Topiramate does not Alter the Kinetics of Arachidonic or Docosahexaenoic Acid in Brain Phospholipids of the Unanesthetized Rat

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Interest in the potential therapeutic utility of topiramate for treating bipolar disorder was stimulated by published reports of investigator-initiated open label clinical studies. Because chronic lithium, carbamazepine and valproate decrease the turnover of arachidonic acid (AA) but not docosahexaenoic acid (DHA) in brain phospholipids of the awake rat, we tested if topiramate would produce similar results. Rats received either topiramate (20 mg/kg twice per day) or vehicle for 14 days and then while unanesthetized were infused intravenously with either [1-14C] AA or [1-14C] DHA for 5 min while blood was collected from the femoral artery at fixed times. Topiramate did not alter the incorporation rate of AA or DHA from their respective brain acyl-CoA pool into brain phospholipids, nor the turnover of AA and DHA in brain phospholipids. The results of our study indicate that topiramate does not possess a pharmacological property that three drugs with proven efficacy in treating bipolar disorder have in common.

KEY WORDS: Arachidonic acid; brain; bipolar disorder; mood stabilizer; anti-epileptic; topiramate; lithium; mania; turnover; metabolism; kinetics.

INTRODUCTION

Topiramate [2,3:4,5-bis-O-(1-methylethylidene-beta-D-fructopyranose sulfamate] was initially shown to be effective in controlling seizures (1–5), and is now considered a broad spectrum drug also useful for migraine (6). Based on several published reports of investigator-initiated open label clinical studies, interest developed in the potential utility of topiramate for treating bipolar disorder (7–11).

The anti-epileptic properties of topiramate have been ascribed to its ability to inhibit (z-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate receptor-mediated synaptic currents, and to increase GABA receptor-mediated synaptic currents (12–17). In animals, topiramate has been shown to be effective against GluR5 kainate receptor-induced seizures (compared to its effects against seizures produced by total kainate, AMPA and N-methyl-D-aspartic acid (NMDA)) (18). However, no topiramate studies have been

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Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; PC, choline glycerophospholipid; PS, phosphatidylserine; PI, phosphatidylinositol; PE, ethanolamine glycerophospholipid; FAME, fatty acid methyl ester; sn, stereospecifically numbered.
reported relevant to pharmacological properties associated with efficacy in the treatment of bipolar disorder.

A potential target of drugs used to treat bipolar disorder is the brain arachidonic acid (20:4n-6, AA) cascade (19). AA is a polyunsaturated fatty acid (PUFA) that is predominately esterified in the stereospecifically numbered (sn)-2 position of brain phospholipids. Multiple neuroreceptor-mediated processes cleave AA from phospholipids via the activation of the Ca\(^{2+}\)-dependent cytosolic phospholipase A\(_2\) (cPLA\(_2\)) (20). Once cleaved, AA has several metabolic fates, including oxidation to eicosanoids (catalyzed by cyclooxygenase), \(\beta\)-oxidation or re-esterification into the (sn)-2 position of lyso-phospholipids (21–23). Lithium and carbamazepine are used to treat bipolar disorder and both decrease the turnover of AA in brain phospholipids of the awake rat (24), without decreasing the turnover of docosahexaenoic acid (22:6n-3, DHA) (24–27). The decreased AA turnover was ascribed to lithium and carbamazepine’s demonstrated ability to decrease brain cPLA\(_2\) activity, protein levels and transcription (28–30). Lithium and carbamazepine also decreased rat brain PGE\(_2\) levels (28,31). Valproic acid, used to treat bipolar disorder, also decreased turnover of AA but not DHA in brain phospholipids of the awake rat (24), independently of changes in cPLA\(_2\) (26,32,33), and its mechanism for this is not clear. Similar to valproate, topiramate does not alter the activity, expression or transcription of cPLA\(_2\) (34), but whether it affects the turnover of AA by an unknown mechanism as did valproate has not been reported. Thus far, the actions of lithium, valproate and carbamazepine suggest that the turnover (release and/or recycling) of AA in brain phospholipids is a target of drugs effective in bipolar disorder, particularly in the manic phase.

Because of interest in the possible utility of topiramate for treating bipolar disorder, we hypothesized that topiramate would decrease the turnover of AA but not of DHA in brain phospholipids when administered chronically to rats. Therefore, in order to gain insight on whether topiramate shares a common mechanism of action of drugs known to be effective in bipolar disorder, we administered topiramate 20 mg/kg p.o to rats, every 12 h for 2 weeks (40 mg/kg daily dose). This dosing regime produces plasma concentrations of 18 ± 4.7 \(\mu\)M, 5 h after the last oral dose (therapeutic range 3–45 \(\mu\)M), as well this dose decreases body weight and serum leptin levels (34) as previously reported (35). Topiramate was administered for 2 weeks based on evidence that 11 days of topiramate administration has been reported, like chronic carbamazepine and valproic acid, to attenuate quinpirole-induced hyperactivity in rats (36). We then applied our \textit{in vivo} fatty acid model in awake rats in order to measure the turnover of AA and DHA in brain phospholipids (21,37). During the course of our study, preliminary reports of the results of four phase III clinical trials appeared in the literature, the results of which indicate that topiramate is not an effective treatment for acute mania (38–41).

**METHODS AND MATERIALS**

**Chemicals.** Topiramate (2,3;4;5-bis-O-(1-methylethylidene-beta-D-fructopyranose sulfamate) was a gift from J & J Pharmaceutical Research & Development, L.L.C., Raritan, NJ. [1\(^{14}\)C]AA and DHA (50 mCi/mmol and 56 mCi/mmol, respectively >98% pure) were purchased from Moravek Biochemicals (Brea, CA). Scintillation counting and HPLC confirmed tracer-specific radioactivity. Phospholipid and neutral lipid standards were purchased from Nu-Chek-Prep (Elysian, MN). HPLC grade \(n\)-hexane and 2-propanol were from EM Science (Gibbstown, NJ). Reagent grade chloroform and methanol were from Mallinckrodt (Paris, KY). A scintillation cocktail (Ready-Safe, Beckman, Fullerton, CA) containing 1.0% glacial acetic acid was used to determine radioactivity.

**Animals.** The study was conducted according to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Publication No. 80–23), and was approved by the National Institutes of Child Health and Development Animal Care and Use Committee. Male CDF-344 rats, weighing 180–190 g (Charles River; Wilmington, MA, USA) were used. They were acclimatized for 1 week in an animal facility in which temperature, humidity and light cycle were controlled, and had \textit{ad libitum} access to food and water. After this, the rats were randomized to either the topiramate or control groups. Topiramate-treated rats received 20 mg/kg topiramate in 0.5% hydroxymethylpropylcellulose (adjusted to pH 7.5 with sodium hydroxide) by gavage every 12 h for 14 days (40 mg/kg/day). This dosing regime produces plasma concentrations of 18 ± 4.7 \(\mu\)M, 5 h after the last oral dose (therapeutic range 3–45 \(\mu\)M), as well this dose decreases body weight and serum leptin levels (34) as previously reported (35). We administered topiramate for 2 weeks based on evidence that 11 days of topiramate administration has been reported, like chronic carbamazepine and valproic acid, to attenuate quinpirole-induced hyperactivity in rats (36). A control group received the same volume of 0.5% hydroxymethylpropylcellulose (adjusted to pH 7.5) by the same route every 12 h, in parallel. On the morning of day 15, rats were gavaged with their appropriate treatment, then anesthetized with 1–3% halothane, and polyethylene catheters were inserted into a femoral artery and vein, as described previously (24). The rats were allowed to recover from surgery for 3 h, with their