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Review

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Lithium and the Other Mood Stabilizers Effective in Bipolar Disorder Target the Rat Brain Arachidonic Acid Cascade

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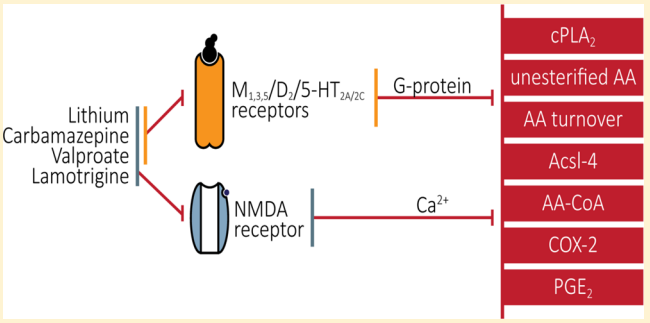
ABSTRACT: This Review evaluates the arachidonic acid (AA, 20:4n-6) cascade hypothesis for the actions of lithium and other FDA-approved mood stabilizers in bipolar disorder (BD). The hypothesis is based on evidence in unanesthetized rats that chronically administered lithium, carbamazepine, valproate, or lamotrigine each downregulated brain AA metabolism, and it is consistent with reported upregulated AA cascade markers in post-mortem BD brain. In the rats, each mood stabilizer reduced AA turnover in brain phospholipids, cyclooxygenase-2 expression, and prostaglandin E_2 concentration. Lithium and carbamazepine also reduced expression of cytosolic phospholipase A_2 (cPLA$_2$) IVA, which releases AA from membrane phospholipids, whereas valproate uncompetitively inhibited in vitro acyl-CoA synthetase-4, which recycles AA into phospholipid. Topiramate and gabapentin, proven ineffective in BD, changed rat brain AA metabolism minimally. On the other hand, the atypical antipsychotics olanzapine and clozapine, which show efficacy in BD, decreased rat brain AA metabolism by reducing plasma AA availability. Each of the four approved mood stabilizers also dampened brain AA signaling during glutamatergic NMDA and dopaminergic D_2 receptor activation, while lithium enhanced the signal during cholinergic muscarinic receptor activation. In BD patients, such signaling effects might normalize the neurotransmission imbalance proposed to cause disease symptoms. Additionally, the antidepressants fluoxetine and imipramine, which tend to switch BD depression to mania, each increased AA turnover and cPLA$_2$ IVA expression in rat brain, suggesting that brain AA metabolism is higher in BD mania than depression. The AA hypothesis for mood stabilizer action is consistent with reports that low-dose aspirin reduced morbidity in patients taking lithium, and that high n-3 and/or low n-6 polyunsaturated fatty acid diets, which in rats reduce brain AA metabolism, were effective in BD and migraine patients.



KEYWORDS: *Lithium, bipolar disorder, arachidonic acid, carbamazepine, mood stabilizers, valproic acid, rat, brain, antidepressant, antipsychotics, biotype*

1. INTRODUCTION

Bipolar disorder (BD) is characterized by recurring cycles of depressive and manic symptoms (Bipolar I) or hypomanic symptoms (Bipolar II). The depressive phase is three times more common than the manic or hypomanic phase, and the lifetime suicide risk is 10−20%. BD is a life-long malady that is not diagnosed on average until 10 years after symptoms appear, and treatment may be delayed for another 10 years.[1] Two serial BD "biotypes" or "biostages" are recognized. An initial one, perhaps explaining presenting symptoms, involves an imbalanced neurotransmission consisting of excessive dopaminergic and glutamatergic transmission, reduced cholinergic muscarinic transmission, with disturbed serotonergic transmission.[1b,2] The later appearing biotype additionally includes cognitive decline, brain atrophy, and symptom worsening, and overlaps with the biotypes of schizophrenia, schizoaffective disorder, and major depressive disorder.[3]

Even with intensive treatment in academic centers, BD therapy is inadequate and produces frequent side effects; on average, patients remain symptomatic for half the year. Thus, major challenges in the field are to identify more effective, less toxic drugs for treatment, and to deal with poor compliance. But drug development has not progressed markedly in the last decades, in part because BD pathology is not sufficiently understood and there is no accepted behavioral animal model for the disease.[1a] Identifying a drug target also is difficult because genomic studies implicate 90 or more risk alleles, each with only a small statistically significant effect.[4] One possible approach, however, is to try to understand mechanisms of action of the already FDA-approved mood stabilizers, using cell or animal biochemical models. These agents, the univalent ion lithium, valproate (2-propylpentanoate), carbamazepine (5*H*-dibenz[*b*,*f*]azepine-5-carboxamide), and lamotrigine (3,5-diamino-6-(2,3-dichloro-

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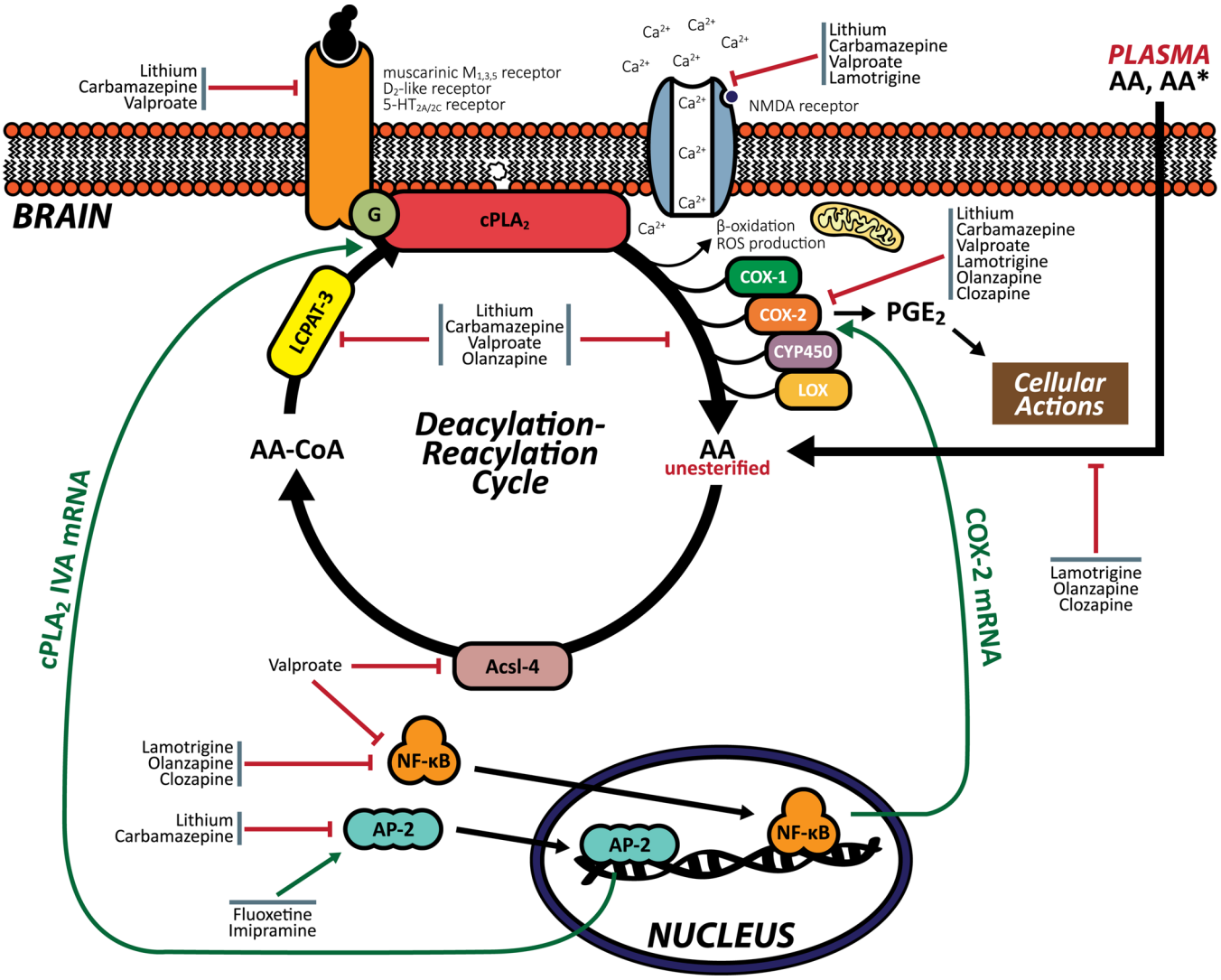

Figure 1. Model of brain arachidonic acid cascade initiated at synapse, with sites of action of mood stabilizers and atypical antipsychotics, based on studies in unanesthetized rats and in vitro. Arachidonic acid (AA), esterified within synaptic membrane phospholipid, is liberated following ligand binding to a neuroreceptor on the outer surface of the plasma membrane, which is coupled $cPLA_2$ activation by a G protein or Ca^{2+}. A fraction of liberated unesterified AA is converted to bioactive eicosanoids (e.g., PGE_2) by COX-2, lipoxygenase (LOX), COX-1, or cytochrome P450 epoxygenase (CYP450), which together with AA produce cellular actions. The larger remaining fraction is converted to AA-CoA by AA-selective acyl-CoA synthetase (Acsl)-4, then is re-esterified into membrane by lysophospholipid choline acyltransferase (LPCAT)-3. When administered chronically to rats, each of the four mood stabilizers interferes with neuroreceptor-mediated activation of $cPLA_2$, reduces COX-2 activity and PGE_2 concentration in the brain. Valproate, lamotrigine, and the antipsychotics olanzapine and clozapine also each reduce COX-2 gene transcription within the nucleus via NF-κB. Lithium and carbamazepine each reduce $cPLA_2$ IVA expression by reducing its gene transcription by AP-2, whereas valproate uncompetitively inhibits AA-selective Acsl-4. Both lithium and carbamazepine increase GRK-3, which may reduce G-protein neuroreceptor coupled activation of $cPLA_2$. The figure also illustrates diffusion of circulating unlabeled unesterified AA and radiolabeled AA* into the cellular unesterified AA pool that is available for reacylation.[18] See text for details. Prepared by Dr. Chuck T. Chen as adapted from Rao et al.[11a]

phenyl)-as-triazine), have no common structure that would suggest a specific common target.[1]

Since the discovery of lithium's efficacy against BD some 65 years ago,[5] multiple hypotheses have been suggested to explain its action,[1] some of which are presented in this volume. In this Review, I present evidence for the arachidonic acid (AA) cascade hypothesis, while other actively investigated hypotheses include the following: (1) Myo-inositol depletion (inhibition of inositol monophosphatase (IMPase) in the phosphatidylinositide cycle).[6] (2) Inhibition of glycogen synthase kinase-3β (GSK-3β).[7] (3) Inhibition of protein kinase C. This hypothesis has been proposed to explain the action of Tamoxifen against bipolar mania.[8] (4) Inhibition of NMDA/AMPA receptors. This hypothesis is consistent with evidence that both the *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine, and the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) antagonist riluzole, showed efficacy in bipolar depression.[9] It overlaps to some extent the AA cascade hypothesis, since each of the mood stabilizers blocks an AA signal caused by injected NMDA in rats (see below).

2. THE ARACHIDONIC CASCADE HYPOTHESIS

2.1. The AA Cascade. Unlike many of the other hypotheses, the AA cascade hypothesis for lithium's action does not identify a single enzyme, protein, or receptor target of lithium. Rather, this hypothesis encompasses a system of ordered metabolic reactions

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Table 1. Effects of Each of Four Chronically Administered Mood Stabilizers in Unanesthetized Rats on Arachidonic Acid Signaling Provoked by Acute Administration of NMDA, Dopaminergic D_2, Cholinergic muscarinic $M_{1,3,5}$ or Serotonergic 5-$HT_{2A/2C}$ agonist, NMDA, Quinpirole, Arecoline, and 2,5-Dimethoxy-4-iodoamphetamine (DOI)[a]

	drug effects on arachidonic acid signal			
receptor subtype	glutamatergic NMDA signal	dopaminergic D_2 signal	cholinergic muscarinic $M_{1,3,5}$ signal	serotonergic 5-$HT_{2A/2C}$ signal
coupling to $cPLA_2$	Ca^{2+} coupled		G-protein coupled	
agonist[b]	NMDA	quinpirole	arecoline	DOI
antagonist[c]	MK-801	raclopride or butaclamol	atropine	mianserin
mood stabilizers				
lithium	↓	↓	↑	↓[d]
carbamazepine	↓	↓	–[e]	–[e]
valproate	↓	↓	–[e]	–[e]
lamotrigine	↓	–[e]	–[e]	–[e]

[a]Specificity of a receptor effect was confirmed by blocking the AA signal in independent experiments with pretreatment with MK-801, raclopride or butaclamol, atropine, or mianserin prior to agonist injection. See text for references. [b]Agonist used to provoke signal in brain. [c]Antagonist that blocked signal in independent experiments. [d]Selective to auditory and visual brain areas. [e]Not tested. See Text for references.

involving AA and its metabolites, which can be modulated by receptor or biochemical events.[10] Within this "system," there can be several points of therapeutic intervention. The hypothesis also applies to each of the four mood stabilizers approved by the FDA for treating BD (but not to potential mood stabilizers proven ineffective), and further suggests related mechanisms of action of certain antidepressants and atypical antipsychotics, of aspirin, and of dietary intervention, with regard to BD symptoms (see below).[1a,11]

AA (20:4n-6) is a long-chain n-6 polyunsaturated fatty acid (PUFA) that, like the n-3 PUFA, docosahexaenoic acid (DHA, 22:6n-3), is esterified in millimolar concentrations in brain phospholipids, triacylglycerols, and cholesteryl esters, or is found in lower μmolar concentrations in its unesterified (free) form within cells, often bound to fatty acid binding proteins. Neither AA nor DHA can be synthesized de novo in vertebrates. Each must be absorbed through the diet or synthesized mainly within the liver by elongation of its shorter-chain nutritionally essential precursor, linoleic acid (LA, 18:2n-6) and α-linolenic acid (α-LNA, 18:3n-3), respectively.[10] AA and DHA are metabolized by separate but interacting metabolic systems or cascades within brain, which are regulated by enzymes often showing specificity for one or the other PUFA and its metabolites.[12] These enzymes often are functionally and transcriptionally coupled within the separate cascades, during brain development and aging.[13] Both AA and DHA and their metabolites have important second messenger actions in brain, affecting gene transcription, membrane fluidity, neurotransmission, electrical excitability, neuroinflammation, excitotoxicity, energy consumption, and other functions.[10]

Figure 1 illustrates some relevant pathways within the AA cascade, superimposed on an outline of synaptic and cell structure.[11a] In this illustration, the cascade is initiated at the outer plasma membrane surface when an agonist binds to a neuroreceptor that is coupled to Ca^{2+}-dependent AA-selective cytosolic phospholipase A_2 ($cPLA_2$).[12a] $cPLA_2$ hydrolyzes esterified AA from the stereospecifically numbered (*sn*)-2 position of synaptic membrane phospholipid, and can be activated via G-protein coupled cholinergic muscarinic $M_{1,3,5}$,[14] dopaminergic D_2-like,[15] or serotonergic 5-$HT_{2A/2C}$ receptors,[16] or following entry of extracellular Ca^{2+} into the cell due to glutamate binding to ionotropic NMDA or AMPA receptors.[17] Of the unesterified AA released into the cytoplasm, the largest fraction (about 95%) is recycled by conversion to AA-CoA by an acyl-CoA synthetase (Acsl) (selectively Acsl-4) with the consumption of 2 ATP,[18] and then is re-esterified by an acyltransferase (selectively lysophospholipid acyltransferase (LPCAT)-3[13,19]) into an available *sn*-2 position of membrane lysophospholipid. The smaller fraction is metabolized through enzymatic oxidation by cyclooxygenase (COX)-2, COX-1, cytochrome P450 epoxygenase (CYP450), or lipoxygenase (LOX), to produce multiple bioactive eicosanoid metabolites, including pro-inflammatory prostaglandin E_2 (PGE_2) and thromboxane B_2 (TXB_2). AA also can be β-oxidized within mitochondria, nonenzymatically converted to reactive oxygen species (ROS), or follow other degradative pathways.

In the pathological condition of neuroinflammation, which is associated with microglial activation, the AA cascade is chronically upregulated by a number of mechanisms. These include secretion of cytokines (e.g., interleukin (IL)-1β or tumor necrosis factor (TNF)-α) that stimulate astrocytic receptors that are coupled to activation of $cPLA_2$ and secretory $sPLA_2$,[20] and excess glutamatergic levels that stimulate neuronal NMDA and AMPA receptors and cause excitotoxicity.[21] Synaptic loss and apoptosis often accompany these changes.[22]

2. EFFECTS OF DRUGS USED IN BIPOLAR DISORDER ON ARACHIDONIC ACID CASCADE

2.1. Lithium and Other Mood Stabilizers Down-regulate Rat Brain AA Cascade. Figure 1 also identifies suggested sites of action of the four FDA-approved mood stabilizers within the AA cascade, as well as of olanzapine and clozapine (see below), based on experiments in unanesthetized rats and in vitro. At the apex of the cascade, each of the mood stabilizers can modulate AA hydrolysis by $cPLA_2$, initiated by agonist activation of certain neuroreceptors. The experimental pattern of modulation is consistent with their ability to rectify the proposed neurotransmission imbalance in early biostage BD (see above).[1a] This was shown by neuroimaging studies in partially restrained unanesthetized rats that had been treated chronically with vehicle or a mood stabilizer, having indwelling femoral vein and artery catheters.[18] Just before acute saline or drug was injected, radiolabeled [1-^{14}C]AA (*AA in Figure 1) was infused intravenously for 5 min, the animal was killed, and its brain was removed, frozen, and sectioned coronally for quantitative autoradiography. Regional incorporation coefficients k^* were quantified as the ratio of brain radioactivity to integrated arterial plasma radioactivity (input function), from which regional rates of incorporation, J_{in}, the product of k^* and unesterified unlabeled plasma AA, were estimated. J_{in} equals the rate of replacement by

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Table 2. Effects of Chronic Administration of Each of Four FDA Approved Mood Stabilizers, and of Topiramate and Gabapentin, on Different Aspects of the Rat Brain Arachidonic Cascade[a]

drug	AA turnover	DHA turnover	$cPLA_2$ activity, protein, mRNA	$iPLA_2$ activity, protein, mRNA	Acsl-4 activity	COX-1 protein	COX-2 protein	COX activity	PGE_2 concentration	TXB_2 concentration	AP-2	NF-κB
lithium	↓	NC[b]	↓	NC	NC	NC	↓	↓	↓	NC	↓	NC[f]
carbamazepine	↓	NC	↓	NC		NC	↓	↓	↓	↓	↓	NC
valproate	↓	NC[b]	NC	NC	↓	↓	↓[d]	↓	↓	↓	NC	↓
lamotrigine	↓[c]		NC	NC		NC	↓[d]	↓	↓	NC		↓
topiramate	NC	NC	NC	NC		NC	NC	NC				
gabapentin			↓[e]	NC		NC	↓	NC	NC	NC		

[a]See text for references. NC, no significant change. [b]Also no effect on palmitate turnover. [c]AA incorporation coefficient decreased. [d]mRNA also reduced. [e]Only mRNA reduced. [f]Chronic lithium did not reduce NF-κB in intact rat, but does so in neuroblastoma SH-SY5Y cells in vitro.

circulating unesterified AA of the AA that has been metabolically lost within brain.[18,23] Both k^* and J_{in} are unaffected by changes in cerebral blood flow, and thus, the imaging method can be used under pathological conditions and with changing functional activity.

As shown in Table 1, chronically administered lithium, valproate, carbamazepine, or lamotrigine, at a therapeutically relevant plasma level, each blocked the AA signal in response to 25 or 50 (lithium) mg/kg i.p. NMDA in unanesthetized rats.[24] Blockage by lithium and carbamazepine is consistent with their in vitro inhibition of NMDA-induced Ca^{2+} influxes,[25] and with therapeutic effects of NMDA or AMPA antagonists in bipolar depression[9] (see above). Lithium, carbamazepine, and valproate each also dampened the AA signal in rats injected with the D_2-like receptor agonist quinpirole,[26] while lithium decreased the signal in brain auditory and visual areas in response to DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride), a serotonergic 5-$HT_{2A/2C}$ receptor agonist.[27] Consistent with the proposed hypocholinergic neurotransmission in BD (see above), and lithium's proconvulsant action with physostigmine, chronic lithium *increased* the rat brain AA signal to the muscarinic $M_{1,3,5}$ agonist arecoline,[28] while also increasing brain glucose metabolism.[29] In separate experiments, each of the acute agonist-induced signals could be blocked by pretreatment with the specific receptor antagonist, confirming its specific receptor origin (Table 2).

Downstream in the cascade at the inner plasma membrane (Figure 1), G-protein receptor kinases (GRKs) modulate homologous desensitization of agonist activated G-protein coupled receptors, like those identified in Table 1. In rat brain, chronic lithium and carbamazepine each significantly increased GRK-3 expression in the membrane but not cytosolic fraction, which might desensitize the AA signal initiated at G-protein coupled D_2-like and other receptors.[30]

It is possible to quantify turnover of long chain fatty acids in brain phospholipids of partially restrained unanesthetized rats by infusing the radiolabeled fatty acid intravenously for 5 min, determining integrated plasma specific activity by repeated arterial sampling, then killing the rat and subjecting its brain to high energy microwaving to prevent post-mortem metabolic changes.[18] Fatty acid specific activity (radioactive/cold concentration) is measured in brain acyl-CoA (Figure 1), the precursor pool for fatty acid incorporation into phospholipid, and in plasma to calculate, as a ratio, a dilution factor λ. A mathematical model then is applied to determine fatty acid turnover in individual phospholipids and other kinetic parameters.[18] Using this approach, we showed that chronic lithium, carbamazepine or valproate each reduced AA turnover (deacylation−reacylation[31] (Figure 1)) in brain phospholipids of unanesthetized rats, while lamotrigine reduced AA incorporation into brain from plasma[32] (Table 2). The reductions were selective for AA, since lithium, valproate, or carbamazepine did not reduce DHA turnover, and lithium or valproate did not reduce palmitate turnover.[1a]

Related to their selective reduction of AA turnover, chronic lithium and carbamazepine each reduced transcription (mRNA level) and activity of AA-selective Ca^{2+}-dependent $cPLA_2$ IVA[12a] in rat brain, and expression of activator protein-2 (AP-2), a $cPLA_2$ IVA transcription factor, without changing expression of DHA-selective Ca^{2+}-independent $iPLA_2$ VIA or of $sPLA_2$ IIA (Table 2).[1a] Valproate did not modify expression of any of the three PLA_2 enzymes, but uncompetitively inhibited AA activation to AA-CoA by recombinant AA-selective Acsl-4 and by a microsomal rat brain preparation.[33] Lithium did not produce such inhibition. On this basis, we are testing in our turnover rat model proven nonteratogenic inhibitors of recombinant Acsl-4, such as the valproate amide derivative valnoctamide,[34] as potential new mood stabilizers for treating BD via their effect on the AA cascade.[35]

Further downstream in the cascade (Figure 1), COX-2 colocalizes and is functionally coupled with $cPLA_2$ IVA at postsynaptic sites in brain.[36] Each of the four mood stabilizers reduced brain COX-2 activity and PGE_2 concentration when given chronically to rats (Table 2). Chronic lithium did not reduce NF-κB in the intact rat,[37] but does so in neuroblastoma SH-SY5Y cells in vitro.[38] Valproate and lamotrigine additionally reduced COX-2 protein and mRNA and the COX-2 transcription factor, NF-κB.[1a] Chronic valproate also reduced brain mRNA levels for 87 genes and increased levels for 34 genes by at least 40%, indicating that its AA cascade effects are embedded in multiple other brain changes.[37a] Lithium's selectivity for the COX-2 pathway was illustrated by showing that it did not change expression of COX-1, 5-LOX, CYP450, or membrane PGE synthase-2 (mPGES-2) in rat brain.[39] At the resting state, it is thought that PGE_2 is produced from AA primarily via COX-2, TXB_2 via COX-1.[40] Lithium, lamotrigine or gabapentin did not change the rat brain TXB_2 concentration, while carbamazepine and VPA each reduced TXB_2.[24c,d,26c,41] Additionally, neither valproate nor carbamazepine altered 5-LOX or its product leukotriene B_4 (LTB_4), and carbamazepine did not change CYP450.[42]

An unexpected and as yet incompletely understood observation was that chronic lithium elevated rat brain concentration of antiinflammatory 17-hydroxy-DHA and other as yet unidentified DHA metabolites.[43] This elevation may contribute to the reported synergy between aspirin and lithium in BD patients, since 17-hydroxy-DHA is formed from DHA by acetylated COX-2 following exposure to aspirin (see below).[44]

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In contrast to the overlapping actions of the four FDA approved mood stabilizers, the antiepileptic topiramate (2,3:4,5-bis-*O*-(1-methylethylidene)-*β*-D-fructopyranose 1-sulfamate), which failed in phase III trials in BD I patients, did not change any measured rat brain AA cascade marker,[45] nor did gabapentin (1-(aminomethyl)cyclohexaneacetic acid), which also lacks efficacy.[41] Thus, the AA cascade model for lithium's action has a strong clinical-experimental correlation based on the rat studies with the six drugs.

2.2. Antidepressants That Switch Bipolar Depression to Mania Increase Rat Brain AA Metabolism. The AA cascade hypothesis has relevance for the effects of certain antidepressants and atypical antipsychotics in BD.[1b,11b] For example, the tricyclic antidepressant imipramine and the selective serotonin reuptake inhibitor (SSRI) fluoxetine are reported to increase "switching" of bipolar depression to mania when used as monotherapy or with mood stabilizers.[46] Bupropion, also an antidepressant but a norepinephrine and dopamine reuptake inhibitor and nicotinic antagonist, does not increase switching.[46] These clinical distinctions correlated with the different effects of the three drugs on rat brain AA metabolism. Thus, chronic fluoxetine and imipramine at therapeutically relevant doses increased AA turnover in rat brain phospholipids, as well as expression (activity, protein, mRNA, and phosphorylation) of cPLA$_2$ IVA and of its transcription factor subunit, AP-2*α*,[47] whereas chronic bupropion had no comparable effect. These results imply that the manic or hypomanic phase of BD has a higher brain AA metabolic rate than does the depression phase, a hypothesis that can be tested directly by PET imaging of brain AA metabolism using [1-^{11}C]AA or [^{18}F]AA.[48]

2.3. Atypical Antipsychotics Used in Bipolar Disorder Indirectly Decrease Rat Brain AA Metabolism. Atypical antipsychotics may act in part in BD by reducing the brain AA cascade, albeit indirectly and secondary to their effect on hepatic PUFA metabolism.[49] Olanzapine is an atypical antipsychotic that is FDA-approved for maintenance therapy in Bipolar I, as well as for bipolar mania, and it can rapidly dampen hyperactive motoric symptoms before mood stabilizers start to act.[1b] Clozapine shows efficacy in acute BD mania, rapid cycling BD, and as maintenance therapy in patients with refractory BD.[1b] As a class, atypical antipsychotics have a high affinity as antagonists for dopaminergic D$_2$ and serotonergic 5-HT$_2$ receptors. They may produce fewer motor side effects than typical antipsychotics such as haloperidol because of their ability to rapidly dissociate from D$_2$ receptors.[50]

Using our in vivo kinetic method, we found that both olanzapine and clozapine when chronically administered to rats reduced brain COX activity and PGE$_2$ concentration, plasma unesterified AA concentration, and AA incorporation into brain from plasma (Figure 1).[51] Olanzapine alone also reduced AA turnover within brain phospholipids, while clozapine alone also increased expression of DHA-selective iPLA$_2$ VIA and COX-1.

3. ADDITIONAL THERAPEUTIC APPROACHES TO BD INVOLVING THE BRAIN ARACHIDONIC ACID CASCADE

3.1. Low Dose Aspirin. In a pharmacoepidemiological study of patients taking lithium for an average duration of 847 days, patients receiving low-dose (30 or 80 mg/day) acetylsalicylic acid (aspirin) were significantly less likely to have a "medication event" (evidence of disease worsening) than patients on lithium alone, independently of use duration.[44] High dose aspirin given for short periods of time, nonselective COX inhibitors, selective COX-2 inhibitors, or glucocorticoids were not beneficial. As low dose aspirin does not increase serum lithium,[52] aspirin's synergistic effect with lithium likely was centrally mediated, particularly because it can enter the brain and inhibit AA metabolism.[53] Clinical trials with aspirin in BD currently are underway.[54]

A central positive effect of aspirin in BD is consistent with a report that aspirin given to men undergoing coronary angiography reduced depression and anxiety.[55] Of relevance, the COX-2 inhibitor celecoxib, although having low brain penetrability,[56] showed significant positive effects as adjunctive therapy in BD patients experiencing depressive or mixed episodes, and in depressed patients.[57]

The clinical data are consistent with the AA cascade hypothesis. Acetylation of COX-2 by aspirin reduces the ability of the enzyme to convert AA to pro-inflammatory PGE$_2$. Additionally, acylated COX-2 can convert AA to anti-inflammatory mediators such as lipoxin A4 and 15-epi-lipoxin A4, as well as DHA to anti-inflammatory 17-(*R*)-OH-DHA.[43a] Lithium similarly reduces rat brain COX-2 activity and PGE$_2$ concentration (Table 2), while increasing brain concentrations of 17-hydroxy-DHA and other potential DHA-derived anti-inflammatory metabolites.[43b]

3.2. Changing Dietary PUFA Composition Can Suppress Brain Arachidonic Acid Cascade. Brain concentrations of AA and DHA can be altered reciprocally by changing dietary PUFA concentrations, since brain AA and DHA concentrations depend on dietary intake and hepatic elongation from nutritionally essential LA and *α*-LNA, respectively.[49] Furthermore, decreases in dietary LA and increases in dietary *α*-LNA have been reported to be neuroprotective in animal models. In rats, reducing dietary *α*-LNA below a level considered to be PUFA "adequate" reduces brain DHA concentration and uptake, expression of DHA-selective iPLA$_2$ VIA, and of brain derived growth factor (BDNF) critical for neuronal integrity,[58] while it increases AA-metabolizing cPLA$_2$ IVA, sPLA$_2$ IIA and COX-2 activities. In contrast, reducing dietary LA below the "adequate" level reduces brain AA concentration, kinetics and enzyme expression, while reciprocally increasing corresponding DHA parameters.[59]

While data are controversial with regard to dietary intervention in the clinic, a cross-national study did identify a significant relation between greater DHA-containing seafood consumption and lower prevalence rates of BD.[60] Also, a review of clinical trials reported that increased dietary n-3 PUFA in combination with standard treatment improved bipolar depression, even taking into account sample bias.[61] In the future, one might maximize effects of dietary intervention by combining dietary n-3 PUFA supplementation with reduced dietary n-6 PUFA, which when compared to a standard diet was effective in a phase III trial in patients with migraine.[62] Migraine occurs in 30% of BD patients.[63]

4. ARACHIDONIC ACID CASCADE AND BIPOLAR DISORDER BRAIN

BD genetics provide minimal evidence if any for the AA cascade hypothesis. A significant association with a calcium-independent iPLA$_2$*β* (VIA) rs3788533 SNP (*PLA2G6*) in BD I has been reported, and the activity of this enzyme was elevated in plasma of patients with a history of psychoses.[64] However, iPLA$_2$ VIA is largely selective for DHA hydrolysis.[12a] Association with the gene for sPLA$_2$ on chromosome 12q23-q24.1 also has been noted.[65]

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On the other hand, there is abundant evidence for general and central inflammation as major contributing factors to BD, and inflammation is classically associated with upregulated AA metabolism and treated with nonsteroidal anti-inflammatory drugs like aspirin and Celebrex (see above).[66] Disturbed circulating concentrations of proinflammatory interleukins, TNF-α, and other cytokines have been reported in BD patients, related to the manic or depressive phase of the disease as well as to response to therapy.[66]

Studies on the post-mortem BD brain do show upregulated AA cascade markers, thus a potential target of the mood stabilizers, accompanied by evidence of neuroinflammation, excitotoxicity, apoptosis, and synaptic loss. The changes are not entirely specific to BD, as similar changes were demonstrated in schizophrenic and Alzheimer's disease brain tissue by the same investigators.[67] The changes may represent common late-stage neurodegeneration in each disease, consistent with evidence of biotype overlap (see above).[3]

In post-mortem BD compared with control prefrontal cortex, mean protein and mRNA levels of AA-selective $cPLA_2$ IVA, $sPLA_2$ IIA, COX-2, and membrane prostaglandin E synthase (mPGES) were significantly elevated, while levels of COX-1 and cytosolic cPGES were reduced. mRNA and protein levels of DHA-selective $iPLA_2$ VIA, of 5-, 12-, and 15-LOX, of thromboxane synthase (TXS) and of CYP450 did not differ from control values.[67a] In relation to the changes, BD compared with control cortex demonstrated decreased expression of antiapoptotic factors B-cell lymphoma (Bcl)-2 and BDNF, but increased expression of pro-apoptotic Bcl-2-associated X protein (BAX), Bcl-2 associated death promoter (BAD), and active caspase-3 and -9.[68] Higher levels also were noted of interleukin (IL)-1β, the IL-1 receptor (IL-1R), and of astrocyte and microglia activation markers, glial fibrillary acidic protein (GFAP), inducible nitric oxide synthase (iNOS), c-fos, and CD11b.[69] Significant synaptic loss, shown as reduced levels of presynaptic synaptophysin and postsynaptic dendritic spine drebrin, might explain the reported cognitive decline in BD.[68] Indeed, synaptic loss often is evident in conditions of neuroinflammation and excitotoxicity, associated with an upregulated AA cascade.[67b,70]

Despite the many similar changes, some differences between changes in post-mortem BD and schizophrenia brain are noteworthy. The dopamine reuptake transporter (DAT) is downregulated in post-mortem schizophrenia and BD frontal cortex,[71] consistent with responsiveness of both diseases to atypical antipsychotics. However, therapeutic responsiveness of BD but not schizophrenia to lithium and the other mood stabilizers that block the AA signal to NMDA in rat brain may relate to an increased glutamate signaling in BD but not schizophrenia brain. More than 90% of released glutamate is cleared from the synaptic cleft by the excitatory amino acid (reuptake) transporter (EAAT)2.[72] EAAT2 is downregulated in the BD cortex, as are NMDA receptor (NR) subunits NR1 and NR2, consistent with increased glutamatergic activity (see above).[9,69] EAAT3 and EAAT4 are unchanged while EAAT1 is elevated in BD.[71] In schizophrenic cortex, EAAT1, EAAT3 and EAAT4 are each upregulated while EAAT2 expression is unchanged,[71] consistent with decreased NMDA function in schizophrenia.[73] Decreased binding of cholinergic muscarinic receptors in BD[74] is consistent with lithium having an effect by upregulating the cholinergic muscarinic AA signal (Table 1).

5. DISCUSSION

BD represents a complex set of symptoms that evolve over time, incompletely characterized pathophysiology, with multiple contributing genetic factors of low effects, and without an agreed-on behavioral animal model. There appear to be two biostages, an initial one involving imbalance in neurotransmission—hyperglutamatergic and hyperdopaminergic transmission, reduced cholinergic and altered serotonergic transmission, and a later appearing stage with superimposed neurodegenerative components associated with cognitive decline, symptom worsening and brain atrophy, which overlaps with biotypes of other neuropsychiatric disorders.

In this section, I review the AA hypothesis for the action of lithium, and show that the hypothesis extrapolates to the actions of the other FDA-approved mood stabilizers carbamazepine, valproate and lamotrigine, but not to topiramate or gabapentin, each of which failed phase III trials in BD patients.

The AA cascade hypothesis proposes that lithium and the other mood stabilizers downregulate brain AA metabolism at different entry points (Figure 1). This suggested target of mood stabilizers is consistent with studies showing upregulated cascade markers in post-mortem BD prefrontal cortex, and can be tested further in patients using PET to image brain AA metabolism. The AA cascade hypothesis also may explain high switching rates of BD depression to mania caused by the antidepressants fluoxetine and imipramine, and some treatment effects of olanzapine and clozapine. Further, it is supported by a pharmacoepidemiological study showing that chronic low dose aspirin reduced morbidity of patients taking lithium, and by evidence that high n-3 and/or low n-6 PUFA diets are helpful in BD and migraine patients.

Lithium and the other mood stabilizers have proven effective in BD, but they do not always work, and they may work in some individuals based on genetic specificity.[75] Each also has unwanted side effects, leading to incomplete compliance and polypharmacy.[1] Based on the AA cascade hypothesis and the suggested targets of the current drugs at different sites within the cascade, future research should aim to develop less toxic and more effective mood stabilizers. This Review suggests that this aim might be promoted by prescreening potential drug candidates for their ability to reduce brain AA turnover and metabolic markers in unanesthetized rodents, using our established kinetic methods and model.[1a,18] Our data also suggest that the animal model for drug screening need not be a behavioral model, but rather might be the intact unanesthetized rodent on which brain lipid metabolic measurements can be performed. In the future, combining mood stabilizers with low dose aspirin or dietary intervention (high n-3/low n-6 PUFAs) may provide synergistic amelioration of BD symptoms and reduce disease progression.

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■ ABBREVIATIONS

AA, arachidonic acid; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP, activator protein; BAD, Bcl-2 associated death promoter; BAX, Bcl-2-associated X protein; BD, bipolar disorder; BDNF, brain derived neurotrophic factor; Bcl-2, B-cell lymphoma-2; COX, cyclooxygenase; CSF, cerebrospinal fluid; CYP450, cytochrome P450 epoxygenase; DAT, dopamine reuptake transporter; DHA, docosahexaenoic acid; DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride; EAAT, excitatory amino acid (reuptake) transporter; GFAP, glial fibrillary acidic protein; GRK, G-protein receptor kinase; GSK, glycogen synthase kinase; 5-HT, 5-hydroxytryptamine (serotonin); IL-1, interleukin-1; IL-1R, interleukin receptor; IMPase, inositol monophosphatase; iNOS, inducible nitric oxide synthase; LPCAT, lysophospholipid acyltransferase; LOX, lipoxygenase; LTB_4, leukotriene B_4; m-PGES-2, membrane PGE synthase-2; NMDA, *N*-methyl-D-aspartic acid; NF-κB, nuclear factor kappa B; NR, NMDA receptor; PET, positron emission tomography; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2; $cPLA_2$, cytosolic PLA_2; $iPLA_2$, calcium independent PLA_2; $sPLA_2$, secretory PLA_2; PUFA, polyunsaturated fatty acid; *sn*, stereospecifically numbered; SSRI, selective serotonin reuptake inhibitor; TNFα, tumor necrosis factor alpha; TXS, thromboxane synthase

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