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Chronic carbamazepine administration reduces NMDA receptor-initiated signaling *via* arachidonic acid in rat brain

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Abstract

Background—Lithium and carbamazepine (CBZ) are used to treat mania in bipolar disorder. When given chronically to rats, both agents reduce brain arachidonic acid (AA) turnover in brain phospholipids and downstream AA metabolism. Lithium administration to rats also attenuates N-methyl-D-aspartic acid receptor (NMDAR) signaling *via* AA.

Hypothesis—Chronic CBZ administration to rats, like chronic lithium, will reduce NMDAR-mediated signaling *via* AA.

Methods—We used our fatty acid method with quantitative autoradiography to image the regional brain incorporation coefficient k* of AA, a marker of AA signaling, in unanesthetized rats that had been given 25 mg/kg/day i.p. CBZ or vehicle for 30 days, then injected with NMDA (25 mg/kg i.p.) or saline. We also measured brain concentration of two AA metabolites, prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2).

Results—In chronic vehicle-treated rats, NMDA compared with saline increased k* significantly in 69 of 82 brain regions examined, but did not change k* significantly in any region in the CBZ-treated rats. In vehicle- but not CBZ-treated rats, NMDA also increased brain concentration of PGE_2 and TXB_2.

Conclusions—Chronic CBZ administration to rats blocks the brain NMDAR-mediated AA signal k* and the increments in PGE_2 and TXB_2 that are seen in vehicle-treated rats. The clinical action of antimanic drugs may involve inhibition of brain NMDAR-mediated signaling involving AA and its metabolites.

Keywords

carbamazepine; NMDA; arachidonic acid; bipolar disorder; phospholipase A_2; prostaglandin E_2

Introduction

Glutamatergic neurotransmission is thought to play a role in the pathophysiology and treatment of bipolar disorder (BD) (1,2). An elevated brain glutamate/glutamine ratio has been reported using magnetic resonance spectroscopy in children and adults with BD (3,4), and gene variants of the NR1 and NR2 subunits of the N-methyl-D-aspartate receptor (NMDAR) have been linked to the disease (5–7). Furthermore, NMDAR density and levels of NR1, NR2A and NR3A

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mRNA were decreased in post-mortem BD brain, as were densities of the NMDAR-associated post-synaptic proteins, PSD-95 and SAP102 (8,9). It has been shown that NMDAR stimulation by glutamate or NMDA decreases NR-1 expression (10), and that the NR3A subunit co-assembles with other subunits (NR1, NR2A or NR2B) to form NMDAR with decreased activity and calcium influx (11,12). Decreased NR1 and NR3A expression observed in BD is consistent with upregulated NMDAR function, and with the observation that mice lacking the NR-3A subunit have increased brain NMDAR activity (13).

Binding of glutamate or NMDA to NMDAR will allow extracellular Ca^{2+} into the cell, thereby activating a number of Ca^{2+}-dependent enzymes. One of these, cytosolic phospholipase A_2 ($cPLA_2$), selectively releases arachidonic acid (AA, 20:4*n*-6) from synaptic membrane phospholipid, to initiate the AA signaling cascade (14–18). A fraction of the released AA may be converted by cyclooxygenase (COX), lipoxygenase and cytochrome P-450 enzymes to eicosanoids, or lost *via* other metabolic pathways, whereas the remainder will be reincorporated into brain phospholipid (19–21). Markers of the AA cascade have been reported to be abnormal in BD (22–24).

Three chemically disparate agents, carbamazepine (CBZ), lithium and valproic acid, have proven effective against acute mania and mixed episodes in BD (25–28). When administered chronically to rats to produce clinically relevant plasma concentrations, each was reported to downregulate the brain AA cascade -- AA turnover in brain phospholipids, $cPLA_2$ or acyl-CoA synthetase activity as well as COX activity, and the concentration of prostaglandin E_2 (PGE_2), an AA metabolite produced preferentially *via* COX-2 (18,29–37).

In addition, chronic administration of lithium to rats and pretreatment with the NMDAR antagonist MK-801 reduced the brain AA signal following acute NMDA administration (38). This signal was measured using quantitative autoradiography as a regional AA incorporation coefficient k* (brain radioactivity/integrated plasma radioactivity) following the intravenous injection of radiolabeled AA. It represents AA lost by metabolism following its release from synaptic membrane phospholipid by $cPLA_2$ activation, due to Ca^{2+} entry into the cell at NMDARs (17,20,38). Suppression by chronic lithium of the NMDA-initiated AA signal suggested that the lithium acts in BD by reducing this signal, consistent with evidence for upregulated glutamatergic transmission in the disease (1,2).

In the present study we hypothesized that effective mood stabilizers might generally interfere with NMDA-initiated signaling *via* AA, and tested this hypothesis by measuring effects of chronic administration of CBZ to rats on the AA signal, and on the concentrations of AA metabolites, PGE_2 and thromboxane B_2 (TXB_2). In this regard, CBZ has been reported to block NMDA-induced currents in cultured spinal cord neurons (39), to inhibit NMDA-induced depolarization in cortical wedges (40), to prevent NMDA-initiated convulsions in mice (41), inhibit NMDA-evoked Ca^{2+} influx in cultured neurons (42,43), and protect against NMDA-mediated neurotoxicity (44,45).

In the present study, we measured regional k* responses and brain concentrations of PGE_2 and TXB_2 after giving 25 mg/kg i.p. NMDA or saline to unanesthetized rats that were injected daily for 30 days with 25 mg/k.g. i.p. CBZ or vehicle. This NMDA dose does not produce convulsions, but causes widespread increments in k* that can be prevented by chronic LiCl feeding or pretreatment with MK-801 (38). The CBZ regimen, which we have used before, produces a mean plasma concentration of 54 μM, in the high end of concentrations reported in CBZ-treated BD patients (51 μM) (31,34,46,47).

Methods and Materials

Animals and Diets

The National Institutes of Health (NIH) Animal Care and Use Committee approved the study in accordance with NIH guidelines on the care and use of laboratory animals. Two-month-old male Fischer CDF (F-344)/CrlBR rats (Charles River Laboratories, Wilmington, MA, USA) were acclimatized for 1 week in an animal facility with regulated temperature, humidity and light cycle, and had *ad libitum* access to food (NIH-31 diet, Zeigler, Gardners, PA, USA) and water. The diet contained (as percent of total fatty acids) 20.1% saturated, 22.5% monounsaturated, 47.9% linoleic, 5.1% linolenic, 0.02% AA, 2.0% eicosapentaenoic, and 2.3% docosahexaenoic acid.

Drugs

[1-^{14}C]AA in ethanol (53 mCi/mmol, >98% pure, Moravek Biochemicals, Brea, CA, USA) was evaporated and resuspended in HEPES buffer, pH 7.4, containing 50 mg/ml fatty acid-free bovine serum albumin (Sigma-Aldrich, St Louis, MO, USA). CBZ-treated rats received 25 mg/kg intraperitoneally once daily for 30 days (Sigma-Aldrich). The CBZ was dissolved in a 50:50 (v/v) dimethyl sulfoxide (DMSO, ≥99.9% Sigma-Aldrich): saline (0.9% NaCl) mixture and kept at 37°C (31,34). A control group received the same volume of DMSO: saline (vehicle) under parallel conditions.

Surgical Procedures and Tracer Infusion

On the morning following the 30th CBZ or vehicle injection, a rat was anesthetized with 2–3% halothane in O_2, and PE 50 polyethylene catheters were inserted into the right femoral artery and vein as described previously (38). The wound was closed with surgical clips and the hindquarters of the rat were wrapped loosely, with its upper body remaining free, in a fast-setting plaster cast taped to a wooden block. The rat was allowed to recover from anesthesia for 3–4 hours in an environment maintained at 25°C. Body temperature was kept at 36.4–37.1° C using a feedback-heating device and rectal thermometer. Arterial blood pressure and heart rate were measured with a blood pressure recorder (CyQ 103/302; Cybersense, Inc., Nicholasville, KY, USA). Arterial blood pH, pO_2 and pCO_2 were measured with a blood gas analyzer (Model 248, Bayer Health Care, Norwood, MA, USA).

Ten min after injecting NMDA or saline (about 4 hours after the last of the chronic CBZ or saline dose), 2 ml [1-^{14}C]AA (170 μCi/kg) was infused into the femoral vein for 5 min at a rate of 400 μl/min using an infusion pump (Harvard Apparatus Model 22, Natick, MA, USA). Twenty min after beginning infusion, the rat was euthanized with an overdose of Nembutal® (100 mg/kg, intravenously) and decapitated. The brain was rapidly removed, frozen in 2-methylbutane maintained at −40°C with dry ice, and stored at −80°C until sectioned.

Chemical Analysis

Thirteen arterial blood samples collected before, during and after [1-^{14}C]AA infusion were centrifuged immediately (30 s at 18,000 g). Total lipids were extracted from 30 μl of plasma with 3 ml chloroform:methanol (2:1, by vol) and 1.5 ml 0.1 M KCl (48). As reported, following [^{14}C]AA infusion, greater than 97% of plasma radioactivity was radiolabeled AA at 5 min (49). Concentrations of unesterified fatty acids were determined in 100–150 μl of frozen arterial plasma. Total lipids were extracted (48) and separated by thin layer chromatography on silica gel 60 plates (Whatman, Clifton, NJ, USA) using the solvent system heptane:diethylether:glacial acetic acid (60:40:3, by vol). Unesterified fatty acids were scraped from the plate and methylated with 1% H_2SO_4 in anhydrous methanol for 3 hours at 70°C

(50). Fatty acid methyl esters were separated and quantified by gas chromatography using heptadecanoic acid (17:0) as an internal standard (31).

Quantitative Autoradiography

Frozen brains were cut in serial 20-μm thick coronal sections in a cryostat at −20°C. The sections were placed for 5 weeks with calibrated [^{14}C]methylmethacrylate standards on Kodak Ektascan C/RA film (Eastman Kodak Company, Rochester, NY, USA). Eighty-two brain regions from autoradiographs were identified from a stereotaxic brain atlas (51), and were sampled in both hemispheres. The average of bilateral measurements for each region from three consecutive brain sections was used to calculate regional radioactivity (nCi/g of brain) by digital quantitative densitometry, using a Macintosh computer and the public domain NIH Image program 1.62 (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Regional incorporation coefficients k* (ml plasma/sec/g brain) of AA were calculated as (21),

$$k^* = \frac{c^*_{brain}(20\text{min})}{\int_0^{20} c^*_{plasma}\,dt} \quad \text{(Eq. 1)}$$

c*$_{plasma}$ equals plasma radioactivity determined by scintillation counting (nCi/ml), c*$_{brain}$(20 min) equals brain radioactivity (nCi/g of brain) at 20 min after staring infusion, and t equals time (min) after beginning [1-^{14}C]AA infusion. Rates of incorporation of unesterified AA from plasma into brain, J_{in} (nmol/sec/g), were calculated as,

$$J_{in} = k^* c_{plasma} \quad \text{(Eq. 2)}$$

where c_{plasma} is the plasma concentration of unlabeled unesterified AA (nmol/ml).

Brain prostaglandin E_2 and thromboxane B_2 concentrations

In separate experiments, vehicle- and CBZ-treated rats were given i.p. NMDA (25 mg/kg) or saline. Ten min later, they were anesthetized with Nembutal® (100 mg/kg, i.p.) and subjected to head-focused microwave irradiation (5.5 kW, 3.4 s then 1.4 s; Cober Electronics, Stamford, CT, USA) to stop post-mortem changes, such as release of fatty acids and formation of prostaglandins (52). Extraction was performed by Radin's method (53). Briefly, half-brains were weighed, then extracted in 18 volumes of hexane:isopropanol (3:2, by vol) using a glass Tenbroeck homogenizer. The extract was concentrated to dryness under nitrogen and resuspended in an enzyme immunoassay buffer provided with a polyclonal PGE_2 or TXB_2 assay kit (Oxford Biochemical Research, Oxford, MI, USA).

Statistical Analyses

An unpaired two-tailed *t*-test was used to compare mean physiological parameters in CBZ- and vehicle-treated rats, using GraphPad Prism version 4.0b (GraphPad Software, San Diego, CA, www.graphpad.com). A standard two-way ANOVA, comparing CBZ administration (CBZ vs. vehicle) with drug (NMDA vs. saline) was performed with regard to arterial plasma radioactivity, plasma unesterified fatty acid concentrations, brain PGE_2 and TXB_2 concentrations, and regional values of k* using SPSS 11.0 (SPSS Inc., Chicago, IL, USA, http://www.spss.com). Where interactions between CBZ and NMDA were statistically significant, probabilities of main effects of CBZ and NMDA were not reported as they cannot be interpreted (54,55). Instead, unpaired two-tailed *t*-tests were used to compare NMDA and saline responses between vehicle and CBZ-treated rats, as well as saline responses in CBZ-compared with vehicle-treated rats. Other comparisons were not considered relevant. A *post-*

hoc test was not used to avoid a correction for multiple comparisons. However, when a Bonferroni's *post-hoc* test with correction for three comparisons was performed, statistical significances of differences were not changed. Data are reported as means ± SD, with statistical significance taken as $p \leq 0.05$.

Results

Physiology, behavior and arterial plasma radioactivity

After 30 days of treatment, body weight in the chronic CBZ- and vehicle-treated rats did not differ significantly [276.4 ± 14.6 (n = 15) vs. 276.1 ± 16.1 (n = 16)]. Compared with acute saline, acute NMDA did not significantly affect arterial pH, pCO_2, pO_2 or blood pressure, but significantly decreased heart rate by 20–29% in both groups (Table 1), as reported (38). NMDA produced repeated cycles of head weaving and body movements following by a resting period. Cycling continued and then ceased after 90–110 sec (Table 1). The mean cycling period did not differ between CBZ- and vehicle-treated rats.

Neither CBZ nor NMDA modified the time course of arterial plasma radioactivity, the input function in Eq. 1, following [1-^{14}C]AA infusion. Mean integrated radioactivity in the plasma organic fraction, in units of (nCi x sec)/ml (n = 7–8), did not differ significantly between groups: vehicle plus saline, 183981 ± 38623; vehicle plus NMDA, 190501 ± 38459; CBZ plus saline, 194895 ± 24409; CBZ plus NMDA, 202102 ± 17701.

Plasma unesterified fatty acid concentrations

A two-way ANOVA did not show a significant interaction between CBZ and NMDA in any measured plasma unesterified fatty acid concentration (Table 2). CBZ compared with vehicle had no significant main effect on any concentration, whereas NMDA compared with saline significantly increased concentrations of unlabeled unesterified palmitic, palmitoleic, stearic, oleic, linoleic, α-linolenic, AA and docosahexaenoic acid in both vehicle- and CBZ-treated rats (Table 2).

Regional brain AA incorporation coefficients, k*

Effects of NMDA in vehicle-treated rats—Mean AA incorporation coefficients, k*, in each of 82 brain regions (Eq. 1), were subjected to a two-way ANOVA (Table 3, Fig. 1). Interactions between CBZ and NMDA were statistically significant in 68 of the 82 regions. In these 68 regions, *t*-tests showed that NMDA compared with saline significantly increased mean k* by 29–122% in the chronic vehicle-treated rats. Affected were prefrontal, frontal, anterior cingulate, motor, somatosensory, auditory and visual cortical areas (33–122%), preoptic area (44%), globus pallidus (60%), olfactory tubercle (57%), diagonal band (52–72%), amygdala (41%), hippocampus (29–87%), nucleus accumbens (83%), caudate-putamen (58–88%), septal nuclei (62–64%), habenular nuclei (67–68%), lateral geniculate nucleus dorsal (56%), medial geniculate nucleus (67%), regions of the thalamus (46–104%), areas in the hypothalamus (46–86%), interpeduncular nucleus (35%), substantia nigra (77%), superior and inferior colliculi (46–79%), and cerebellar gray matter (32%). Where CBZ x NMDA interactions were statistically insignificant, NMDA increased k* significantly in the pyriform cortex (33%). Thus, NMDA increased k* significantly in 69 (68 + 1) of the 82 brain regions.

Effects of chronic CBZ—In the 68 regions in which CBZ x NMDA interactions were statistically significant, *t*-tests showed that chronic CBZ vs. vehicle did not significantly change mean baseline (response to acute saline) k* in any region. In the 14 regions with statistically insignificant CBZ x NMDA interactions, CBZ did not have any main effect (Table 3). NMDA compared with saline did not change k* significantly in any of the 68 regions in which CBZ x NMDA interactions were statistically significant, nor did it affect k* in the pyriform cortex,

where the CBZ x NMDA interaction was insignificant. Thus, chronic CBZ blocked each of the 69 NMDA-induced k* increments that were seen in the chronic vehicle rats.

Regional rates of incorporation of unlabeled unesterified plasma AA

Rates of incorporation of unlabeled unesterified AA from plasma into brain, J_{in}, for AA (Table were calculated by Eq. 2 using regional values of k* (Table 3) and c_{plasma} did not differ significantly 2) (results not shown). Because baseline values of c_{plasma}between chronic CBZ- and vehicle-treated rats (Table 2), the baseline relations of regional *Jin* corresponded to respective relations in regional values of k* between the two groups. In vehicle-treated rats, baseline *Jin* ranged from 3.3 fmol/sec/g in the periventricular area of the hypothalamus to 28.9 fmol/sec/g in the choroid plexus. by 2.1–2.4 fold (Table 2), a steady-However, because NMDA acutely increased c_{plasma} state plasma (and likely brain) AA concentration was unavailable to calculate J_{in} in response to NMDA, and thus we did not calculate or compare J_{in} responses to acute NMDA and saline.

Brain PGE_2 and TXB_2 concentrations

A two-way ANOVA demonstrated significant interactions between CBZ and NMDA for brain PGE_2 ($p = 0.031$) and TXB_2 ($p = 0.014$) concentrations. Consequent *t*-tests showed that chronic CBZ decreased both basal concentrations, PGE_2 by 45% ($p = 0.023$) and TXB_2 by 51% ($p = 0.0014$) (Table 4). NMDA administration to chronic saline-treated rats significantly increased concentrations of PGE_2 ($p = 0.009$) and TXB_2 ($p = 0.0009$) by 1.8 and 2.2 fold, respectively, whereas NMDA had no significant effect in the chronic CBZ-treated rats.

Discussion

In this study, acute NMDA in chronic vehicle-treated unanesthetized adult rats increased k* for AA, a marker of $cPLA_2$ activation and AA release from phospholipids, in widespread brain regions, and also increased brain concentrations of the AA metabolites, PGE_2 and TXB_2. Chronic CBZ blocked the NMDA-induced increments of k* as well as of PGE_2 and TXB_2. As chronic lithium also blocks NMDA-induced increments in k* for AA in unanesthetized rats (38), taken together the results suggest that effective antibipolar agents generally block NMDA-mediated signaling *via* AA. Supporting this interpretation, valproic acid, another effective agent, has been reported to interfere with NMDA neurotransmission (56,57), but it remains to be seen whether chronic valproate also blocks the NMDA-initiated AA signal in unanesthetized rats and, furthermore, whether abnormal NMDAR-mediated signaling, suggested to exist in BD (see Introduction), might be modified in humans by the agents (58).

Mechanisms underlying attenuation of NMDA-induced increments in k* by chronic CBZ or lithium are uncertain. Inhibition of AA cascade enzymes (e.g., $cPLA_2$ and COX), as well as direct interference with the NMDAR, may be involved. Both chronic lithium and CBZ reduce rat brain $cPLA_2$ activity, protein and mRNA levels, without changing activities of calcium-independent $iPLA_2$ or secretory $sPLA_2$ (33,34,59). $cPLA_2$ downregulation by both drugs is accompanied by reduced expression and DNA-binding of activator protein 2 (AP-2), a $cPLA_2$ transcription factor (47,60). Both drugs also reduce the brain basal (this paper and (34)) PGE_2 concentration, and in this paper we show that CBZ also reduces brain basal TXB_2 concentration and increments in PGE_2 and TXB_2 concentrations in response to acute NMDA. PGE_2 and TXB_2 are AA metabolites produced preferentially *via* COX-2 and COX-1, respectively, and their reductions agree with evidence of a 40% reduction in net brain COX activity caused by chronic CBZ (34,61–65).

Reduced k* responses to NMDA also might arise from chronic CBZ's effect on the NMDAR receptor itself, causing reduced entry of Ca^{2+} into the cell and reduced activation of $cPLA_2$

and other Ca^{2+}-dependent intracellular enzymes. Indeed, CBZ has been reported to interfere with multiple NMDAR signaling processes in *in vitro* studies (see Introduction) (39–43,66). Additionally, chronic CBZ is reported to inhibit brain cyclic AMP-dependent protein kinase A, which phosphorylates the NMDAR (47,67). NMDAR activation can increase acetylation of histones H3 and H2A.X *via* the PKC/ERK signaling cascade, and CBZ can inhibit histone deacetylases. Thus, CBZ may affect histones as well as NMDAR transcription factors that are regulated by acetylation, such as specificity protein-1 (SP-1) (68–71). CBZ's blocking of NMDAR-mediated intracellular events is consistent with evidence that it has neuroprotective properties (44,72–74). Neuroprotection may involve pro-apoptotic proteins p53, cytoprotective protein Bcl-2, and cell survival protein kinase Akt, and would be interesting to know the effects of CBZ on these proteins.

The ability of chronic CBZ (this paper) and of chronic lithium (38) to attenuate k* responses to AA in unanesthetized rats is consistent with proposals that inhibition of glutamatergic neurotransmission would have an antimanic effect in BD (1,2,75). In this regard, chronic lithium and valproic acid administration to rats reduced expression of the glutamatergic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit, GluR1, an effect that been associated with reduced AMPA receptor function (76).

We did not determine if any of the CBZ effects arose from short-term brain exposure to drug. This is unlikely, as chronic CBZ exposure is accompanied by significant changes in $cPLA_2$ mRNA and in the AP-2 transcription that modulates $cPLA_2$ transcription, as well as by other neuroplastic changes that likely require long term exposure (34,47,77–79). Acute CBZ effects include altering serotonergic transmission *in vivo* and *in vitro* and reducing high frequency firing in cultured neurons (80–82). The brains in the present study were sampled within 4 hours after the last of the daily CBZ doses, and pharmacokinetic data show that, following an i.p. CBZ in rats, the brain concentration peaks at 100 min and then declines with a half-life of 205-min (83). Thus, the brain would have been exposed to a therapeutic level of CBZ for only 1–2 hours.

Our baseline values in Table 3 of k* (after acute saline in chronic vehicle-treated rats) ranged from 2.69 to 7.14×10^{-4} ml/sec/g brain, agreeing with published values (38,84,85). NMDA 25 mg/k.g. i.p. significantly increased k* in 69/82 brain structures that have high NMDAR densities, including the cerebral cortex, caudate-putamen, globus pallidus, hippocampus, thalamus, hypothalamus, superior and inferior colliculi, and substantia nigra (86,87). In a prior study, 49/83 regions were significantly activated by the same NMDA dose (38). The discrepancy may be due to the lower variance of k* in the present study, a DMSO (vehicle) effect, or just to experimental variation.

Chronic CBZ did not significantly affect baseline values of k*, consistent with its lack of effect on baseline values of k* in individual phospholipid classes in microwaved rat brain (31). Regional baseline values for J_{in} in vehicle-treated rats, $33–289 \times 10^{-4}$ nmol/sec/g, agree with a published global value of 65.7×10^{-4} nmol/sec/g (31). Given that J_{in} and k* represent regional rates of AA metabolic loss from brain (88–90), our data indicate comparable baseline rates of AA loss, but reduced incremental rates following NMDA, in chronic CBZ- compared with vehicle-treated rats. The reduced incremental rates likely reflect the reduced formation of PGE_2 and TXB_2. In this regard, k* responses in rats to the cholinergic agonist arecoline, which activates PLA_2 *via* muscarinic cholinergic receptors, were absent in COX-2 knockout compared with wild-type mice (89), and in rats treated with a mixed COX-1/2 inhibitor (Basselin et al., unpublished data).

The behavioral effects of NMDA noted in Table 1 were not altered by chronic CBZ, and also are not altered by chronic lithium (38). Consistent with the lack of effect of both agents, neither

modifies the seizure threshold to NMDA in rodents (91–93). Thus, the behavioral and seizure responses to NMDA do not appear to directly involve the AA cascade.

Acute NMDA significantly increased many unesterified fatty acid concentrations in plasma in both the chronic vehicle and CBZ groups (Table 2), likely through by stimulating corticosterone secretion (94,95). The inability of CBZ treatment to block these increments agrees with our previous report (31), and is consistent with evidence in normal human volunteers that CBZ increases cortisol blood levels (96).

If disturbed NMDAR-initiated signaling *via* AA contributes to the mania of BD, as has been suggested (see above), then our finding in this paper that CBZ suppresses the NMDA signal, and elsewhere that chronic LiCl feeding does so as well, may mean that the efficacy of both agents in BD involves such an effect. Experiments with valproic acid help to test this possibility, as valproic acid, like lithium and CBZ, downregulates the AA cascade in rat brain and has been shown to interfere with NMDAR function (29,36,56,57). Additionally, chronic lithium has been reported to reduce k* for AA in response to quinpirole, an agonist of dopaminergic D_2-like receptors coupled to $cPLA_2$ by a G-protein (84,97). It would be worthwhile to see if CBZ and VPA do so as well, which would suggest a more general receptor action of these agents on $cPLA_2$-mediated AA signaling.

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Abbreviations

AA
: arachidonic acid (20:4*n*-6)

AP-2
: activator protein-2

BD
: bipolar disorder

CBZ
: carbamazepine

COX
: cyclooxygenase

NMDA
: N-methyl-D-aspartic acid

NMDAR
: NMDA receptor

cPLA$_2$
: cytosolic phospholipase

sPLA$_2$
: secretory PLA$_2$

iPLA$_2$
: calcium independent PLA$_2$

PGE$_2$

prostaglandin E_2

TXB_2

thromboxane B_2

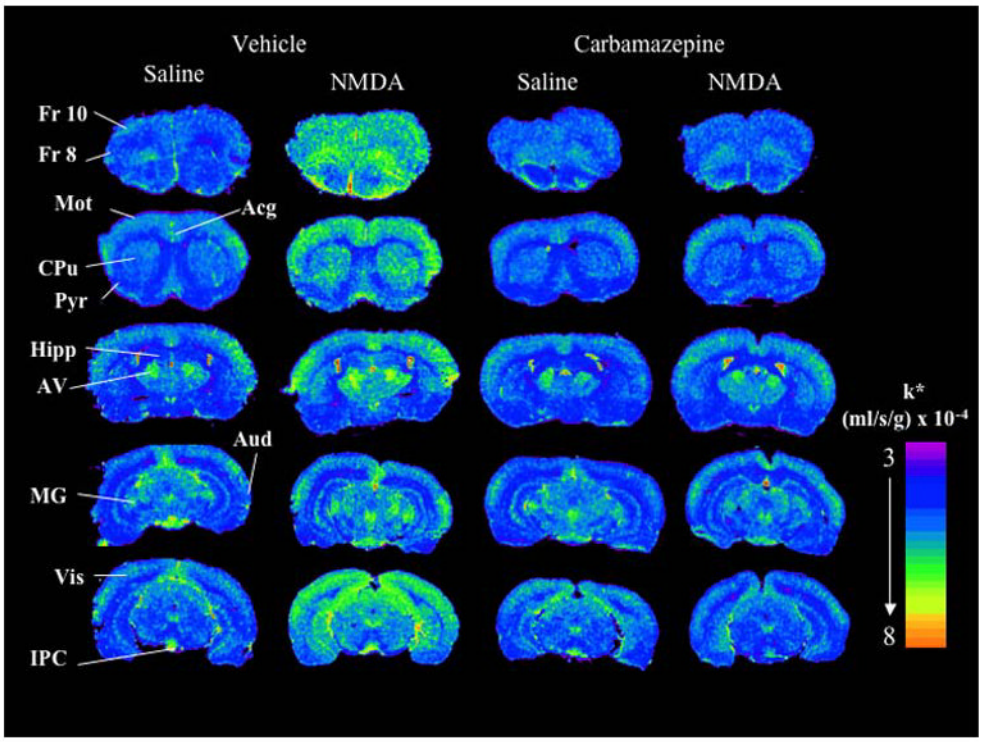

Figure 1. Coronal autoradiographs of brain showing effects of NMDA and carbamazepine on regional AA incorporation coefficients k* in rats

Values of k* (ml/sec/g brain × 10^{-4}) are given on a color scale from 3 (blue) to 8 (yellow-orange). Abbreviations: Acg, anterior cingulate cortex; Aud, auditory cortex; AV, anteroventral thalamus nucleus; CPu, caudate-putamen; Fr, frontal cortex; Hipp, hippocampus; IPC, interpeduncular nucleus; MG, medial geniculate nucleus; Mot, motor cortex; Pyr, pyriform cortex; Vis, visual cortex.

Table 1

Effects of chronic carbamazepine and acute NMDA on physiological parameters

	Vehicle				Carbamazepine (CBZ)			
	Saline		NMDA		Saline		NMDA	
	Before	After	Before	After	Before	After	Before	After
Body weight (g)	277 ± 14	-	275 ± 19	-	275 ± 13	-	278 ± 18	-
Body temperature (°C)	36.5 ± 0.3	36.7 ± 0.2	36.4 ± 0.3	36.6 ± 0.3	36.6 ± 0.3	36.8 ± 0.4	36.3 ± 0.5	36.6 ± 0.2
Heart rate (beats/min)	461 ± 40	451 ± 33	461 ± 24	326 ± 35***	442 ± 38	436 ± 42	446 ± 32	356 ± 21***
Arterial blood pressure (mm Hg)								
Systolic	158 ± 15	148 ± 13	149 ± 11	139 ± 7	153 ± 7	152 ± 5	159 ± 6	159 ± 6
Diastolic	104 ± 9	107 ± 7	104 ± 9	103 ± 6	94 ± 6	96 ± 6	94 ± 4	97 ± 4
pH	7.460 ± 0.010	7.463 ± 0.021	7.440 ± 0.039	7.453 ± 0.021	7.475 ± 0.019	7.461 ± 0.027	7.450 ± 0.020	7.443 ± 0.027
pO_2 (mm Hg)	83.6 ± 6.2	85.5 ± 3.0	81.7 ± 7.3	85.3 ± 1.1	87.6 ± 4.1	88.6 ± 5.6	91.3 ± 5.8	92.0 ± 3.2
pCO_2 (mm Hg)	33.2 ± 2.8	36.1 ± 3.2	36.7 ± 2.7	36.0 ± 1.0	36.2 ± 3.2	40.0 ± 4.9	37.8 ± 2.7	40.0 ± 1.3
Behavior duration (s)								
Cycle								
Activity				5 ± 1				4 ± 1
Calm				10 ± 2				9 ± 1
Net cycling				90 ± 20				110 ± 10

Values are means ± SD (n = 7–8) measured before surgery (body weight) or before [1-^{14}C]AA infusion.

*** $p < 0.001$, significant effect of 25 mg/kg i.p. NMDA in chronic vehicle- or CBZ-treated rats; -, not measured

Table 2

Effects of NMDA and carbamazepine on plasma unesterified fatty acid concentrations

	Vehicle		CBZ		CBZ x NMDA interaction	CBZ effect	NMDA effect
Fatty Acid	**saline (n = 8)**	**NMDA (n = 8)**	**saline (n = 7)**	**NMDA (n = 7)**	**p value**	**p value**	**p value**
	Concentration, nmol/ml plasma						
Palmitic (16:0)	183.5 ± 33.7	375.7 ± 234.5	175.5 ± 41.7	399.8 ± 167.5	0.769	0.884	0.001
Palmitoleic (16:1)	21.9 ± 5.0	39.9 ± 24.5	18.9 ± 5.1	37.6 ± 13.6	0.950	0.629	0.002
Stearic (18:0)	52.3 ± 6.9	60.7 ± 16.0	54.4 ± 11.4	67.3 ± 16.8	0.648	0.381	0.039
Oleic (18:1 n-9)	130.2 ± 34.7	313.7 ± 207.7	134.4 ± 42.2	394.6 ± 167.9	0.453	0.405	< 0.001
Linoleic (18:2 n-6)	134.7 ± 31.6	363.6 ± 262.7	134.0 ± 44.4	394.6 ± 168.0	0.789	0.798	< 0.001
α-Linolenic (18:3 n-3)	8.5 ± 2.6	19.8 ± 13.0	7.8 ± 3.1	20.5 ± 9.1	0.814	0.988	0.001
Arachidonic (20:4 n-6)	12.8 ± 3.6	27.1 ± 18.6	13.1 ± 4.7	32.3 ± 10.7	0.602	0.469	0.001
Docosahexaenoic (22:6 n-3)	12.8 ± 5.7	53.9 ± 44.0	14.6 ± 7.5	64.8 ± 30.0	0.657	0.532	< 0.001

Values are means ± SD measured in arterial plasma collected before [1-^{14}C]AA infusion..

Table 3

Effect of chronic carbamazepine on NMDA-induced regional AA incorporation coefficients k^* in rat brain

	Vehicle		Carbamazepine (CBZ)		CBZ x NMDA Interaction	CBZ effect	NMDA effect
	k^*, ml/sec/gram brain × 10^{-4}						
Brain region	**saline (n = 8)**	**NMDA (n = 8)**	**saline (n = 7)**	**NMDA (n = 7)**	**p-value**	**p-value**	**p-value**
Prefrontal cortex layer I	3.30 ± 0.33	5.46 ± 0.37***	3.60 ± 0.38	3.55 ± 0.14	< 0.001		
Prefrontal cortex layer IV	3.76 ± 0.49	6.08 ± 0.33***	3.75 ± 0.34	3.93 ± 0.60	< 0.001		
Primary olfactory cortex	3.28 ± 0.25	5.47 ± 0.59***	3.33 ± 0.61	3.68 ± 0.33	< 0.001		
Frontal cortex (10)							
Layer I	3.26 ± 0.28	5.82 ± 0.47***	3.51 ± 0.56	3.64 ± 0.20	< 0.001		
Layer IV	3.66 ± 0.34	6.49 ± 0.17***	3.98 ± 0.44	3.90 ± 0.38	< 0.001		
Frontal cortex (8)							
Layer I	3.59 ± 0.34	6.28 ± 0.34***	3.72 ± 0.52	3.97 ± 0.46	< 0.001		
Layer IV	4.13 ± 0.39	6.96 ± 0.50***	4.27 ± 0.48	4.32 ± 0.45	< 0.001		
Pyriform cortex	3.13 ± 0.30	4.15 ± 0.74	3.04 ± 0.80	3.39 ± 0.34	0.189	NS	0.002
Anterior cingulate cortex	5.33 ± 1.02	7.77 ± 0.41***	4.60 ± 0.52	4.46 ± 0.73	< 0.001		
Motor cortex							
Layer I	3.50 ± 0.18	6.24 ± 0.63***	3.73 ± 0.38	3.62 ± 0.40	< 0.001		
Layer II –III	3.52 ± 0.18	6.53 ± 0.72***	3.98 ± 0.24	3.42 ± 0.52	< 0.001		
Layer IV	3.97 ± 0.30	7.09 ± 0.93***	4.39 ± 0.33	4.18 ± 0.53	< 0.001		
Layer V	3.00 ± 0.15	5.35 ± 0.45***	3.22 ± 0.33	3.52 ± 0.68	< 0.001		
Layer VI	2.91 ± 0.19	5.13 ± 0.34***	3.12 ± 0.43	3.10 ± 0.24	< 0.001		
Somatosensory cortex							
Layer I	3.66 ± 0.44	6.55 ± 1.27***	3.81 ± 0.33	3.95 ± 0.48	< 0.001		
Layer II – III	3.48 ± 0.21	6.45 ± 0.92***	3.98 ± 0.25	3.90 ± 0.45	< 0.001		
Layer IV	4.34 ± 0.57	7.32 ± 0.93***	4.41 ± 0.27	4.52 ± 0.68	< 0.001		
Layer V	3.55 ± 0.23	6.31 ± 0.98***	3.98 ± 0.40	4.04 ± 0.53	< 0.001		
Layer VI	3.46 ± 0.15	6.14 ± 0.91***	3.82 ± 0.31	4.11 ± 0.61	< 0.001		
Auditory cortex							
Layer I	3.86 ± 0.19	7.86 ± 0.97***	4.29 ± 0.77	4.27 ± 0.94	< 0.001		
Layer IV	3.72 ± 0.32	8.26 ± 0.88***	4.46 ± 0.45	4.59 ± 1.10	< 0.001		
Layer VI	4.09 ± 0.15	6.86 ± 0.70***	4.18 ± 0.56	4.01 ± 0.74	< 0.001		
Visual cortex							
Layer I	3.92 ± 0.14	6.57 ± 0.49***	4.06 ± 0.50	3.98 ± 0.82	< 0.001		
Layer IV	4.09 ± 0.16	6.99 ± 0.43***	4.53 ± 0.40	4.58 ± 1.07	< 0.001		
Layer VI	3.82 ± 0.32	6.58 ± 0.68***	4.03 ± 0.48	4.18 ± 0.78	< 0.001		
Preoptic area (LPO/MPO)	3.28 ± 0.24	4.74 ± 0.79***	3.07 ± 0.56	3.23 ± 0.15	0.002		
Suprachiasmatic nu	3.06 ± 0.33	3.47 ± 0.25	2.60 ± 0.55	3.39 ± 0.33	NS	NS	NS
Globus pallidus	3.04 ± 0.42	4.86 ± 0.68***	2.56 ± 0.62	3.11 ± 0.29	0.003		
Bed nu stria terminalis	3.06 ± 0.20	5.17 ± 0.64***	2.87 ± 0.57	3.20 ± 0.30	< 0.001		
Olfactory tubercle	3.84 ± 0.14	6.02 ± 0.33***	3.71 ± 0.46	3.75 ± 0.55	< 0.001		
Diagonal band Dorsal	3.62 ± 0.11	5.49 ± 0.90***	3.67 ± 0.76	3.68 ± 0.23	< 0.001		
Ventral	3.45 ± 0.14	5.94 ± 1.16***	3.41 ± 0.70	3.66 ± 0.21	< 0.001		
Amygdala basolateral/med ial	3.60 ± 0.29	5.07 ± 0.41	3.04 ± 0.99	3.65 ± 0.44	0.050		
Hippocampus							
CA1	3.58 ± 0.38	4.65 ± 0.40***	3.03 ± 0.41	2.94 ± 0.19	< 0.001		
CA2	3.59 ± 0.38	4.63 ± 0.43***	3.27 ± 0.56	3.11 ± 0.26	0.001		
CA3	3.55 ± 0.35	4.96 ± 0.59***	3.28 ± 0.56	3.29 ± 0.28	< 0.001		
Dentate gyrus	3.12 ± 0.24	5.84 ± 0.54***	3.88 ± 0.95	3.58 ± 0.20	< 0.001		

	Vehicle		Carbamazepine (CBZ)		CBZ x NMDA Interaction	CBZ effect	NMDA effect
	k*, ml/sec/gram brain × 10^{-4}						
Brain region	**saline (n = 8)**	**NMDA (n = 8)**	**saline (n = 7)**	**NMDA (n = 7)**	**p-value**	**p-value**	**p-value**
SLM	3.48 ± 0.16	6.03 ± 1.34***	4.71 ± 0.67	4.34 ± 0.88	< 0.001		
Accumbens nucleus	2.99 ± 0.13	5.48 ± 1.23***	3.45 ± 0.38	3.70 ± 0.43	< 0.001		
Caudate putamen							
Dorsal	3.47 ± 0.10	5.49 ± 0.41***	3.67 ± 0.51	3.75 ± 0.38	< 0.001		
Ventral	3.33 ± 0.19	5.68 ± 0.66***	3.65 ± 0.46	3.85 ± 0.31	< 0.001		
Lateral	3.30 ± 0.17	5.68 ± 0.82***	3.63 ± 0.50	3.84 ± 0.47	< 0.001		
Medial	3.35 ± 0.22	6.30 ± 0.34***	3.57 ± 0.53	3.72 ± 0.31	< 0.001		
Septal nu lateral	3.11 ± 0.29	5.05 ± 0.83***	2.76 ± 0.67	3.27 ± 0.16	0.002		
Septal nu medial	3.37 ± 0.17	5.51 ± 0.62***	3.46 ± 0.68	3.85 ± 0.44	< 0.001		
Habenular nu lateral	5.20 ± 0.21	8.75 ± 1.26***	4.97 ± 0.48	5.43 ± 0.83	< 0.001		
Habenular nu medial	5.11 ± 0.20	8.52 ± 1.00***	5.31 ± 0.99	5.26 ± 0.88	< 0.001		
Lateral geniculate nu dorsal	3.89 ± 0.19	6.07 ± 0.47***	4.21 ± 0.48	4.65 ± 0.32	< 0.001		
Medial geniculate nu	4.27 ± 0.16	7.15 ± 1.08***	4.78 ± 0.54	5.02 ± 0.62	< 0.001		
Thalamus							
Ventroposterior lateral nu	3.75 ± 0.15	6.20 ± 1.44***	4.09 ± 0.80	4.11 ± 0.52	0.001		
Ventroposterior medial nu	3.76 ± 0.14	6.69 ± 0.89***	4.03 ± 0.47	4.32 ± 0.34	< 0.001		
Paratenial nu	3.52 ± 0.29	5.50 ± 0.52***	3.89 ± 1.00	4.01 ± 0.23	< 0.001		
Anteroventral nu	5.33 ± 0.70	7.87 ± 0.89***	4.69 ± 0.37	5.02 ± 1.12	0.007		
Anteromedial nu	3.50 ± 0.21	7.15 ± 1.21***	4.22 ± 1.19	4.15 ± 0.50	< 0.001		
Reticular nu	3.53 ± 0.37	6.13 ± 0.35***	3.72 ± 0.56	4.41 ± 0.61	< 0.001		
Paraventricular nu	3.41 ± 0.22	6.07 ± 0.50***	3.55 ± 0.61	3.90 ± 0.50	< 0.001		
Parafascicular nu	3.26 ± 0.16	6.31 ± 0.42***	3.51 ± 0.45	3.25 ± 0.49	< 0.001		
Subthalamic nu	5.14 ± 0.49	7.52 ± 1.61**	4.21 ± 0.65	4.32 ± 0.50	< 0.001		
Hypothalamus							
Supraoptic nu	3.14 ± 0.13	3.92 ± 0.67	3.46 ± 0.89	3.08 ± 0.60	NS	NS	NS
Lateral	2.68 ± 0.23	4.99 ± 0.93***	2.64 ± 0.62	3.12 ± 0.39	< 0.001		
Anterior	3.16 ± 0.30	4.98 ± 0.89***	2.77 ± 0.69	3.36 ± 0.26	0.010		
Periventricular	2.56 ± 0.27	4.42 ± 0.41***	2.35 ± 0.43	2.90 ± 0.26	< 0.001		
Arcuate	3.19 ± 0.29	4.66 ± 0.51***	3.66 ± 0.56	3.93 ± 0.82	< 0.001		
Ventromedial	3.09 ± 0.31	4.96 ± 0.73***	2.94 ± 0.56	3.51 ± 0.38	0.002		
Posterior	5.38 ± 0.21	6.20 ± 1.37	5.24 ± 0.54	5.65 ± 0.68	NS	NS	NS
Mammillary nu	3.04 ± 0.14	4.05 ± 1.82	3.90 ± 0.55	4.05 ± 0.94	NS	NS	NS
Interpeduncular nu	6.05 ± 0.72	8.15 ± 0.80***	5.57 ± 0.81	5.56 ± 0.60	0.028		
Substantia ni gra	3.59 ± 0.31	6.34 ± 1.24***	3.77 ± 0.49	3.56 ± 0.49	< 0.001		
Pretectal area	3.77 ± 0.62	5.44 ± 1.09**	4.38 ± 0.45	4.36 ± 0.26	0.003		
Grey layer superior colliculus	4.00 ± 0.15	7.17 ± 1.50***	4.49 ± 0.52	4.23 ± 0.59	< 0.001		
Superior colliculus	3.89 ± 0.45	5.99 ± 0.51***	4.03 ± 0.56	4.20 ± 0.84	0.009		
Inferior colliculus	6.18 ± 0.51	9.04 ± 0.41***	5.82 ± 1.24	5.79 ± 0.77	0.006		
Flocculus	4.56 ± 0.13	5.30 ± 1.11	4.67 ± 1.04	4.81 ± 0.95	NS	NS	NS
Cerebellar gray matter	4.20 ± 0.26	5.55 ± 0.48***	3.98 ± 0.31	3.82 ± 0.29	< 0.001		
Molecular layer cerebellar gray	7.14 ± 0.65	7.13 ± 1.23	7.01 ± 0.77	7.09 ± 0.70	NS	NS	NS
White matter							
Corpus callosum	3.18 ± 0.50	3.57 ± 0.29	2.90 ± 0.41	3.12 ± 0.35	NS	NS	NS
Zone incerta	3.08 ± 0.31	3.83 ± 1.15	3.40 ± 0.61	3.45 ± 0.53	NS	NS	NS
Internal capsule	2.69 ± 0.31	3.09 ± 0.35	2.43 ± 0.41	2.66 ± 0.56	NS	NS	NS
Cerebellar white matter	3.00 ± 0.09	3.31 ± 0.98	3.12 ± 0.78	3.24 ± 0.55	NS	NS	NS
Non-blood-brain barrier regions							

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	Vehicle		Carbamazepine (CBZ)		CBZ x NMDA Interaction	CBZ effect	NMDA effect
	k*, ml/sec/gram brain × 10^{-4}						
Brain region	**saline (n = 8)**	**NMDA (n = 8)**	**saline (n = 7)**	**NMDA (n = 7)**	**p-value**	**p-value**	**p-value**
Subfornical organ	5.45 ± 0.20	5.67 ± 1.26	5.39 ± 0.75	3.75 ± 0.67	NS	NS	NS
Median eminence	3.30 ± 0.12	3.83 ± 0.40	3.52 ± 0.67	3.81 ± 0.50	NS	NS	NS
Choroid plexus	22.6 ± 2.49	22.8 ± 2.69	24.0 ± 5.46	22.8 ± 4.0	NS	NS	NS

* Abbreviations: nu, nucleus; Values of k are means ± S.D.

Main effects are not reported if statistically significant CBZ x NMDA interaction. In cases of statistically significant CBZ x NMDA interaction, unpaired *t*-tests were determined:

** $p < 0.01$;

*** $p < 0.001$; vehicle plus NMDA versus vehicle plus saline, CBZ plus saline versus vehicle plus saline, and CBZ plus NMDA versus CBZ plus sali

Table 4

Effect of NMDA on brain PGE_2 and TXB_2 concentrations in chronic vehicle- and CBZ-treated rats

	Vehicle		CBZ		CBZ x NMDA interaction
	Saline	NMDA	Saline	NMDA	p-value
PGE_2 (ng/g brain)	7.8 ± 1.6	13.8 ± 4.7**	4.3 ± 1.7 **	3.2 ± 1.4	0.031
TXB_2 (pg/g brain)	43.4 ± 2.6	97.8 ± 17.6***	21.2 ± 7.6**	38.2 ± 17.2	0.014

Each value is a mean ± S.D (n = 4).

** $p < 0.01$;

*** $p < 0.001$; Vehicle plus NMDA versus vehicle plus saline, CBZ plus saline versus vehicle plus saline, and CBZ plus NMDA versus CBZ plus saline