Chronic N-methyl-D-aspartate administration increases the turnover of arachidonic acid within brain phospholipids of the unanesthetized rat

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Abstract Whereas antibipolar drug administration to rats reduces brain arachidonic acid turnover, excessive N-methyl-D-aspartate (NMDA) signaling is thought to contribute to bipolar disorder symptoms and may increase arachidonic acid turnover in rat brain phospholipids. To determine whether chronic NMDA would increase brain arachidonic acid turnover, rats were daily administered NMDA (25 mg/kg, ip) or vehicle for 21 days. In unanesthetized rats, on day 21, [1-^{14}C]arachidonic acid was infused intravenously and arterial blood plasma was sampled until the animal was euthanized at 5 min and its microwaved brain was subjected to chemical and radiotracer analysis. Using equations from our in vivo fatty acid model, we found that compared with controls, chronic NMDA increased the net rate of incorporation of plasma unesterified arachidonic acid into brain phospholipids (25–34%) as well as the turnover of arachidonic acid within brain phospholipids (35–58%). These changes were absent at 3 h after a single NMDA injection.▮▮ The changes, opposite to those after chronic administration of antimanic drugs to rats, suggest that excessive NMDA signaling via arachidonic acid may be a model of upregulated arachidonic acid turnover in brain phospholipids.—Lee, H-J., J. S. Rao, L. Chang, S. I. Rapoport, and R. P. Bazinet. **Chronic N-methyl-D-aspartate administration increases the turnover of arachidonic acid within brain phospholipids of the unanesthetized rat.** *J. Lipid Res.* 2008. **49:** 162–168.

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Glutamatergic synapses are highly abundant in the central nervous system, constituting >70% of synapses (1, 2). Glutamate receptors are classified into two major classes, ionotropic and metabotropic. Ionotropic glutamate receptors are further classified based on their selectivity into α-amino-3 hydroxy-5-methyl-4-isoxazolprorionic acid, kainate, or N-methyl-D-aspartate (NMDA) receptors.

NMDA receptor function is believed to be altered in several brain disorders (3), including Alzheimer's disease (4, 5), schizophrenia (6, 7), major depression (8), and bipolar disorder (9–15).

Activation of NMDA receptors induces an influx of extracellular calcium into neurons, stimulating many calcium-dependent signals (16), including arachidonic acid (20:4n-6) release from membrane phospholipids (17–19). Arachidonic acid is a polyunsaturated fatty acid found mainly in the sn-2 position of brain phospholipids (20). Multiple neuroreceptor-mediated processes, including ionotropic NMDA receptors, release arachidonic acid via coupling to the activation of calcium-dependent cytosolic phospholipase A_2 (cPLA$_2$) (21, 22). A portion of the released unesterified arachidonic acid can be converted to eicosanoids (23, 24) or β-oxidized (25), whereas the majority is recycled into membrane phospholipid via the Lands pathway (20, 26). Arachidonic acid signaling has been implicated in bipolar disorder (27), because chronic antimanic drug administration (lithium, carbamazepine, or valproate) to rats decreases arachidonic acid turnover in their brain phospholipids (28–30). Furthermore, chronic lithium or carbamazepine administration to rats decreases the expression of brain cPLA$_2$ (31, 32), whereas chronic NMDA administration increases brain cPLA$_2$ expression (33). Thus, chronic NMDA administration also may increase arachidonic acid turnover in rat brain phospholipids.

Drugs effective in the manic phase of bipolar disorder decrease the turnover of arachidonic acid in rat brain phospholipids (27, 34–36). Because NMDA receptor activation can stimulate brain cPLA$_2$, we hypothesized that chronic NMDA administration to rats would increase

Abbreviations: ChoGpl, choline glycerophospholipid; cPLA$_2$, calcium-dependent cytosolic phospholipase A$_2$; EtnGpl, ethanolamine glycerophospholipid; NMDA, N-methyl-D-aspartate; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine.

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brain arachidonic acid turnover, opposite to what has been reported with chronic antimanic drug administration (28–30). Thus, chronic NMDA-treated rats may be a model of upregulated brain arachidonic acid turnover. To test this hypothesis, rats were administered NMDA (25 mg/kg/day, ip, for 21 days), a dose shown to be subconvulsive (37) and to increase the incorporation coefficient of arachidonic acid into brain phospholipids at 10 min after administration (38). We applied our in vivo brain fatty acid turnover technique (20, 39) to rats after repeated (chronic) or a single NMDA injection and found that only chronic NMDA increased arachidonic acid turnover in their brain phospholipids.

MATERIALS AND METHODS

Animals

This study was conducted according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (Publication 80-23) and was approved by the National Institute of Child Health and Human Development Animal Care and Use Committee. Male CDF-344 rats, weighing 180–190 g (Charles River, Wilmington, MA), were acclimatized for 1 week in an animal facility in which temperature, humidity, and light cycle were controlled and had ad libitum access to food (NIH-31) and water. They were randomized to either chronic or single NMDA treatment (n = 15) or a control group (n = 12). Chronic NMDA-treated rats received 25 mg/kg/day (ip) NMDA (Sigma Chemical Co., St. Louis, MO) dissolved in 0.9% saline once daily for 21 days, whereas single NMDA-injected animals (n = 11) received the same volume of vehicle (0.9% saline) once daily, intraperitoneally, also for 20 days, and then on day 21 received one intraperitoneal injection of 25 mg/kg NMDA. Controls received the same volume of vehicle (0.9% saline) once daily for 21 days. We reported that this chronic NMDA dosing regimen increases brain $cPLA_2$ activity, protein, and mRNA (33). On day 21, rats were anesthetized with 1–3% halothane. Polyethylene catheters were inserted into a femoral artery and vein, as reported (30). Rats were allowed to recover from surgery, and 30 min after the removal of halothane anesthesia they were injected with the appropriate treatment with their hindquarters loosely wrapped and taped to a wooden block. During recovery, arterial blood pressure was monitored and temperature was maintained at 37°C by means of a rectal probe and heating element (Indicating Temperature Controller; Yellow Springs Instrument Co., Yellow Springs, OH).

Infusion of [1-^{14}C]arachidonic acid and tissue sampling

[1-^{14}C]arachidonic acid (50 mCi/mmol, >98% pure; Moravek Biochemicals, Brea, CA) was prepared in saline containing 3% fatty acid-free BSA (Sigma) as described previously (30, 40). Three hours after the last injection, unanesthetized rats were infused intravenously for 5 min with 1 ml containing 170 μCi/kg arachidonic acid at a rate of 0.223 (1-e$^{-1.92t}$) ml/min with a computer-controlled variable-rate infusion pump (No. 22; Harvard Apparatus, South Natick, MA), to achieve a steady-state plasma specific activity within 1 min (29, 30, 41). Arterial blood samples were collected at 0, 15, 30, 45, 90, 180, 240, and 300 s during infusion to determine the radioactivity and unlabeled concentrations of nonesterified arachidonic acid in plasma. Five minutes after starting infusion, the rat was anesthetized with sodium pentobarbital (20 mg/kg, iv) and subjected to

head-focused microwave irradiation to stop brain metabolism (5.5 kW, 3.4 s; Cober Electronics, Stamford, CT) (42, 43). The brain was excised, bisected sagittally, and stored at −80°C for further analysis.

Brain lipid extraction and chromatography

Total lipids were extracted from frozen plasma and from one brain hemisphere by the method of Folch, Lees, and Sloane Stanley (44). Heptadecanoic acid (17:0) was added as an internal standard to plasma before extraction. The extracts were separated by thin-layer chromatography on silica gel plates (Whatman, Clifton, NJ). Unesterified fatty acids were separated using a mixture of heptane-diethyl ether-glacial acetic acid (60:40:2, v/v) (45), and phospholipids [choline glycerophospholipid (ChoGpl), phosphatidylserine (PtdSer), phosphatidylinositol (PtdIns), and ethanolamine glycerophospholipid (EtnGpl)] were separated in chloroform-methanol-water-glacial acetic acid (60:50:4:1, v/v) (46) and identified with unlabeled standards in separate lanes. Phospholipid and standard bands were visualized with 6-p-toluidine-2-naphthalene-sulfonic acid (Acros, Fairlawn, NJ) under ultraviolet light. Each band was removed and analyzed for radioactivity by liquid scintillation counting. Phospholipid bands also were individually scraped and 200 μl of toluene was added with a known amount of di-17:0-PtdCho for quantification before methylation. Fatty acid methyl esters were formed by heating the phospholipid scrapings in 1% H_2SO_4 in methanol at 70°C for 3 h (47). The methyl esters were separated on a 30 m × 0.25 mm inner diameter capillary column (SP-2330; Supelco, Bellefonte, PA) using gas chromatography with a flame ionization detector (model 6890N; Agilent Technologies, Palo Alto, CA). Runs were initiated at 80°C, with a temperature gradient to 160°C (10°C/min) and 230°C (3°C/min) in 31 min, and held at 230°C for 10 min. Peaks were identified by retention times of fatty acid methyl ester standards (Nu-Chek-Prep, Elysian, MN). Fatty acid concentrations (nmol/g brain or nmol/ml plasma) were calculated by proportional comparison of gas chromatography peak areas to that of the 17:0 internal standard. Tracer identification and separation were performed on fatty acid methyl esters of pooled plasma samples (at the end of the infusion) and pooled brain total lipid extracts as described above. The fatty acid methyl esters were separated as described previously with slight modifications (48) using an HPLC system (Beckman, Fullerton, CA) equipped with an in-line ultraviolet/visible light detector (λ = 242 nm; Gilson, Middleton, WI) and an in-line scintillation counter (β-RAM; IN/US System, Tampa, FL) with a Luna C18 column (Phenomenex, Torrance, CA). Initial conditions were set to a 1 ml/min gradient system composed of 100% water (A) and 100% acetonitrile (B). The gradient started with 85% B for 30 min, then increased to 100% B over 10 min, where it was held for 20 min before returning to 85% B over 5 min.

Quantification of labeled and unlabeled acyl-CoA

Acyl-CoA species were isolated from the remaining half-brain by the method of Deutsch et al. (49). Weighed brain and a known amount of 17:0-CoA as an internal standard were placed in a 15 ml conical vial before sonicating the brain in 25 mM potassium phosphate. Isopropanol (2 ml) was added to the vial, and the homogenate was sonicated again. Saturated ammonium sulfate (0.25 ml) was added, and the sample was lightly shaken by hand. Acetonitrile (4 ml) was added, and the sample was vortexed for 10 min before centrifugation. The upper phase was extracted, and 10 ml of 25 mM potassium phosphate was added. Each sample was run three times through an activated oligonucleotide purification cartridge (Applied Biosystems,

Foster City, CA), washed with 10 ml of 25 mM potassium phosphate, and eluted with 400 μl of isopropanol-1 mM glacial acetic acid (75:25, v/v). Samples were dried under nitrogen and reconstituted in 100 μl of isopropanol-1 mM glacial acetic acid (75:25, v/v) for HPLC analysis. Acyl-CoA species were separated using HPLC (Beckman) with a Symmetry C-18, 5 μm column (250 × 4.6 mm; Waters-Millipore Corp., Milford, MA). Conditions were set to a 1 ml/min gradient system composed of 75 mM potassium phosphate (A) and 100% acetonitrile (B). The gradient started with 44% B, increased to 49% over 25 min and then to 70% over 5 min, remained at 70% for 9 min, and returned to 44% over 4 min and was held there for 4 min (end of run). Concentrations of acyl-CoA species and their associated radioactivity were measured using peak area analysis from HPLC chromatograms relative to 17:0-CoA and liquid scintillation counting. These values were used to calculate the specific activities of arachidonoyl-CoA.

Calculations

The model for determining the in vivo kinetics of brain fatty acids in rats has been described in detail elsewhere (20, 50). Briefly, unidirectional incorporation coefficients (k_i^*; ml/s/g) of [1-^{14}C]arachidonic acid, representing incorporation from plasma into brain phospholipid compartments i, were calculated as:

$$k_i^* = \frac{c^*_{br,i}(T)}{\int_O^T c^*_{pl}\, dt} \qquad Eq.\ 1$$

where $C^*_{br,i}(T)$ (nCi/g) is the radioactivity of brain lipid i at time T of 5 min (time of termination of experiment), t is time after beginning the infusion, and c^*_{pl} (nCi/ml) is the plasma concentration of labeled unesterified arachidonic acid during infusion. Integrals of plasma radioactivity were determined by trapezoidal integration. The de novo brain synthesis of arachidonic acid from its dietary precursor linoleic acid (18:2n-6) represents <0.5% of the arachidonic acid within the brain (51); thus, net rates of incorporation of nonesterified arachidonic acid from plasma into brain phospholipid i ($J_{in,i}$), and from brain arachidonoyl-CoA into brain phospholipid i ($J_{FA,i}$), were calculated as follows:

$$J_{in,i} = k_i^* c_{pl} \qquad Eq.\ 2$$

$$J_{FA,i} = \frac{J_{in,i}}{\lambda} \qquad Eq.\ 3$$

where c_{pl} (nmol/ml) is the concentration of unlabeled nonesterified arachidonic acid in plasma. The "dilution factor" λ is defined as the steady-state ratio during [1-^{14}C]arachidonic acid infusion of the specific activity of the brain arachidonoyl-CoA pool to plasma specific activity of unesterified arachidonic acid:

$$\lambda = \frac{c^*_{br,CoA}/c_{br,CoA}}{c^*_{pl}/c_{pl}} \qquad Eq.\ 4$$

The steady state is reached within 1 min after infusion begins (41, 52). The fractional turnover rate of arachidonic acid within phospholipid i ($F_{FA,i}$; %/h) is defined as:

$$F_{FA,i} = \frac{J_{FA,i}}{c_{br,i}} \qquad Eq.\ 5$$

Data and statistics

Data are presented as means ± SD. One-way ANOVA with Tukey's pairwise posthoc test was used to compare means between chronic NMDA-treated, single NMDA-injected, and control animals (SAS 9.0; Cary, NC). Statistical significance was taken as $P \leq 0.05$.l

RESULTS

Body weight

Body weights did not differ significantly between groups after 21 days of treatment (270 ± 8, 263 ± 11, and 264 ± 9 g for chronic NMDA, single NMDA, and control rats, respectively).

Plasma and brain fatty acids

As in our previous reports (34, 53), HPLC separation of fatty acid methyl ester derivatives from pooled plasma total lipid extracts confirmed that >97% of total lipid plasma radioactivity represented labeled arachidonic acid after 5 min of infusion of [1-^{14}C]arachidonic acid across treatment groups. HPLC separation of fatty acid methyl ester derivatives from pooled brain total lipid extracts also showed that >94% of total brain radioactivity was in the form of [1-^{14}C]arachidonic acid across treatment groups. Percentage radioactivities from plasma and brain total lipid extracts were comparable between the treatment groups and control rats and are similar to those reported in other studies (28, 34, 54).

Chronic or a single NMDA injection did not significantly change the plasma concentration of unlabeled unesterified arachidonic acid, or of other measured plasma unesterified fatty acids (data not shown), compared with control concentrations (**Table 1**). There was no significant difference in the concentration of brain arachidonoyl-CoA or any measured acyl-CoA species between chronic or single NMDA-injected and control rats (Table 1). Chronic NMDA significantly increased the unlabeled esterified arachidonic acid concentration in ChoGpl and EtnGpl, by 10% and 18%, respectively, compared with control values (Table 1). A single NMDA injection did not alter the unlabeled esterified arachidonic acid concentration in brain phospholipids compared with controls. There was no significant difference in any other measured unlabeled esterified fatty acid concentration in brain phospholipids between the treatment groups and compared with controls (Table 1).

Kinetics

The integral of the steady-state plasma radioactivity ($\int_0^T c^*_{pl}\, dt$; plasma input function) after the 5 min [1-^{14}C] arachidonic acid infusion was significantly higher in chronic NMDA-treated rats (269,606 ± 83,668 nCi/s/ml) compared with rats receiving a single NMDA injection (124,213 ± 15,000 nCi/s/ml) and control rats (124,468 ± 12,296 nCi/ s/ml). Chronic but not a single NMDA injection increased k_i^* (equation 1) of arachidonic acid in ChoGpl, PtdSer, PtdIns, EtnGpl, and total phospholipids by 22, 25, 38, 39, and 29%, respectively, compared with controls (**Table 2**). Chronic but not a single NMDA injection increased $J_{in,i}$ (equation 2) in ChoGpl, PtdSer, PtdIns, EtnGpl, and total phospholipids by 26, 25, 30, 25, and 34%, respectively,

TABLE 1. Arterial plasma unesterified arachidonic acid, brain arachidonoyl-CoA, and brain arachidonic acid concentrations in major glycerophospholipid classes in control, chronic NMDA, and single NMDA-injected rats

Arachidonic Acid Pool	Control (n = 12)	Chronic NMDA (n = 15)	Single NMDA (n = 11)
Plasma unesterified arachidonic acid (nmol/ml)	29.2 ± 5.78	31.3 ± 8.73	31.7 ± 7.10
Brain (nmol/g)			
Arachidonoyl-CoA	1.8 ± 0.69	2.0 ± 0.9	1.9 ± 0.44
ChoGpl arachidonic acid	1,753 ± 163 a	1,932 ± 124 b	1,816 ± 118 a, b
PtdSer arachidonic acid	551 ± 72	596 ± 46	545 ± 43
PtdIns arachidonic acid	1,145 ± 134	1,239 ± 175	1,130 ± 103
EtnGpl arachidonic acid	4,336 ± 313 a	5,097 ± 847 b	4,527 ± 276 a

ChoGpl, choline glycerophospholipid; EtnGpl, ethanolamine glycerophospholipid; NMDA, N-methyl-D-aspartate; PtdIns, phosphatidylinositol; PtdSer, phosphatidylserine. Control rats received saline for 21 days, chronic NMDA rats received 25 mg/kg NMDA for 21 days, and single NMDA-injected rats received saline for 20 days and 25 mg/kg NMDA at day 21. Data are means ± SD. Means within a row not sharing a common lowercase letter are statistically different ($P < 0.05$).

compared with controls (Table 2). λ (equation 4) was not significantly different between chronic (0.029 ± 0.008) or single NMDA-injected (0.036 ± 0.014) and control (0.036 ± 0.019) rats. Chronic but not a single injection of NMDA increased $J_{FA,i}$ (equation 3) in ChoGpl, PtdSer, PtdIns, EtnGpl, and total phospholipids by 48, 40, 70, 55, and 56%, respectively, compared with controls (**Table 3**). The turnover rate of arachidonic acid ($F_{FA,i}$; equation 5) was significantly higher within brain ChoGpl (35%), PtdSer (36%), PtdIns (58%), EtnGpl (50%), and total phospholipids (41%) of rats chronically receiving NMDA compared with controls (Table 3).

DISCUSSION

The baseline concentrations of plasma unesterified fatty acids, brain esterified fatty acids, and brain acyl-CoA species as well as the arachidonic acid kinetics in controls are similar to those in previous reports (28, 34, 54). We previously reported that chronic but not a single NMDA injection to rats increases $cPLA_2$ activity, protein, and mRNA expression as well as the activity of the $cPLA_2$ transcription factor, activator protein-2 (33). Consistent with these findings, chronic but not single NMDA injection increased the net rates of incorporation of arachidonic acid into brain phospholipids from the brain arachidonoyl-CoA pool ($J_{FA,i}$) and of arachidonic acid

turnover ($F_{FA,i}$). Although decreased $cPLA_2$ activity correlates with reduced arachidonic acid turnover in rats treated chronically with lithium or carbamazepine (28, 30–32), future mechanistic studies are needed to determine whether the increase in $cPLA_2$ activity upon chronic NMDA administration is directly responsible for the increase in brain arachidonic acid turnover.

Collectively, the effects of chronic NMDA are opposite to those in our previous reports, in which chronic administration of lithium, carbamazepine, or valproate decreased arachidonic acid $J_{FA,i}$ and $F_{FA,i}$ in unanesthetized rats (28–30, 45, 55). The reduction in arachidonic acid turnover in rats chronically administered lithium or carbamazepine was associated with decreases in the activity, protein, and mRNA of the arachidonic acid-selective $cPLA_2$ (31, 32) as well as its transcription factor, activator protein-2 (56–58), whereas valproate likely targeted an arachidonic acid-selective fatty acyl-CoA synthetase (59). Topiramate, a drug that initial nonrandomized trials suggested was effective in bipolar disorder (60, 61) and that was later found to have no therapeutic effect (62, 63), upon chronic administration to rats did not alter arachidonic acid turnover in brain phospholipids (54, 64). Because increased NMDA receptor-mediated signaling has been implicated in bipolar disorder (10, 65, 66) and because chronic NMDA has the opposite effect on brain arachidonic acid kinetics compared with each of the three effective antimanic drugs in rats, chronic NMDA-treated

TABLE 2. Incorporation coefficients and net rates of incorporation from plasma unesterified arachidonic acid into major brain glycerophospholipid classes in control, chronic NMDA, and single NMDA-injected rats

Phospholipid	Incorporation Coefficient			Net Rate of Incorporation		
	Control (n = 12)	Chronic NMDA (n = 15)	Single NMDA (n = 11)	Control (n = 12)	Chronic NMDA (n = 15)	Single NMDA (n = 11)
	$ml/g/s \times 10^{-5}$			$nmol/g/s \times 10^{-4}$		
Total phospholipids	18.2 ± 0.96 a	23.5 ± 6.51 b	17.1 ± 2.75 a	53.0 ± 9.95 a	70.8 ± 16.73 b	53.4 ± 10.60 a
ChoGpl	8.2 ± 0.64 a	10.0 ± 2.49 b	7.6 ± 1.39 a	23.9 ± 4.57 a	30.1 ± 7.23 b	23.7 ± 4.65 a
PtdSer	1.6 ± 0.20 a, b	2.0 ± 0.63 b	1.5 ± 0.27 a	4.8 ± 1.11	6.0 ± 1.44	4.8 ± 1.11
PtdIns	6.1 ± 0.46 a	8.4 ± 2.29 b	5.7 ± 1.03 a	17.6 ± 3.17 a	25.3 ± 5.90 b	17.9 ± 4.04 a
EtnGpl	2.3 ± 0.22 a	3.2 ± 1.21 b	2.2 ± 0.37 a	6.6 ± 1.56 a	9.5 ± 2.70 b	7.0 ± 1.61 a

Infusion of [1-^{14}C]arachidonic acid (170 µCi/kg) over 5 min. Control rats received saline for 21 days, chronic NMDA rats received 25 mg/kg NMDA for 21 days, and single NMDA-injected rats received saline for 20 days and 25 mg/kg NMDA at day 21. Data are means ± SD. Means within a row not sharing a common lowercase letter are statistically different ($P < 0.05$).

TABLE 3. Net incorporation of arachidonic acid from brain arachidonoyl-CoA and turnover of arachidonic acid in major glycerophospholipid classes in control, chronic NMDA, and single NMDA-injected rats

| | Net Incorporation of Arachidonic Acid | | | Turnover of Arachidonic Acid | | |
Phospholipid	Control (n = 12)	Chronic NMDA (n = 15)	Single NMDA (n = 11)	Control (n = 12)	Chronic NMDA (n = 15)	Single NMDA (n = 11)
	$nmol/g/s \times 10^{-2}$			%/h		
Total phospholipids	16.2 ± 4.84 a	25.3 ± 7.63 b	16.7 ± 5.09 a	7.4 ± 2.07 a	10.4 ± 3.19 b	7.4 ± 2.23 a
ChoGpl	7.3 ± 2.31 a	10.8 ± 3.51 b	7.4 ± 2.29 a	14.9 ± 4.45 a	20.1 ± 6.21 b	14.6 ± 4.49 a
PtdSer	1.5 ± 0.55 a	2.1 ± 0.57 b	1.5 ± 0.53 a	9.4 ± 3.28 a	12.8 ± 3.48 b	10.1 ± 3.69 a, b
PtdIns	5.3 ± 1.49 a	9.0 ± 2.59 b	5.7 ± 1.79 a	16.8 ± 4.76 a	26.6 ± 8.20 b	18.0 ± 6.17 a
EtnGpl	2.0 ± 0.63 a	3.4 ± 1.10 b	2.2 ± 0.64 a	1.6 ± 0.48 a	2.4 ± 0.82 b	1.7 ± 0.48 a

Infusion of [1-^{14}C]arachidonic acid (170 μCi/kg) over 5 min. The dilution factors were 0.036 ± 0.019, 0029 ± 0.008, and 0.036 ± 0.014 for controls, chronic NMDA, and single NMDA injection, respectively. Control rats received saline for 21 days, chronic NMDA rats received 25 mg/kg NMDA for 21 days, and single NMDA-injected rats received saline for 20 days and 25 mg/kg NMDA at day 21. Data are means ± SD. Means within a row not sharing a common lowercase letter are statistically different ($P < 0.05$).

rats may represent a model of upregulated brain arachidonic acid metabolism. It would also be of interest to test whether brain arachidonic acid turnover is increased in other animal models of bipolar disorder (67).

In this regard, dietary n-3 polyunsaturated fatty acid deprivation for 15 weeks after weaning in rats was reported to increase scores on tests of aggression and depression, symptoms found in bipolar disorder (68). This deprivation also increased cPLA$_2$ activity, protein, and mRNA in the rat frontal cortex (35). It would be of interest to apply tests of aggression and depression as well as other potential bipolar disorder-related behaviors (67) in rats chronically administered NMDA to compare the results with the n-3 polyunsaturated fatty acid deprivation regimen.

Clinical studies suggest that arachidonic acid signaling is altered in patients with mood disorders, including bipolar disorder (69, 70). Genetic studies have suggested that alterations in the secretory PLA$_2$ gene increase the risk of bipolar disorder (71, 72) and that patients with bipolar disorder have increased serum PLA$_2$ activity (73, 74). Increased phospholipid hydrolysis has been reported in patients with affective disorders (70, 75–77), including increased prostaglandin E$_2$ levels in plasma (77), cerebrospinal fluid (78), and saliva (79), whereas a postmortem study has reported decreased cortical cytosolic prostaglandin E$_2$ synthase in treated patients with bipolar disorder (80).

In the current study, chronic but not a single injection of NMDA increased the net rate of incorporation of unesterified plasma arachidonic acid into brain phospholipids ($J_{in,i}$). $J_{in,i}$ represents the net rate of plasma unesterified arachidonic acid incorporation into brain phospholipids and at a steady state approximates the rate of loss from brain (81). The finding that chronic NMDA administration increased $J_{in,i}$ suggests that arachidonic acid metabolism was increased, possibly via conversion to prostaglandin E$_2$ or other oxidative species, and future studies examining brain cyclooxygenase and prostaglandin E$_2$ are warranted.

In conclusion, chronic NMDA administration to rats increases the turnover of arachidonic acid in their brain phospholipids. The effect is opposite to that of drugs effective in the manic phase of bipolar disorder. Thus, the chronic NMDA-treated rat may represent a model of increased arachidonic acid turnover in brain phospholipids.

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