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Chronic Valproate Treatment Blocks D_2-like Receptor-Mediated Brain Signaling via Arachidonic Acid in Rats

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Abstract

Background and Objective—Hyperdopaminergic signaling and an upregulated brain arachidonic acid (AA) cascade may contribute to bipolar disorder (BD). Lithium and carbamazepine, FDA-approved for the treatment of BD, attenuate brain dopaminergic D_2-like (D_2, D_3, and D_4) receptor signaling involving AA when given chronically to awake rats. We hypothesized that valproate (VPA), with mood-stabilizing properties, would also reduce the D_2-like-mediated signaling via AA.

Methods—An acute dose of quinpirole (1 mg/kg) or saline was administered to unanesthetized rats that had been treated for 30 days with a therapeutically relevant dose of VPA (200 mg/kg/day) or vehicle. Regional brain AA incorporation coefficients, k*, and incorporation rates, J_{in}, markers of AA signaling and metabolism, were measured by quantitative autoradiography after intravenous [1-^{14}C]AA infusion. Whole brain concentrations of prostaglandin (PG)E_2 and thromboxane (TX)B_2 also were measured.

Results—Quinpirole compared to saline significantly increased k* in 40 of 83 brain regions, and increased brain concentrations of PGE_2 in chronic vehicle-treated rats. VPA treatment by itself reduced concentrations of plasma unesterified AA and whole brain PGE_2 and TXB_2, and blocked the quinpirole-induced increments in k* and PGE_2.

Conclusion—These results further support our hypothesis that similar to lithium and carbamazepine, VPA downregulates brain dopaminergic D_2-like receptor-signaling involving AA.

Keywords

arachidonic acid; phospholipase A_2; valproate; D_2-like receptor; quinpirole; bipolar disorder

1. Introduction

Valproate (2-propylpentanoate, VPA) has a wide clinical spectrum of use in both psychiatric and neurological disorders. It is one of the most frequently used antiepileptic drugs, has mood-stabilizing properties in the treatment of acute mania (Bowden, 2009), and might be

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effective for the reduction of depressive symptoms of acute bipolar depression (Smith et al., 2010; Wang et al., 2010). Despite more than 40 years of clinical use, the mechanism(s) of action of VPA in bipolar disorder (BD) is still not fully understood. It is well known that VPA exerts multiple pharmacological effects, and has been found to affect glycogen synthase kinase-3 (GSK-3), the Wnt/β-catenin pathway, the extracellular signal-regulated kinase (ERK) pathway, γ-aminobutyric acid (GABA)ergic neurotransmission, N-methyl-D-aspartate (NMDA) glutamatergic signaling, pre- and post-synaptic dopamine (DA) neurotransmission, voltage-gated sodium and T-type calcium channels, histone acetylation, and brain lipids and their metabolism (Basselin et al., 2008a; Montezinho et al., 2006; Phiel et al., 2001; Rapoport et al., 2009; Yatham et al., 2002). In addition, VPA is neuroprotective in several models of neurodegenerative diseases (Monti et al., 2010; Monti et al., 2009).

Hyperdopaminergic neurotransmission is suggested to be involved in the pathophysiology of mania in BD (Berk et al., 2007; Cousins et al., 2009; Diehl and Gershon, 1992; Goetz, 1997). Reports show that administration of drugs that inhibit DAergic transmission (haloperidol, chlorpromazine) has antimanic action whereas drugs that stimulate DA synthesis (levodopa), bind to D_2-receptors (bromocriptine) or reduce DA reuptake (amphetamine) often precipitate mania (Anand et al., 2000; Cipriani et al., 2006; Peet and Peters, 1995). In this context, psychotic BD brains show higher D_2 receptor expression in the caudate and prefrontal cortex (Feng, 2008; Pearlson et al., 1995) and genetic studies have linked the DA reuptake transporter (DAT) and BD (Greenwood et al., 2001; Greenwood et al., 2006), with a DAT mutation causing inhibition of the transporter cell surface expression being associated with BD (Horschitz et al., 2005). Furthermore, analysis of postmortem cortex from BD patients shows significantly elevated levels of the neuronal calcium sensor-1 (NCS-1), which inhibits D_2 desensitization/internalization (Kabbani et al., 2002; Koh et al., 2003), changes in the levels of DA and cyclic adenosine 3':5'-monophosphate-regulated phosphoprotein of relative molecular mass 32,000 (DARPP-32) (Ishikawa et al., 2007; Zhan et al., 2011), and decreased protein and mRNA levels of DAT (Rao JS and Rapoport SI, unpublished data).

Dopaminergic D_2-like (D_2, D_3, and D_4) receptors in brain can be coupled *via* a Gαi/o-protein to Ca^{2+}-dependent cytosolic phospholipase ($cPLA_2$, EC 3.1.1.4), which when activated releases arachidonic acid (AA, 20:4n-6) from the stereospecifically numbered (*sn*)-*2* position of synaptic membrane phospholipid (Clark et al., 1995; Nilsson et al., 1998; Ong et al., 1999; Vial and Piomelli, 1995). AA is an important second messenger in brain with multiple effects, and a precursor of bioactive eicosanoids such as prostaglandin E_2 (PG)E_2 (Rapoport, 2008). Markers of the AA cascade have been reported to be abnormal in BD (Kim et al., 2011; Noponen et al., 1993). The brain AA signaling can be measured in unanesthetized rodents by infusing radiolabeled AA intravenously, quantifying integrated plasma radioactivity, using quantitative autoradiography to determine regional brain radioactivity due to tracer AA incorporated in membrane phospholipid, then applying a mathematical model to calculate AA incorporation coefficients and rates, k* and J_{in}, respectively (Rapoport et al., 2001; Robinson et al., 1992). Since the AA lost after release and metabolism cannot be synthesized *de novo* from 2-carbon fragments, or elongated significantly (< 1%) from its shorter chain polyunsaturated precursor, linoleic acid (18:2n-6) (Demar et al., 2006; Holman, 1986), k* and J_{in} for AA represent net AA consumption following release from phospholipid.

We previously showed using the intravenous infusion method that acute administration to unanesthetized rats of quinpirole (1 mg/kg, D_2-like receptor agonist) (Seeman and Van Tol, 1994), amphetamine or apomorphine (D_1/D_2 receptor agonist), but not the D_1-like receptor agonist, SKF-38393, increased k* and J_{in} for AA in many brain regions rich in D_2-like receptors, and that the increases could be blocked by pre-administration of a D_2-receptor

antagonist (e.g. butaclamol, raclopride) or of each of the two FDA-approved antimanic mood stabilizers, lithium and carbamazepine when given chronically (Basselin et al., 2005; Basselin et al., 2008b; Bhattacharjee et al., 2005; Bhattacharjee et al., 2006, 2008; Hayakawa et al., 2001). Each mood stabilizer downregulated brain AA turnover and/or reduced levels and activities of essential enzymes and metabolites of the brain AA cascade (Bazinet et al., 2006; Bosetti et al., 2002; Chang et al., 1996; Ghelardoni et al., 2004; Rao et al., 2007; Rao et al., 2005).

VPA also is approved as an antimanic mood stabilizer for BD, and when given chronically reduces AA turnover within brain phospholipids and decreases activity and concentrations of cyclooxygenase (COX) and its metabolites, respectively (Bosetti et al., 2003; Chang et al., 2001). We hypothesized that chronic administration of VPA to produce therapeutically relevant plasma levels, also would block the quinpirole-initiated AA signal and other AA cascade markers in rat brain. We applied our established *in vivo* fatty acid and activity methods, and measured AA incorporation coefficients ,k*, and rates, J_{in}, in each of 83 brain regions after acutely giving saline or quinpirole (1 mg/kg) to unanesthetized rats that had chronically received VPA (200 mg/kg/day, i.p) or vehicle for 30 days. Whole brain concentrations of prostaglandin PGE_2 and thromboxane $(TX)B_2$ were also measured.

2. Material and methods

2.1. Animals and Diets

Two-month-old male Fischer CDF 344 rats (Charles River Laboratories, Wilmington, MA) were acclimated for 1 week in an animal facility with regulated temperature, humidity and light cycle, and had free access to food and water. The diet (Rodent NIH-31 auto 18-4 diet, Zeigler Bros, Gardens, PA) contained (as % of total fatty acid) 20.1% saturated, 22.5% monounsaturated, 47.9% linoleic, 5.1% α-linolenic, 0.02% AA, 2.0% eicosapentaenoic, and 2.3% docosahexaenoic acid (Demar et al., 2006). Experiments were conducted following the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health Publication No. 86-23) and were approved by the Animal Care and Use Committee of *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. All efforts were made to reduce the number of animals used and to minimize animal suffering.

2.2. Drugs and Tracers

Radiolabeled [1-^{14}C]AA in ethanol (53 mCi/mmol, >98% pure, Moravek Biochemicals, Brea, CA) 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). VPA (sodium salt, Sigma-Aldrich)-treated rats received 200 mg/kg intraperitoneally (i.p) once daily for 30 days. VPA was dissolved in saline (0.9% NaCl, Hospira, Lake Forest, IL) as described previously (Basselin et al., 2008a; Bazinet et al., 2005; Bosetti et al., 2003; Chang et al., 2001). A control group received the same volume of saline (vehicle) under parallel conditions. An acute 1 mg/kg i.v. dose of (−)-quinpirole hydrochloride dissolved in saline (Sigma-Aldrich) was chosen because it produces widespread significant increments in k* for AA in the brain of unanesthetized rats that can be blocked by D_2-like receptor antagonists, butaclamol or raclopride, without causing convulsions (Basselin et al., 2005; Bhattacharjee et al., 2005).

2.3. Surgical Procedures and Tracer Infusion

On the morning of day 30, a rat was injected with the last VPA or vehicle dose, and then anesthetized with halothane (2–3% v/v in O_2). Polyethylene (PE 50) catheters were surgically inserted into the right femoral artery and vein as described previously (Basselin et al., 2005). The wound was closed with surgical clips and the rat was wrapped loosely, with

its upper body remaining free, in a fast-setting plaster cast taped to a wooden block. Surgery lasted 20–25 min. Rats were allowed to recover from anesthesia for 3–4 h in an environment maintained at 25°C. Rectal temperature was maintained 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, Nicholasville, KY). One minute after an i.v. injection of quinpirole or saline, [1-^{14}C]AA (170 μCi/kg, 2 ml) 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). Twenty min after beginning tracer infusion, the rat was euthanized with an overdose of Nembutal® (90 mg/kg, i.v.) and decapitated. The brain was removed (<30 s), frozen in 2-methylbutane maintained at −40°C in dry ice, and stored at −80°C until sectioned.

2.4. Chemical Analysis

Blood samples, collected before, during or after [1-^{14}C]AA infusion, were centrifuged immediately at 18,000 *g* for 30 s. Total lipids were extracted from plasma (30 μl) using a modified Folch procedure (Folch *et al*, 1957). One hundred μl of the lower organic phase was used to determine the radiolabeled unesterified plasma AA concentration by liquid scintillation counting. As previously reported (DeGeorge *et al*, 1989), greater than 95–98% of total plasma and brain radioactivity at 5 min following [1-^{14}C]AA infusion is radiolabeled AA. Concentrations of unlabeled, unesterified fatty acids were determined from frozen/thawed arterial plasma. Total lipids were extracted and separated by thin layer chromatography on 60 silica gel plates (Whatman, Clifton, NJ) using the solvent system heptane: diethylether:glacial acetic acid (60:40:3, v/v/v). Unesterified fatty acids (identified under UV light) were scraped from the plate and methylated with 1% H_2SO_4 (by vol) in anhydrous methanol (3 h at 70°C), then separated and quantified by gas-liquid chromatography using heptadecanoic acid (17:0) as an internal standard.

2.5. Quantitative Autoradiography

Quantitative autoradiography was performed as described earlier (Basselin et al., 2006a). A total of 83 brain regions from autoradiographs of coronal brain sections were identified from a stereotaxic rat brain atlas (Paxinos and Watson, 1987), 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 wet brain) by digital quantitative densitometry, using the public domain 1.62 Analysis NIH Image program. Regional brain incorporation coefficients k* (ml plasma/s/g wet brain) of AA were calculated as (Robinson *et al*, 1992),

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

c^*_{brain} (nCi/g wet brain wt) is brain radioactivity 20 min after beginning infusion, c^*_{plasma} (nCi/ml plasma) is arterial labeled unesterified AA, and t (min) is time after beginning [1-^{14}C] AA infusion. Integrated plasma radioactivity (input function) was determined by trapezoidal integration and used to calculate k* for each experiment. The regional rate of incorporation of unesterified AA from plasma into brain phospholipids, J_{in} (pmol/s/g), was calculated as follows:

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

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

2.6. Brain PGE_2 and TXB_2 Concentrations

In a separate experiment and after the last of 30 daily administrations of VPA or vehicle, a rat was injected with quinpirole (1 mg/kg, i.p) or saline. Twenty-one minutes later (Basselin et al., 2008b), it was anesthetized with Nembutal® (45 mg/kg, i.p.), and immediately subjected to head-focused microwave irradiation (5.5 kW, 3.8 s; Cober Electronics, Stamford, CT) to stop postmortem brain lipid metabolism (Farias et al., 2008; Poddubiuk et al., 1982). A half-brain was weighed, homogenized with 18 volumes of hexane:isopropanol (3:2, by volume) using a glass Tenbroeck homogenizer and the homogenates were centrifuged (800 g, 5 min). Tissue residues were rinsed with 3 × 2 volumes of the same solvent. The resultant lipid extracts were concentrated to dryness under N_2 and resuspended in the enzyme immunoassay buffer provided by the polyclonal PGE_2 and TXB_2 immunoassay kits (Oxford Biochemical Research, Oxford, MI).

2.7. Statistical Analyses

A paired *t* test using GraphPad Prism version 4.0b (GraphPad Software, San Diego, CA) was applied to compare mean physiological parameters in the same animal before and after drug injection. A standard two-way analysis of variance (ANOVA) was performed to compare chronic VPA and vehicle treatment with acute quinpirole vs. saline administration with regard to: integrated arterial plasma radioactivity input functions, plasma unesterified fatty acid concentrations, brain PGE_2 and TXB_2 concentrations, and regional values of k* and J_{in} for AA. If interactions between VPA and quinpirole were statistically insignificant, probabilities of effects of VPA and quinpirole were reported. If interactions were statistically significant, probabilities of main effects of VPA and quinpirole were not reported (Tabachnick and Fidell, 2001). Alternatively, a one-way ANOVA with Bonferroni's post-test was used to compare quinpirole and saline responses between chronic VPA- and vehicle-treated rats, as well as saline responses in VPA-treated compared with vehicle-treated rats. Data are reported as the mean ± SD, with statistical significance taken as $p \leq 0.05$.

3. Results

3.1. Physiology, behavior and Arterial Plasma Radioactivity

After 30 days of treatment, the mean body weight of VPA-treated rats was significantly lower than that of vehicle-treated rats (294.1 ± 25.9 g [n = 14] vs. 263.4 ± 21.0 g [n = 14], p = 0.002), as previously reported (Basselin et al., 2008a; Daoud et al., 2004; Hassel et al., 2001). There was no significant difference between rats chronically injected with VPA or saline with regard to rectal temperature, heart rate or arterial blood pressure (Table 1). Acute quinpirole provoked repeated cycles of an "active" period of repetitive head and mouth movements and sniffing, followed by a "calm" period (Horvitz et al., 2001). No significant difference in mean cycling periods was observed in VPA-treated compared to vehicle-treated rats (Table 1).

Neither chronic VPA nor acute quinpirole modified the time course of arterial plasma radioactivity (Eq. 1) following intravenous [^{14}C]AA infusion. The mean integral of radioactivity in the plasma organic fraction (nCi × s)/ml (n = 7), the input function, did not differ significantly among groups: chronic vehicle + saline, 149,317 ± 30,502; chronic vehicle + quinpirole, 152,433 ± 32,473; chronic VPA + saline, 121,565 ± 8,959; chronic VPA + quinpirole, 144,614 ± 24,116.

3.2. Plasma Concentrations of Unlabeled Unesterified Fatty Acids

A two-way ANOVA showed a significant VPA and quinpirole interaction for the plasma concentrations of unesterified stearic and AA but not for unesterified palmitic, oleic,

linoleic, α-linolenic, or docosahexaenoic acids (Table 2). A one-way ANOVA with Bonferroni's post-test showed that chronic VPA compared to vehicle significantly reduced plasma concentrations of stearic acid and AA by 39% and 66%, respectively. Compared with vehicle, chronic VPA had a significant main negative effect (−57% to −70%) on each of the remaining six unesterified fatty acids concentrations, while acute quinpirole had no main effect on any of these concentrations.

3.3. Regional Brain AA Incorporation Coefficients, k*

Figure 1 presents coronal autoradiographs of brains from rats treated chronically (30 days) with vehicle (control) or VPA, then acutely injected with saline or quinpirole. k* for AA, calculated by Eq. 1, is color-coded. The figure shows no difference in regional values of k* in response to saline between VPA- and vehicle-treated rats. Acute quinpirole increased k* in multiple brain regions of the chronic vehicle- but not of the VPA-treated rats. Data obtained from such autoradiographs are summarized in Table 3.

Values of the mean AA incorporation coefficients, k*, determined in each of 83 brain regions were subjected to a two-way ANOVA. Statistically significant interactions between VPA and quinpirole were found in 40 regions belonging primarily to the nigrostriatal and mesocorticolimbic systems, which comprise the DAergic circuits of the basal ganglia (Baldessarini and Tarazi, 1996) (Table 3, Fig 1). In all 40 regions, a one-way ANOVA with Bonferroni's post-test showed that chronic VPA did not significantly change mean baseline (after saline) k* in any region (Table 3). The same one-way ANOVA showed that acute quinpirole compared with saline increased k* by 22% to 58% in chronic vehicle-treated rats. Affected regions included caudate-putamen (36–43%), globus pallidus (45%), subthalamic nucleus (33%), substantia nigra (41%), prefrontal cortex (39–50%), primary olfactory cortex (35%), frontal cortex (29–38%), pyriform and anterior cingulated cortex (22%), motor (31–44%), somatosensory, auditory (29–33%), visual (41–48%) cortical areas (26–39%), bed nucleus of the stria terminalis (53%), amygdala (58%), nucleus accumbens (42%), ventral tegmental area (44%), arcuate nucleus of the hypothalamus (28%), ventroposterior thalamic nuclei (40–45%) and zona incerta (29%). Quinpirole compared to saline did not significantly increase k* in any of the 40 regions in chronic VPA-treated rats.

In the 43 regions where VPA and quinpirole interaction was statistically insignificant, neither VPA nor quinpirole had any significant main effect on k* for AA (data not shown). Thus, chronic VPA blocked each of the 40 quinpirole-induced k* increments that were observed in the chronic vehicle-treated rat.

3.4. Regional Rates of Incorporation of Unlabeled Unesterified AA into Brain

Rates of incorporation of unlabeled unesterified AA from plasma into brain, J_{in}, (data not shown) were calculated by Eq. 2 from regional k* (Table 3) and c_{plasma} for AA (Table 2). A two-way ANOVA showed no statistically significant interaction between VPA and quinpirole in any of the 83 brain regions examined. Chronic VPA compared with vehicle had a significant main negative effect in each of the 83 brain regions while acute quinpirole had no main effect on any. In vehicle-treated rats J_{in} ranged from 5.9 pmol/s/g in the internal capsule to 28 pmol/s/g in the choroid plexus, whereas J_{in} ranged from 2.2 pmol/s/g to 11 pmol/s/g in the respective areas of the VPA-treated rats.

3.5. Brain PGE_2 and TXB_2 Concentrations

As shown in Table 4, a two-way ANOVA demonstrated a statistically significant interaction between VPA and quinpirole with regard to the brain PGE_2 concentration. Consequently, a one-way ANOVA with Bonferroni's post-test showed that chronic VPA reduced basal brain concentrations of PGE_2 by 59 % ($p < 0.05$). Acute quinpirole significantly increased the

PGE_2 ($p < 0.01$) concentration by 1.7-fold in vehicle- but not VPA-treated rats. Chronic VPA reduced TXB_2 concentration at baseline and in response to quinpirole (significant VPA main effect) by 42%.

4. Discussion

In this study, we showed that daily administration of VPA (200 mg/kg, i.p.) to rats for 30 days, at a dose that produces a plasma VPA concentration relevant to BD, prevented the statistically significant increases in AA incorporation coefficients k*, and in whole brain PGE_2 concentration, that were produced by an acute dose of quinpirole in chronic vehicle-treated rats. To the extent that DAergic signaling *via* D_2-like receptors and the AA cascade are pathologically upregulated in BD patients, for which evidence exists (see "Introduction") (Berk et al., 2007; Cousins et al., 2009; Diehl and Gershon, 1992; Goetz, 1997; Kim et al., 2011), these results suggest that the efficacy of VPA in the disease treatment is due in part to its ability to dampen upregulated D_2-like signaling involving AA and its downstream metabolites. In agreement, chronic administration to rats of a therapeutically relevant plasma concentration of lithium or carbamazepine also dampens D_2-induced elevations in k* for AA and in brain eicosanoids (Basselin et al., 2005; Basselin et al., 2008b; Bosetti et al., 2002; Bosetti et al., 2003). Taken together, reduced D_2-like signal involving AA and its metabolites may be common to the therapeutic action of mood stabilizers effective in BD. In contrast, topiramate, which appeared effective in Phase II trials in BD, but later failed Phase III placebo-controlled trials (Kushner et al., 2006), did not change markers of the rat brain AA cascade (Ghelardoni et al., 2005; Lee et al., 2005). Topiramate has not been tested with regard to the D_2-like signal.

Similar to lithium and carbamazepine, chronic VPA significantly decreased baseline PGE_2 and TXB_2 concentrations as previously reported (Basselin et al., 2008a; Bosetti et al., 2002; Bosetti et al., 2003; Ghelardoni et al., 2004). We ascribe this to VPA selectively decreasing the binding activity of the transcription factor NF-κB that regulates neuronal COX-2 gene expression, as well as reducing COX-1 and COX-2 protein levels and whole brain COX activity (Bosetti et al., 2003; Kaltschmidt et al., 2002; Rao et al., 2007).

Acute quinpirole significantly increased k* for AA in 40 brain regions, most of which are rich in D_2-like receptors (Levant et al., 1992; Lidow et al., 1989) and are related to the topographical distribution of DAergic innervation in the brain (mesocorticolimbic, nigrostriatal, and tuberoinfundibular pathways). The zona incerta, located in the ventral thalamus, and cerebral cortical areas (layers I to VI) including auditory and visual cortex also contain DA neurons (Berger et al., 1985; Bjorklund and Lindvall, 1975; Lidow et al., 1989; Rivera and Chun, 2008). The globus pallidus, subthalamic nucleus, and ventrobasal thalamus also receive DAergic innervation and express DA receptors (Baldessarini and Tarazi, 1996; Govindaiah et al., 2010).

The mechanisms underlying VPA's ability to block the D_2-like-receptor-induced increases in k* for AA and to reduce PGE_2 and TXB_2 concentrations in rat brain are not clear. VPA could have acted by reducing COX activity, and COX-1 and COX-2 protein levels (Bosetti et al., 2003). When COX enzymes are pharmacologically inhibited or knocked out in rodent brain, k* responses to drugs acting at $cPLA_2$-coupled neuroreceptors are reduced or lost, as are the increases in brain PGE_2 and/or TXB_2 concentrations (Basselin et al., 2006b; Basselin et al., 2007b). VPA also may have interfered with the DAergic system and D_2-like receptors. Consistent with altered gene expression of histone deacetylases and increased in histone H3 and H4 acetylation in BD patients (Hobara et al., 2010; Sharma et al., 2006), VPA, a direct histone deacetylase inhibitor (Phiel et al., 2001), may modify the transcription of the rate-limiting enzyme in DA biosynthesis, tyrosine hydroxylase (D'Souza et al., 2009), or of Sp1

(Marinova et al., 2009), a transcription factor of the D_2 receptor (Yajima et al., 1998), and/or DAT gene acetylation (Wang and Bannon, 2005). Consistent with its property, VPA has been shown to decrease D_2 receptor protein in the rat prefrontal cortex (Montezinho et al., 2006), lower presynaptic DA function in the striatum of patients with mania (Yatham et al., 2002), and increase DAT gene expression in rat midbrain DA neurons (Wang et al., 2007), thus decreasing extracellular DA concentration at the synaptic cleft. Although VPA has been reported to inhibit GSK-3 (Chen G et al., 1999), which can be regulated by DA via the Akt signaling pathway (Beaulieu et al., 2004; Beaulieu, 2011), this effect is indirect and has been attributed to inhibition of activation of Akt and inactivation of GSK-3 following inhibition of histone deacetylase (Phiel et al., 2001; De Sarno et al., 2002).

In addition, a therapeutically relevant concentration of VPA has been shown to decrease the activity and protein level of protein kinase C (Chen et al., 1994), which mediates phosphorylation, desensitization and trafficking of the D_2 receptor (Namkung and Sibley, 2004). Alternatively, chronic VPA may have indirectly attenuated the D_2-mediated AA signaling by (i) enhancing GABAergic transmission, which participates in regulating the activity of DA release and inhibiting DAergic activity (Agmo et al., 1996) and/or by (ii) reducing excitatory neurotransmission and blocking the AA signaling mediated by glutamatergic-NMDA receptors (Basselin et al., 2008a), as D_2-like and NMDA receptors are often functionally coupled and colocalized on the same neurons in the brain (Cepeda and Levine, 1998; Wang et al., 2003). Together with our previous reports (Basselin et al., 2005, 2006a; Basselin et al., 2008b; Basselin et al., 2007a), we strongly infer that antimanic mood stabilizers effective in BD suppress AA signaling coupled to both NMDA and D_2-like receptors. Combined, these data are consistent with VPA protecting DA neurons in lipopolysaccharide-induced neurotoxicity (Peng et al., 2005). In agreement, VPA was neuroprotective in experimental models of cerebral ischemia, Parkinson's disease and glutamate-induced excitotoxicity *via* histone deacetylase inhibition (Monti et al., 2010; Monti et al., 2009; Ren et al., 2004; Wang et al., 2010; Chuang et al., 2009).

VPA increased brain-derived neurotrophic factor (BDNF) (Einat et al., 2003; Yasuda et al., 2009), hippocampal neurotrophin-3 (Walz et al., 2008), anti-apoptotic factor B-cell lymphoma-2 (Bcl-2) (Chen et al., 1999), and restored amphetamine-induced downregulation of BDNF and of neurotrophin-3 in rat brain (Frey et al., 2006; Walz et al., 2008). Given that the brain and serum in BD have reduced BDNF and other neurotrophic factors (Kauer-Sant'Anna et al., 2009; Kim et al., 2010; Knable et al., 2004; Tramontina et al., 2009), these actions may contribute to VPA's neuroprotective effect in BD, a disease characterized by progression and apoptosis (Benes et al., 2006; Kim et al., 2010; Rapoport et al., 2009).

Consistent with our previous studies, chronic VPA significantly decreased the plasma concentration of unlabeled unesterified fatty acids including AA (Bazinet et al., 2005; Chang et al., 2001), indicating a widespread effect on whole body fatty acid metabolism. A similar reduction in plasma unesterified fatty acids has been found with other mood stabilizers and antipsychotics used to treat BD, such as lamotrigine, olanzapine and clozapine (Ramadan et al., 2011; Cheon et al., unpublished observation) suggesting a common peripheral effect of these drugs. The decrease in plasma unesterified fatty acids may be due to (i) reduced liver secretion of lipoprotein-bound esterified fatty acids, the main source of unesterified fatty acids in plasma, or (ii) reduced release of unesterified fatty acids from adipose tissue by lipases. Although the effects of VPA on free fatty acid release have not been investigated, evidence of impaired secretion of esterified fatty acids has been demonstrated with a marked reduction in triglyceride secretion following VPA treatment (Bellringer et al., 1988).

The baseline values of k* and J_{in} in this study agree with previous reports (Basselin et al., 2005; Basselin et al., 2008a; Bhattacharjee et al., 2005; Bhattacharjee et al., 2006, 2008). Values of baseline k* were not altered by chronic VPA, which is consistent with our previous data (Basselin et al., 2008a), and supports the finding that chronic VPA does not affect basal cPLA$_2$-IV expression (Bosetti et al., 2003). Chronic VPA had a significant main negative effect on all J_{in} values at baseline compared to vehicle-treated rats, indicating that the regional rate of metabolic AA loss from brain is lower in the VPA-treated animals. We ascribe this to VPA's significant reduction of the plasma concentration of unlabeled unesterified AA and of brain PGE$_2$ and TXB$_2$ concentrations, and to its selective inhibition of acyl-CoA synthetase 4-mediated activation of AA to AA-CoA (Bazinet et al., 2006a; Shimshoni et al., 2011).

Chronic VPA, like chronic carbamazepine but unlike chronic lithium, did not prevent quinpirole-induced hyperactivity or stereotypy (Basselin et al., 2005; Basselin et al., 2008b; Beaulieu et al., 2004; Shaldubina et al., 2002). As each of the three mood stabilizers downregulates the brain AA cascade, their different effects on quinpirole-induced behaviors suggest that these behaviors do not involve AA signaling, and that the quinpirole-induced activity cycles are not modeling BD. In contrast, VPA attenuated the hyperactivity and preservative locomotor behavior in the DAT knockdown mice (Ralph-Williams et al., 2003).

We investigated the effects only of chronic VPA in this study, mood stabilization properties in BD patients only appears after 10 days of treatment with VPA. An acute injection of VPA (200–300 mg/kg) in rats caused no/very transient change in the brain DA level (Ahmad et al., 2005; Mitsikostas et al., 1993).

In conclusion, chronic VPA pretreatment prevented the statistically significant increases in k* for AA and in PGE$_2$ concentrations that were observed in response to quinpirole in chronic vehicle-treated rats. These and observations in rats administered chronic lithium or carbamazepine support the hypothesis that mood stabilizers commonly downregulate brain AA signaling *via* D$_2$-like receptors, and are consistent with evidence that some BD symptoms arise from excessive DAergic neurotransmission (Goetz, 1997). It would be worthwhile to see if atypical antipsychotics (e.g. clozapine, olanzapine), which are D$_2$-like receptor antagonists, do so as well, which would suggest a more general receptor action of these agents on cPLA$_2$-mediated AA signaling (Liauw and McIntyre, 2010). Additionally positron emission tomography using [1-^{11}C]AA might be employed in BD patients under or without chronic VPA treatment, before and after drug induced D$_2$-like receptor activation, to see if VPA has a similar transient effect on AA signaling BD (Giovacchini et al., 2004; Goetz, 1997; Hosey et al., 2005).

Highlights

- The research identifies VPA's ability to downregulate dopamine-D$_2$ receptor signaling via AA.
- Quinpirole increases AA signaling and metabolism in vehicle-treated rats.
- Chronic VPA blocks increments in AA signaling and metabolism induced by quinpirole.
- Mood stabilizers attenuate hyperdopaminergic neurotransmission.
- Possible implication for the efficacy of mood stabilizers against bipolar disorder.

Abbreviations

AA	arachidonic acid
BD	bipolar disorder
BDNF	brain-derived neurotrophic factor
cPLA$_2$	Ca^{2+}-dependent cytosolic phospholipase A_2
COX	cyclooxygenase
DA	dopamine
DAT	dopamine reuptake transporter
GSK-3	glycogen synthase kinase-3
VPA	valproate
PGE$_2$	prostaglandin E_2
TXB$_2$	thromboxane B_2

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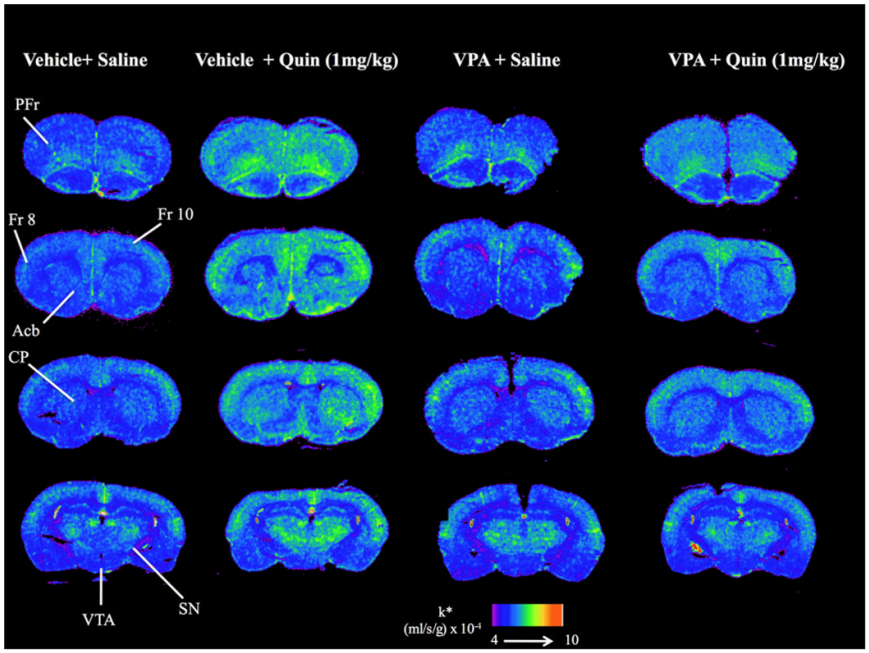

Figure 1.

Coronal autoradiographs of brain showing effects of quinpirole and valproate on regional arachidonic acid incorporation coefficients k* in rats. Values of k* (ml/s/g wet brain × 10^{-4}) are on a color scale from 4 (blue) to 10 (orange). Acb, nucleus accumbens; CP, caudate putamen; Fr 8, frontal cortex (8); Fr 10, frontal cortex (10); PFr, prefrontal cortex; SN, substantia nigra; VTA, ventral tegmantal area. VPA, valproate; Quin, quinpirole.

Table 1

Physiological parameters following drug administration in unanesthetized rats

	Chronic Vehicle				Chronic Valproate			
	Saline		Quinpirole		Saline		Quinpirole	
	Before	After	Before	After	Before	After	Before	After
Rectal Temperature (°C)	36.8 ± 0.5	36.7 ± 0.4	36.8 ± 0.3	36.9 ± 0.2	36.9 ± 0.4	37.0 ± 0.3	36.8 ± 0.4	37.0 ± 0.2
Heart Rate (beats/min)	414 ± 26	402 ± 48	409 ± 42	430 ± 30	425 ± 37	434 ± 27	441 ± 31	433 ± 53
Arterial blood pressure								
(mmHg)								
Systolic	178 ± 13	166 ± 7	174 ± 10	181 ± 13	175 ± 10	166 ± 8	162 ± 14	162 ± 11
Diastolic	109 ± 8	101 ± 7	109 ± 7	103 ± 7	109 ± 6	96 ± 11	102 ± 7	100 ± 6
Orofacial activity duraion (s)								
Orofacial activity				10 ± 2			11 ± 3	
Calm period				17 ± 4			15 ± 3	

Values are means ± SD (n= 7) measured before drug injection (quinpirole 1mg/kg, i.v.) and 10 minutes after [^{14}C] AA infusion. Paired t-tests were used to compare means in the same animal before and after drug injection.

Table 2

Effects of Valproate and Quinpirole on plasma unesterified fatty acid concentrations.

	Chronic Vehicle		Chronic VPA		VPA X Quin Interaction	VPA Effect	Quin Effect
Fatty Acid	**Saline**	**Quin**	**Saline**	**Quin**	**P-value**	**P-value**	**P-value**
			(nmol/ml plasma)				
Palmitic(16:0)	199.9 ± 82.8	164.3 ± 43.1	75.7 ± 20.4	70.6 ± 15.9	0.4133	< 0.0001	0.2768
Stearic(18:0)	49.2 ± 10.1	36.6 ± 14.3	30.0 ± 4.8**	33.7 ± 5.9	0.0334		
Oleic(18:1n-9)	124.0 ± 50.8	89.9 ± 42.4	53.2 ± 15.8	45.2 ± 11.1	0.3264	0.0002	0.1192
Linoleic (18:2n-6)	181.9 ± 73.3	146.5 ± 68.1	70.2 ± 19.4	72.0 ± 16.9	0.3505	< 0.0001	0.3977
α-Linolenic (18:3n-3)	11.9 ± 5.3	10.3 ± 4.4	4.1 ± 1.5	3.8 ± 1.5	0.6485	< 0.0001	0.4819
Arachidonic (20:4n-6)	16.0 ± 6.1	12.6 ± 2.9	5.5 ± 1.0***	7.9 ± 1.9	0.0411		
Docosahexaenoic (22:6n-3)	19.5 ± 10.2	15.7 ± 4.8	5.8 ± 1.6	6.4 ± 1.3	0.3199	< 0.0001	0.4665

Values are means ± SD (n=7) measured from arterial plasma collected at 19 min after the beginning of [1-^{14}C] AA infusion.

** P< 0.01,

*** P < 0.001;

vehicle plus quinpirole vs vehicle plus saline, VPA plus saline vs vehicle plus saline, and VPA plus quinpirole vs VPA plus saline (one-way ANOVA Bonferroni's tests). VPA, valproate; Quin, quinpirole.

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Table 3

Chronic Valproate blocked Quinpirole-induced increments in arachidonic acid incorporation coefficients, k*, in dopaminergic brain regions

	Chronic Vehicle		Chronic VPA		VPA × Quin Interaction
	Saline	Quin	Saline	Quin	
Brain region					**P-value**
Nigrostiatal system					
Caudate putamen					
Dorsal	5.65 ± 0.82	7.69 ± 1.30***	6.37 ± 0.21	5.97 ± 0.90	0.0015
Ventral	5.52 ± 0.76	7.90 ± 1.38***	6.25 ± 0.28	5.96 ± 0.97	0.0009
Lateral	5.69 ± 0.93	7.83 ± 1.38**	6.42 ± 0.22	6.12 ± 0.95	0.0027
Medial	5.52 ± 0.91	7.83 ± 1.15***	6.08 ± 0.31	5.56 ± 0.88	0.0002
Globus pallidus	5.01 ± 0.69	7.25 ± 1.15***	5.55 ± 0.42	4.55 ± 1.06	<0.0001
Subthalamic nucleus	5.87 ± 0.89	7.79 ± 1.37**	6.58 ± 0.48	6.05 ± 1.05	0.0035
Substantia nigra	4.87 ± 0.91	6.88 ± 1.46**	6.16 ± 0.52	5.59 ± 0.96	0.0028
Mesocorticolimbic					
Cortical					
Prefrontal cortex					
layer I	4.91 ± 0.45	7.34 ± 1.50***	5.68 ± 0.35	5.06 ± 0.39	<0.0001
layer IV	5.55 ± 0.78	7.74 ± 1.79**	6.27 ± 0.21	5.86 ± 0.45	0.0023
Primary olfactory cortex					
	5.34 ± 0.80	7.23 ± 1.21**	5.67 ± 0.47	5.16 ± 0.74	0.0010
Frontal cortex (10)					
Layer I	5.41 ± 0.81	7.48 ± 1.16***	5.77 ± 0.25	5.49 ± 0.55	0.0005
Layer IV	5.94 ± 1.06	8.22 ± 1.53***	6.30 ± 0.23	6.09 ± 0.66	0.0029
Frontal cortex (8)					
Layer I	5.86 ± 0.94	7.85 ± 1.20***	6.13 ± 0.24	5.69 ± 0.57	0.0007
Layer IV	6.64 ± 1.23	8.57 ± 1.42**	6.71 ± 0.35	6.93 ± 1.05	0.0489
Motor cortex					
Layer I	5.46 ± 0.94	7.35 ± 1.09**	5.74 ± 0.38	4.89 ± 1.22	0.0009

	Chronic Vehicle		Chronic VPA		VPA × Quin Interaction
	Saline	Quin	Saline	Quin	
Brain region					**P-value**
Layer II-III	5.85 ± 1.03	7.65 ± 1.35*	6.20 ± 0.31	5.47 ± 1.32	0.0051
Layer IV	6.44 ± 0.95	8.51 ± 1.73*	6.70 ± 0.27	6.06 ± 1.32	0.0062
Layer V	5.76 ± 1.28	7.98 ± 1.53**	6.42 ± 0.35	5.68 ± 1.35	0.0037
Layer VI	5.50 ± 1.23	7.91 ± 1.58**	6.13 ± 0.31	5.62 ± 1.17	0.0030
Somatosensory cortex					
Layer I	5.78 ± 0.85	7.29 ± 1.20*	5.91 ± 0.63	4.99 ± 1.18	0.0055
Layer II-III	6.12 ± 0.93	7.92 ± 1.39*	6.35 ± 0.27	5.54 ± 1.19	0.0028
Layer IV	6.41 ± 0.74	8.56 ± 1.51**	7.07 ± 0.26	6.15 ± 1.17	0.0006
Layer V	6.35 ± 0.82	8.13 ± 1.55*	6.52 ± 0.27	5.80 ± 1.17	0.0144
Layer VI	6.15 ± 0.89	8.03 ± 1.51**	6.41 ± 0.31	5.66 ± 1.09	0.0028
Pyriform cortex					
	4.55 ± 0.54	5.55 ± 1.09*	5.20 ± 0.45	4.82 ± 0.56	0.0163
Anterior cingulate cortex					
	6.91 ± 1.09	8.42 ± 2.57*	7.33 ± 0.40	6.39 ± 0.77	0.0363
Auditory cortex					
Layer I	5.40 ± 1.00	6.99 ± 1.18*	6.14 ± 0.52	5.78 ± 0.83	0.0095
Layer IV	5.88 ± 0.88	7.81 ± 1.23**	6.99 ± 0.49	6.90 ± 0.97	0.0084
Layer VI	5.57 ± 1.06	7.29 ± 1.05*	6.36 ± 0.62	6.32 ± 0.99	0.0216
Visual cortex					
Layer I	4.58 ± 0.61	6.77 ± 1.59**	5.79 ± 0.36*	5.25 ± 0.98	0.0014
Layer IV	5.43 ± 0.60	7.67 ± 1.35**	6.12 ± 0.73	5.97 ± 0.88	0.0024
Layer VI	4.86 ± 0.55	7.11 ± 1.39***	6.04 ± 0.72	5.62 ± 0.89	0.0010
Subcortical					
Bed nucleus stria terminalis					
	4.49 ± 0.59	6.89 ± 1.16***	5.14 ± 0.38	4.47 ± 0.62	<0.0001
Amygdala basolateral/medial					

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	Chronic Vehicle		Chronic VPA		VPA × Quin Interaction
	Saline	Quin	Saline	Quin	
Brain region					**P-value**
	4.38 ± 0.89	6.92 ± 0.98****	5.26 ± 0.71	5.02 ± 1.15	0.0007
Nucleus accumbens	4.99 ± 0.59	7.08 ± 0.90***	5.74 ± 0.32	4.99 ± 0.74	<0.0001
Ventral tegmental area	4.44 ± 0.93	6.40 ± 1.46**	5.67 ± 0.27	5.16 ± 0.89	0.0028
Tuberoinfundibular					
Arcuate nucleus hypothalamus					
	4.88 ± 0.45	6.22 ± 1.02*	5.39 ± 0.41	5.04 ± 1.03	0.0089
Other dopaminergic					
Zona Incerta	6.04 ± 0.79	7.82 ± 1.69**	6.93 ± 0.85	6.42 ± 1.44	0.0237
Ventroposterior					
lateral nucleus	5.28 ± 1.37	7.40 ± 1.46*	6.85 ± 0.84	6.34 ± 1.37	0.0126
medial nucleus	5.25 ± 1.16	7.61 ± 1.38**	6.51 ± 0.84	6.24 ± 1.41	0.0088

Each k* (ml/s/g wet brain) × 10^{-4} value is a mean ± SD (n = 7). Quinpirole administration: 1 mg/kg i.v., 1min.

* P< 0.05;

** P< 0.01;

*** P< 0.001;

vehicle plus quinpirole vs vehicle plus saline, VPA plus saline vs vehicle plus saline, and VPA plus quinpirole vs VPA plus saline (oneway ANOVA with Bonferroni tests). VPA, valproate; Quin, quinpirole.

Table 4

Effects of Quinpirole and Valproate on brain PGE_2 and TXB_2 concentrations in rats

	Chronic Vehicle		Chronic VPA		VPA × Quin Interaction	VPA Effect	Quin Effect
	Saline	Quin	Saline	Quin	P-value	P-value	P-value
PGE_2 (ng/g brain)	10.3 ± 3.1	17.7 ± 6.8**	4.2 ± 1.1*	5.3 ± 0.8	0.0348		
TXB_2 (pg/g brain)	41.3 ± 11.9	31.4 ± 9.1	22.2 ± 6.8	20.2 ± 9.0	0.2710	0.0002	0.1034

Each value is a mean ± SD (n = 7). Bonferroni's multiple comparison tests were performed.

* P< 0.05;

** P < 0.01;

vehicle plus quinpirole vs vehicle plus saline, VPA plus saline vs vehicle plus saline, and VPA plus quinpirole vs VPA plus saline. VPA, valproate; Quin, quinpirole.