Is the brain arachidonic acid cascade a common target of drugs used to manage bipolar disorder?

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Abstract

Although lithium has been used therapeutically to treat patients with bipolar disorder for over 50 years, its mechanism of action, as well as that of other drugs used to treat bipolar disorder, is not agreed upon. In the present paper, I review studies in unanesthetized rats using a neuropharmacological approach, combined with kinetic, biochemical and molecular biology techniques, demonstrating that chronic administration of three commonly used mood stabilizers (lithium, valproic acid and carbamazepine), at therapeutically relevant doses, selectively target the brain arachidonic acid cascade. Upon chronic administration, lithium and carbamazepine decrease the binding activity of activator protein-2 and, in turn, the transcription, translation and activity of its arachidonic acid-selective calcium-dependent phospholipase A2 gene product, whereas chronic valproic acid non-competitively inhibits long-chain acyl-CoA synthetase. The net overlapping effects of the three mood stabilizers are decreased turnover of arachidonic acid, but not of docosahexaenoic acid, in rat brain phospholipids, as well as decreased brain cyclo-oxygenase-2 and prostaglandin E2. As an extension of this theory, drugs that are thought to induce switching to mania, especially when administered during bipolar depression (fluoxetine and imipramine), up-regulate enzymes of the arachidonic acid cascade and turnover of arachidonic acid in rat brain phospholipids. Future basic and clinical studies on the arachidonic acid hypothesis of bipolar disorder are warranted.

Overview of arachidonic acid uptake and metabolism in the brain

Arachidonic acid (C20:4, n-6) is an ω-6 polyunsaturated fatty acid that must either be consumed in the diet or synthesized from its precursor linoleic acid (C18:2, n-6) in the liver. The brain contains relatively low levels of linoleic acid, and very little linoleic acid is converted into arachidonic acid within the brain [1]. Thus the brain relies on a steady supply of arachidonic acid from the plasma. Although several pools, including plasma lipoproteins and lysophospholipids, may contribute to brain arachidonic acid levels, their quantitative contribution remains unclear [2,3]. Plasma unesterified arachidonic acid enters the brain at a rate of 2–5 pmol/s per g of brain in rodents and 17.8 mg/day per whole brain in adult humans [4]. Upon its entry into the brain, arachidonic acid is activated by an Acsl (long-chain acyl-CoA synthetase) and can be esterified into the sn-2 position of neural phospholipids. Various phospholipase A2s which are activated via coupling to serotonergic (5-hydroxytryptaminergic) [5,6], glutamatergic [7,8], dopaminergic [9,10] and cholinergic [11,12] receptors release arachidonic acid from the sn-2 position of neural phospholipids. In general, cPLA2 (calcium-dependent cytosolic phospholipase A2), which is located at postsynaptic terminals, is thought to be selective for releasing arachidonic acid from the sn-2 position of neural phospholipids, whereas iPLA2 (calcium-independent phospholipase A2) is thought to release DHA (docosahexaenoic acid) (C22:6, n-3) from neural phospholipids [13,14]. Upon its release, a portion of the unesterified arachidonic acid is converted into oxygenated derivatives, including prostaglandins, leukotrienes and lipoxins, a portion can be subjected to β-oxidation, and the remainder (approx. 97% under basal conditions) is activated by Acsl and ultimately recycled and re-esterified into the sn-2 position of neural phospholipids [2,15]. In rodents, the net rate of arachidonic acid release from brain phospholipids is 200–400 pmol/s per g of brain, leading to brain phospholipid turnover rates of 10%/h or complete turnover just over twice per day. We have estimated previously that the turnover of DHA consumes approx. 0.1% of the brain’s 208 nmol/s per g of brain ATP [13]. To maintain the turnover of arachidonic acid within brain phospholipids would require approx. 0.3% of the brain’s ATP consumption.

Although arachidonic acid has been extensively studied for its role in growth and development [16], it is now also recognized that arachidonic acid and its derivates act as secondary messengers where they are ligands for several key transcriptional regulators, including PPARs (peroxisomal-proliferator-activator receptors) [17], HNF-4α (hepatic nuclear factor-4α) [18], prostaglandin receptors [19] and LXR (liver X receptor) [20]. Within the brain,
arachidonic acid is released from the sn-2 position of neural phospholipids by PLA₂ activation coupled to dopaminergic, cholinergic, glutamatergic and serotonergic stimulation, via G-proteins or calcium [21]. Although the signals that arachidonic acid and its derivates relay are not completely understood, with regard to the bipolar disorder, they have been reported to play a role in regulating blood flow [22], neuroinflammation [23,24], excitotoxicity [25], the sleep/wake cycle [26] and neurogenesis [27].

**Measuring brain arachidonic acid turnover *in vivo***

Because arachidonic acid from the plasma unesterified pool rapidly enters the brain, it is possible to trace the uptake and metabolism of arachidonic acid, or other fatty acids, from this pool into brain phospholipids with radiotracers. The short infusion (5 min) of albumin-bound arachidonic acid into the femoral vein of the rat upon achieving steady state along with measuring brain phospholipid radioactivity at the end of the infusion, is used to calculate a plasma/brain incorporation coefficient. This incorporation coefficient when multiplied by the concentration of non-labelled arachidonic acid in the plasma unesterified pool is used to calculate the net rate of arachidonic acid entry from the plasma unesterified fatty acid pool into brain phospholipids. Because fatty acids pass through their respective acyl-CoA pool before entering brain phospholipids, correcting the previously calculated net rate of entry into brain phospholipids from the plasma unesterified pool by the ratio of plasma unesterified and brain acyl-CoA specific activities gives the net rate of entry into brain phospholipids from the brain acyl-CoA pool [15,28,29]. With the advent of positron (¹¹C) labelled arachidonic acid, it is now possible to image aspects of brain arachidonic acid metabolism in humans [30,31].

**Approaches to studying bipolar disorder**

Several approaches including epidemiological, genetic and clinical studies have suggested that bipolar disorder is likely to be a multifactorial highly polygenic disorder [32,33]. Owing to a lack of an accepted animal model of bipolar disorder, pre-clinical approaches have attempted to elucidate the mechanism of action of drugs used to manage bipolar disorder symptoms in model systems [34]. Potential targets of bipolar disorder drugs currently under examination include myo-inositol [35,36] and GSK3 (glycogen synthase kinase 3) [37]. In 1996, a study published from Stanley Rapoport’s laboratory suggested that the turnover of arachidonic acid may be a therapeutic target of lithium [38]. Over the last decade, this initial observation has expanded to become what is known as the ‘arachidonic acid hypothesis of bipolar disorder’ [28,39–41]. The present review focuses on how administering therapeutically relevant doses of drugs used to manage bipolar disorder symptoms to rodents target the brain arachidonic acid cascade. Readers interested in other aspects of the arachidonic acid hypothesis of bipolar disorder, including implications for drug development, imaging bipolar disorder and neurotransmission, should consult other reviews [39–41].

**Lithium and the arachidonic acid cascade**

Lithium (Li⁺) (Figure 1A) was first used to treat bipolar disorder over 50 years ago [42] and is still one of the most commonly used mood stabilizers [43]. In 1996, Chang et al. [38] reported that rats, upon consuming a chow containing lithium chloride for 6 weeks, to produce a therapeutically relevant plasma level (~0.7 mM) [38,44] of lithium, had decreased arachidonic acid turnover within their brain phospholipids (Figure 2). This effect was specific for arachidonic acid, as turnover rates of DHA and of palmitic acid (C₁₆:₀) were not altered [45]. The decrease in arachidonic acid turnover after chronic lithium was ascribed to lithium’s ability to reduce brain expression (mRNA, protein, activity) of cPLA₂, sparing sPLA₂ (secretory PLA₂) and iPLA₂ [46,47], a finding that has been reproduced by chronic, but not acute, lithium administration (2 mM) to astrocyte cultures [48]. Because cPLA₂ is selective for arachidonic acid release from brain phospholipids [49], a reduction in its activity was thought to explain how chronic lithium selectively reduced the turnover of arachidonic acid, but not DHA or palmitic acid. Chronic lithium also decreased brain COX (cyclo-oxygenase)-2 activity and protein level, and PGE₂ (prostaglandin E₂) concentration [46], without altering the protein level of 5-lipoxygenase or cytochrome P450 [50]. Further investigation into the mechanism by which lithium decreased cPLA₂ transcription revealed a down-regulation of AP (activator protein)-2 DNA-binding activity, probably by the down-regulation of its AP-2α and AP-2β protein subunits as well as decreased PKC (protein kinase C) activity [51]. This effect was specific for AP-2, as there were no changes in the binding activity of four other transcription factors that regulate cPLA₂ gene expression, AP-1, GRE (glucocorticoid-response element), PEA3 (polyoma enhancer activator 3) and NF-κB (nuclear factor κB).

**Valproic acid and the arachidonic acid cascade**

As discussed above, in an attempt to test whether a down-regulation of the brain arachidonic acid cascade might be therapeutically relevant to lithium’s mode of action, Chang et al. [52] tested whether valproic acid would also decrease the turnover or arachidonic acid in unanesthetized rats. Valproic acid (valproate or 2-propylpentanoic acid) (Figure 1B) is a branched-chain carboxylic acid with demonstrated mood-stabilizing properties in the treatment of acute mania [53] and in mixed episodes [54]. Similar to chronic lithium, 30 days of valproic acid administration to rats, to produce therapeutically relevant plasma levels (0.2 mM) [52], was shown to decrease the turnover rate of arachidonic acid [52], but not of DHA [55], in rat brain phospholipids (Figure 2). However, unlike lithium, valproic acid did not down-regulate cPLA₂, AP-2-binding activity or any measured PLA₂ [52,56,57].
Because of valproic acid’s structural similarity to unesterified fatty acids, we assessed whether it would compete as a substrate for activation by Acsl. By isolating Acsl from rat brain microsomes, we were able to demonstrate that valproic acid inhibited Acsl activity and that its $K_i$ for inhibiting arachidonoyl-CoA formation was lower than that for inhibiting formation of docosahexaenoyl-CoA or palmitoyl-CoA [58], possibly explaining how valproic acid selectively down-regulates brain arachidonic acid turnover. Similar to lithium, chronic valproic acid decreased rat brain COX-2 activity and expression and PGE2 concentrations [56,57], without altering 5-lipoxygenase or cytochrome P450 protein levels and leukotriene B4 or thromboxane B2 concentrations [60]. Whereas lithium decreased PKC and the AP-2α and AP-2β subunits, carbamazepine selectively decreased cAMP-dependent PKA (protein kinase A) activity and the expression and phosphorylation of the AP-2α subunit. Carbamazepine did not alter other cPLA2 gene-regulating transcription factors (AP-1, NF-κB, GRE and PEA3) [62].

**Carbamazepine and the arachidonic acid cascade**

As a third test of the hypothesis that drugs used to treat bipolar disorder target the brain arachidonic acid cascade, we examined carbamazepine (5$H$-dibenz[b,f]azepine-5-carboxamide) (Figure 1C) as it is known to be beneficial in bipolar disorder [59]. Similar to lithium and valproic acid, chronic (30 days) carbamazepine administration, producing therapeutic relevant plasma levels (53.6 μM) [62], decreased the turnover of arachidonic acid, but not of DHA, in brain phospholipids of unanaesthetized rats [61] (Figure 2). Also, similar to lithium, the decreased arachidonic acid turnover was accompanied by decreased brain mRNA, protein and activity of cPLA2, but no change in sPLA2 or iPLA2 expression or activity [60]. Furthermore, chronic carbamazepine, like lithium and valproic acid, decreased brain COX-2 activity and PGE2 [46,60], without altering 5-lipoxygenase or cytochrome P450 protein levels and leukotriene B4 or thromboxane B2 concentrations [60]. Whereas lithium decreased PKC and the AP-2α and AP-2β subunits, carbamazepine selectively decreased cAMP-dependent PKA (protein kinase A) activity and the expression and phosphorylation of the AP-2α subunit. Carbamazepine did not alter other cPLA2 gene-regulating transcription factors (AP-1, NF-κB, GRE and PEA3) [62].

**Lamotrigine and the arachidonic acid cascade**

Lamotrigine [6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine] (Figure 1D) does not appear to delay the onset of mania patients with bipolar disorder, although it does delay the onset of depressive symptoms [63,64] and is effective in rapid cycling bipolar disorder [65,66]. Lamotrigine also decreases locomotive hyperactivity in the amphetamine model of mania [67]. Upon chronic administration to produce therapeutically relevant plasma levels in rats, lamotrigine did not decrease the turnover of arachidonic acid in their brain phospholipids; however, it did decrease the unidirectional incorporation coefficient for arachidonic acid into brain phospholipids [68]. Furthermore, similar to lithium, valproic acid and
Bipolar disorder drugs target the brain arachidonic acid cascade

Lithium and carbamazepine decrease the activity of AP-2, which in turn decreases the transcription of cPLA₂, which leads to decreased translation and activity of cPLA₂, while valproic acid decreases the activity of an Acsl. The net effect of lithium, carbamazepine and valproic acid is a decrease in the turnover of arachidonic acid. Lithium, valproic acid, carbamazepine and lamotrigine also decrease COX-2 expression. Whereas fluoxetine increases AUF-1 and imipramine increases AP-2, they both increase cPLA₂ and the turnover of arachidonic acids within brain phospholipids. Modified from [2] with permission.

Topiramate and the arachidonic acid cascade

To extend our hypothesis, we examined topiramate [2,3:4,5-bis-O-(1-methylethylidene)-β-D-fructopyranose sulfamate] (Figure 1E), a drug that Phase I clinical trials [70] and the quinpirole model of mania [71] had suggested to be effective in bipolar disorder, on brain arachidonic acid turnover. Despite achieving a therapeutically relevant plasma topiramate level of 18.1 μM after chronic treatment, and a decrease in body weight and serum leptin, as reported by others [72], topiramate did not alter cPLA₂ expression or any of the measured enzymes in the arachidonic acid cascade, nor did it alter the turnover of arachidonic acid or DHA in brain phospholipids of the unanaesthetized rat [73,74]. Supporting our negative findings, the results of four recent double-blind placebo-controlled trials demonstrate that topiramate is not an effective antibipolar disorder drug [75], a finding that was predicted by the arachidonic acid hypothesis [73,74].

Antidepressants and the arachidonic acid cascade

Because antidepressants can induce switching to mania when given bipolar disorder patients [43,76–78], we initially examined the selective serotonin (5-hydroxytryptamine)-re-uptake inhibitor fluoxetine [N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxyl]propan-1-amine] (Figure 1F) in the unaesthetized rat model. In contrast with lithium, valproic acid and carbamazepine, chronic fluoxetine increased the turnover of arachidonic acid in rat brain phospholipids (Figure 2). The increase in arachidonic acid turnover was also associated with increased cPLA₂ activity, protein and mRNA expression [79,80]. Unlike chronic lithium and carbamazepine, fluoxetine did not target AP-2-binding activity, but did increase protein levels of nuclear AUF-1 [AU-rich element/poly(U)-binding/degradation factor-1], possibly increasing the half-life of cPLA₂ mRNA. However, chronic fluoxetine did not alter COX-2 protein or PGE₂ levels [79]. As a second test of this hypothesis, Lee et al. [81] examined chronic administration of antidepressants with a high {[imipramine, 3-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)-N,N-dimethylpropan-1-amine]} (Figure 1G) and low {bupropion, 2-(t-butylamino)-1-(3-chlorophenyl)propan-1-one} (Figure 1H) risk of switching to mania. They reported that only imipramine, upon chronic, but not acute, administration, increased arachidonic acid turnover in rat brain phospholipids [81]. The increase in arachidonic acid turnover, in rats receiving chronic imipramine, was associated with a selective increase in cPLA₂ activity, protein and mRNA, as well as an increase in AP-2 expression. Also, similar to fluoxetine, chronic imipramine did not alter COX expression.

Conclusions

To date, the mechanism of action of mood stabilizers used to treat bipolar disorder is not agreed upon. The arachidonic acid hypothesis appears to predict drugs that will be therapeutic or associated with risk of inducing mania in patients with bipolar disorder. However, similar to other hypotheses, it is not known whether a down-regulation of the brain arachidonic acid cascade is necessary for the therapeutic effects of mood stabilizers. A small randomized clinical trial of adjunctive celecoxib in bipolar patients suggests that targeting the brain arachidonic acid cascade may produce a rapid-onset improvement in mood [82]. The confirmation and extension of these findings in rodents and other model systems as well as further testing of the arachidonic acid hypothesis of bipolar disorder in clinical settings is warranted.

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