is unclear at present. The slow onset of the blocking effect is compatible with an indirect action. Indeed, it has been suggested that topiramate acts on protein kinases to modify the phosphorylation state of receptors, channels, or both (Shank et al., 2000). Angehagen et al. (2004) has provided evidence to support the hypothesis that topiramate binds to phosphorylation sites on AMPA/kainate receptors, but only in the dephosphorylated state, and thereby exerts a negative allosteric modulatory effect on channel activity.

Enhancement of GABA_A Receptor-Mediated Currents by Topiramate. We found that topiramate enhanced the amplitude of stimulation-evoked IPSCs, action potential-dependent sIPSCs, mIPSCs, and muscimol-evoked currents recorded from BLA pyramidal cells. Although topiramate has been shown previously to modulate responses to exogenous GABA (White et al., 2000; Simeone et al., 2006), this is the first demonstration that the drug enhances synaptic GABA-mediated inhibitory responses. The enhancement of evoked IPSCs and sIPSCs could be due to either a presynaptic effect of topiramate on GABA-release mechanisms or a postsynaptic effect on GABA_A receptors. However, a presynaptic action can be excluded because the frequencies of sIPSCs or mIPSCs were not affected by topiramate. Moreover, the finding that topiramate increases the amplitude of mIPSCs and muscimol-evoked currents demonstrates that topiramate acts postsynaptically. The more rapid onset of the topiramate potentiation of GABA responses than the block of GluK1 responses is compatible with the possibility that the effect on GABA responses occurs through a direct action on GABA_A receptors. The conclusion that topiramate acts postsynaptically as a positive modulator of GABA_A receptors is consis-

tent with previous studies in cultured cortical and cerebellar neurons that found that topiramate enhances at least some types of GABA_A receptor currents via an interaction with a site that is distinct from the GABA, benzodiazepine, or barbiturate recognition sites (White et al., 1997, 2000; Simeone et al., 2006). Our observation that topiramate enhances IPSC amplitude but has little effect on IPSC decay time course leads to two conclusions (Frerking et al., 1995). First, GABA synapses of BLA interneurons onto BLA principal neurons seem not to be saturated by transmitter. A similar situation applies for some but not all central GABA synapses (Liu, 2003). Second, even though topiramate has been shown previously not to act via the benzodiazepine recognition site (White et al., 2000), the effect of topiramate is functionally similar to that of benzodiazepines, which also potentiate the amplitude of mIPSCs at nonsaturated synapses without a major effect on the decay time course. The benzodiazepine response pattern is attributed to enhancement of the activation of GABA_A receptors by GABA with minimal effect on mean channel open time. This contrasts with barbiturate-like actions (prolongation of channel open time), in which IPSC decay is prolonged without a change in amplitude.

Although GABA responses of GABA_A receptors on BLA principal neurons were potentiated by topiramate, not all studies have found GABA_A receptor responses to be sensitive to the drug (Gordey et al., 2000; Shank et al., 2000). Indeed, Simeone et al. (2006) recently observed that topiramate modulation of GABA receptor currents is subunit-selective. Strong potentiation requires the $\beta 2$ or $\beta 3$ subunit, whereas $\beta 1$ -containing heteromeric receptors are only minimally affected by topiramate. These authors proposed that the variability seen in previous studies was likely to be explained by the different preparations studied expressing different subunits. It is interesting that, although all β -subunit mRNAs are expressed in the lateral amygdala, $\beta 1$ is of relatively lower abundance (Wisden et al., 1992) so that topiramate-sensitive isoforms are expected to be present.

Modulation of Amygdalar Excitability by Topiramate. The amygdala plays a central role in the pathology and symptomatology of temporal lobe epilepsy (Aroniadou-Anderjaska et al., 2008). The BLA, in particular, is the amygdalar nucleus that is primarily responsible for the generation and spread of limbic seizures. Activation of GluK1 kainate receptors, which are highly expressed in the BLA (Bettler et al., 1990; Li et al., 2001), can trigger epileptiform activity in the nucleus (Rogawski et al., 2003). In the BLA, GluK1 kainate receptors are present on somatodendritic sites of both principal neurons (Gryder and Rogawski, 2003) and interneurons, as well as on presynaptic terminals of GABAergic interneurons (Braga et al., 2003). Activation of principal neuron somatodendritic GluK1 kainate receptors would enhance amygdalar excitability by depolarizing these neurons, leading to glutamate release onto their postsynaptic targets. Activation of somatodendritic GluK1 kainate receptors on interneurons should suppress amygdalar excitability due to interneuronal depolarization and enhanced GABA release. However, presynaptic GluK1 kainate receptors on GABAergic terminals seem to modulate the output of interneurons. These GluK1 kainate receptors facilitate GABA release when activated by basal, low concentrations of glutamate, but they inhibit GABA release when activated intensely (Braga et al., 2003). As expected from the biphasic effects on synaptic function, exposure of amygdala slices to low

concentrations of ATPA ($\leq 1 \mu\text{M}$) (in the absence of pharmacological antagonists) causes a suppression of excitability, as revealed by field potential and intracellular recordings, whereas the net effect of strong activation of GluK1 kainate receptors ($10 \mu\text{M}$ ATPA) is an enhancement of neuronal excitability and the generation of epileptiform activity (Rogawski et al., 2003). Consistent with these in vitro results, systemic and intra-amygdala infusion of ATPA in vivo induces myoclonic and limbic seizure activity (Banks and Rogawski, 2002; Fritsch et al., 2006). It seems plausible that the ability of topiramate to inhibit the activation of GluK1 kainate receptors and to facilitate GABA_A receptor responses may contribute to its ability to protect against seizures, at least at the level of the BLA. Our results do not address whether the antiepileptic actions of topiramate in other brain regions are mediated by similar mechanisms. In this regard, GluK1 kainate receptors seem to play different roles in the hippocampus and elsewhere (Huettner, 2003; Lerma, 2003; Jane et al., 2009). Nevertheless, GluK1 kainate receptor antagonists prevented hippocampal seizures induced by pilocarpine or electrical stimulation, both in vitro and in vivo (Smolders et al., 2002). In addition, topiramate was found to selectively inhibit clonic seizures induced by intravenous infusion of ATPA, which should activate GluK1 kainate receptors (Kaminski et al., 2004; Fritsch et al., 2006). These studies suggest that the blockade of GluK1 kainate receptors by topiramate is not only relevant to the prevention of epileptic discharges in the amygdala but also may contribute to the overall antiepileptic efficacy of the drug.

References

- Alt A, Weiss B, Ogden AM, Knauss JL, Oler J, Ho K, Large TH, and Bleakman D (2004) Pharmacological characterization of glutamatergic agonists and antagonists at recombinant human homomeric and heteromeric kainate receptors in vitro. *Neuropharmacology* **46**:793–806.
- Angehagen M, Ben-Menachem E, Shank R, Rönnbäck L, and Hansson E (2004) Topiramate modulation of kainate-induced calcium currents is inversely related to channel phosphorylation level. *J Neurochem* **88**:320–325.
- Aroniadou-Anderjaska V, Fritsch B, Qashu F, and Braga MF (2008) Pathology and pathophysiology of the amygdala in epileptogenesis and epilepsy. *Epilepsy Res* **78**:102–116.
- Banks MK and Rogawski MA (2002) A role for GluR5 kainate receptors in the convulsant and epileptogenic effects of kainate. *Epilepsia* **43** (Suppl 7):8.
- Bettler B, Boulter J, Hermans-Borgmeyer I, O'Shea-Greenfield A, Deneris ES, Moll C, Borgmeyer U, Hollmann M, and Heinemann S (1990) Cloning of a novel glutamate receptor subunit, GluR5: expression in the nervous system during development. *Neuron* **5**:583–595.
- Braga MF, Aroniadou-Anderjaska V, Xie J, and Li H (2003) Bidirectional modulation of GABA release by presynaptic glutamate receptor 5 kainate receptors in the basolateral amygdala. *J Neurosci* **23**:442–452.
- Clarke VR, Ballyk BA, Hoo KH, Mandelzys A, Pellizzari A, Bath CP, Thomas J, Sharpe EF, Davies CH, Ornstein PL, et al. (1997) A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. *Nature* **389**:599–603.
- Collingridge GL, Olsen RW, Peters J, and Spedding M (2009) A nomenclature for ligand-gated ion channels. *Neuropharmacology* **56**:2–5.
- Dodgson SJ, Shank RP, and Maryanoff BE (2000) Topiramate as an inhibitor of carbonic anhydrase isoenzymes. *Epilepsia* **41** (Suppl 1):S35–S39.
- Fritsch B, Gasior M, Kaminski RM, and Rogawski MA (2006) Role of kainate receptor subunits in seizure induction as assessed with kainate receptor knockout mice. *Epilepsia* **47** (Suppl 4):305.
- Frerking M, Borges S, and Wilson M (1995) Variation in GABA mini amplitude is the consequence of variation in transmitter concentration. *Neuron* **15**:885–895.
- Gibbs JW III, Sombati S, DeLorenzo RJ, and Coulter DA (2000) Cellular actions of topiramate: blockade of kainate-evoked inward currents in cultured hippocampal neurons. *Epilepsia* **41** (Suppl 1):S10–S16.
- Goerdy M, DeLorey TM, and Olsen RW (2000) Differential sensitivity of recombinant GABA_A receptors expressed in *Xenopus* oocytes to modulation by topiramate. *Epilepsia* **41** (Suppl 1):S25–S29.
- Gryder DS and Rogawski MA (2003) Selective antagonism of GluR5 kainate-receptor-mediated synaptic currents by topiramate in rat basolateral amygdala neurons. *J Neurosci* **23**:7069–7074.
- Herrero AI, Del Olmo N, González-Escalada JR, and Solís JM (2002) Two new actions of topiramate: inhibition of depolarizing GABA_A-mediated responses and activation of a potassium conductance. *Neuropharmacology* **42**:210–220.
- Huettner JE (2003) Kainate receptors and synaptic transmission. *Prog Neurobiol* **70**:387–407.
- Jane DE, Lodge D, and Collingridge GL (2009) Kainate receptors: pharmacology, function and therapeutic potential. *Neuropharmacology* **56**:90–113.
- Kaila K (1994) Ionic basis of GABA_A receptor channel function in the nervous system. *Prog Neurobiol* **42**:489–537.
- Kaminski RM, Banerjee M, and Rogawski MA (2004) Topiramate selectively protects against seizures induced by ATPA, a GluR5 kainate receptor agonist. *Neuropharmacology* **46**:1097–1104.
- Lerma J (2003) Roles and rules of kainate receptors in synaptic transmission. *Nat Rev Neurosci* **4**:481–495.
- Li H, Chen A, Xing G, Wei ML, and Rogawski MA (2001) Kainate receptor-mediated heterosynaptic facilitation in the amygdala. *Nat Neurosci* **4**:612–620.
- Li H and Rogawski MA (1998) GluR5 kainate receptor mediated synaptic transmission in rat basolateral amygdala in vitro. *Neuropharmacology* **37**:1279–1286.
- Liu G (2003) Presynaptic control of quantal size: kinetic mechanisms and implications for synaptic transmission and plasticity. *Curr Opin Neurobiol* **13**:324–331.
- May TW, Rambeck B, and Jürgens U (2002) Serum concentrations of topiramate in patients with epilepsy: influence of dose, age, and comedication. *Ther Drug Monit* **24**:366–374.
- Perrais D, Pinheiro PS, Jane DE, and Mulle C (2009) Antagonism of recombinant and native GluK3-containing kainate receptors. *Neuropharmacology* **56**:131–140.
- Perucca E and Bialer M (1996) The clinical pharmacokinetics of the newer antiepileptic drugs. Focus on topiramate, zonisamide and tiagabine. *Clin Pharmacokinet* **31**:29–46.
- Poulsen CF, Simeone TA, Maar TE, Smith-Swintosky V, White HS, and Schousboe A (2004) Modulation by topiramate of AMPA and kainate mediated calcium influx in cultured cerebral cortical, hippocampal and cerebellar neurons. *Neurochem Res* **29**:275–282.
- Rainnie DG, Asprodini EK, and Shinnick-Gallagher P (1993) Intracellular recordings from morphologically identified neurons of the basolateral amygdala. *J Neurophysiol* **69**:1350–1362.
- Rogawski MA, Gryder D, Castaneda D, Yonekawa W, Banks MK, and Li H (2003) GluR5 kainate receptors, seizures, and the amygdala. *Ann N Y Acad Sci* **985**:150–162.
- Rogawski MA and Löscher W (2004) The neurobiology of antiepileptic drugs. *Nat Rev Neurosci* **5**:553–564.
- Sah P, Faber ES, Lopez De Armentia M, and Power J (2003) The amygdaloid complex: anatomy and physiology. *Physiol Rev* **83**:803–834.
- Shank RP, Gardocki JF, Streeter AJ, and Maryanoff BE (2000) An overview of the preclinical aspects of topiramate: pharmacology, pharmacokinetics, and mechanism of action. *Epilepsia* **41** (Suppl 1):S3–S9.
- Simeone TA, Wilcox KS, and White HS (2006) Subunit selectivity of topiramate modulation of heteromeric GABA_A receptors. *Neuropharmacology* **50**:845–857.
- Skradski S and White HS (2000) Topiramate blocks kainate-evoked cobalt influx into cultured neurons. *Epilepsia* **41** (Suppl 1):S45–S47.
- Smith L, Price-Jones M, Hughes K, Egebjerg J, Poulsen F, Wiberg FC, and Shank RP (2000) Effects of topiramate on kainate- and domoate-activated [¹⁴C]guanidinium ion flux through GluR6 channels in transfected BHK cells using Cytostar-T scintillating microplates. *Epilepsia* **41** (Suppl 1):S48–S51.
- Smolders I, Bortolotto ZA, Clarke VR, Warre R, Khan GM, O'Neill MJ, Ornstein PL, Bleakman D, Ogden A, Weiss B, et al. (2002) Antagonists of GLUK5-containing kainate receptors prevent pilocarpine-induced limbic seizures. *Nat Neurosci* **5**:796–804.
- Stensbøl TB, Borre L, Johansen TN, Egebjerg J, Madsen U, Ebert B, and Krosgaard-Larsen P (1999) Resolution, absolute stereochemistry and molecular pharmacology of the enantiomers of ATPA. *Eur J Pharmacol* **380**:153–162.
- Taverna S, Sancini G, Mantegazza M, Franceschetti S, and Avanzini G (1999) Inhibition of transient and persistent Na⁺ current fractions by the new anticonvulsant topiramate. *J Pharmacol Exp Ther* **288**:960–968.
- Twyman RE, Ben-Menachem E, Veloso F, Bombhof MAM, Wu SC, and Pledger G (1999) Plasma topiramate (TPM) concentration vs. therapeutic response during monotherapy. *Epilepsia* **40** (Suppl 7):111–112.
- Washburn MS and Moises HC (1992) Electrophysiological and morphological properties of rat basolateral amygdaloid neurons in vitro. *J Neurosci* **12**:4066–4079.
- White HS, Brown SD, Woodhead JH, Skeen GA, and Wolf HH (1997) Topiramate enhances GABA-mediated chloride flux and GABA-evoked chloride currents in murine brain neurons and increases seizure threshold. *Epilepsy Res* **28**:167–179.
- White HS, Brown SD, Woodhead JH, Skeen GA, and Wolf HH (2000) Topiramate modulates GABA-evoked currents in murine cortical neurons by a nonbenzodiazepine mechanism. *Epilepsia* **41** (Suppl 1):S17–S20.
- Wisden W, Laurie DJ, Monyer H, and Seeburg PH (1992) The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* **12**:1040–1062.
- Yonekawa WE, Li H, and Rogawski MA (2002) GluR5 kainate receptor-mediated epileptiform bursting in rat basolateral amygdala in vitro. *Epilepsia* **43** (Suppl 7):133.
- Zhang X, Velumina AA, Jones OT, and Carlen PL (2000) Modulation of high-voltage-activated calcium channels in dentate granule cells by topiramate. *Epilepsia* **41** (Suppl 1):S52–S60.
- Zona C, Ciotti MT, and Avoli M (1997) Topiramate attenuates voltage-gated sodium currents in rat cerebellar granule cells. *Neurosci Lett* **231**:123–126.

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