Neuropsychopharmacology (2005) 30, 1064–1075

www.neuropsychopharmacology.org

Chronic Lithium Chloride Administration to Unanesthetized Rats Attenuates Brain Dopamine D_2-Like Receptor-Initiated Signaling Via Arachidonic Acid

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We studied the effect of lithium chloride on dopaminergic neurotransmission via D_2-like receptors coupled to phospholipase A_2 (PLA_2). In unanesthetized rats injected i.v. with radiolabeled arachidonic acid (AA, 20:4 n-6), regional PLA_2 activation was imaged by measuring regional incorporation coefficients k^* of AA (brain radioactivity divided by integrated plasma radioactivity) using quantitative autoradiography, following administration of the D_2-like receptor agonist, quinpirole. In rats fed a control diet, quinpirole at 1 mg/kg i.v. increased k^* for AA significantly in 17 regions with high densities of D_2-like receptors, of 61 regions examined. Increases in k^* were found in the prefrontal cortex, frontal cortex, accumbens nucleus, caudate–putamen, substantia nigra, and ventral tegmental area. Quinpirole, 0.25 mg/kg i.v. enhanced k^* significantly only in the caudate–putamen. In rats fed LiCl for 6 weeks to produce a therapeutically relevant brain lithium concentration, neither 0.25 mg/kg nor 1 mg/kg quinpirole increased k^* significantly in any region. Orofacial movements following quinpirole were modified but not abolished by LiCl feeding. The results suggest that downregulation by lithium of D_2-like receptor signaling involving PLA_2 and AA may contribute to lithium's therapeutic efficacy in bipolar disorder.
Neuropsychopharmacology (2005) **30,** 1064–1075, advance online publication, 6 April 2005; doi:10.1038/sj.npp.1300671

Keywords: lithium; phospholipase A_2; dopamine; quinpirole; arachidonic acid; bipolar disorder

INTRODUCTION

Lithium (Li^+) has been used to treat bipolar disorder for about 50 years, but its mechanism of action is not agreed upon (Barchas *et al*, 1994; Cade, 1999). One possibility is that it corrects a neurotransmission imbalance that contributes to the disease. Clinical evidence suggests that this imbalance is due to excessive dopaminergic activity but reduced cholinergic activity (Bunney and Garland-Bunney, 1987; Bymaster and Felder, 2002; Janowsky and Overstreet, 1995; Post *et al*, 1980). Thus, cholinometics as well as drugs that inhibit dopaminergic transmission (eg olanzapine, haloperidol) have an antimanic action in bipolar disorder (Bhana and Perry, 2001; Bymaster and Felder, 2002), whereas drugs that stimulate dopamine synthesis (levodopa), bind to dopamine receptors (bromocriptine), or reduce dopamine reuptake (amphetamine) often precipitate mania (Fisher *et al*, 1991; Peet and Peters, 1995; Sultzer and Cummings, 1989).

Some reported effects in rats of chronically administered lithium are consistent with its ameliorating the suggested cholinergic–dopaminergic imbalance of bipolar disorder. Thus, lithium reduces the convulsant threshold to the nonspecific cholinergic muscarinic agonist arecoline and to anticholinesterases (Evans *et al*, 1990; Jope, 1993; Lerer, 1985; Morrisett *et al*, 1987). In this regard, studies in knockout mice indicate that the agonist-induced convulsions are largely mediated by muscarinic M_1 receptors (Bymaster *et al*, 2003; Hamilton *et al*, 1997). However, lithium's effects on dopaminergic neurotransmission are not agreed upon, as lithium is reported to inhibit (Engel and Berggren, 1980; Friedman and Gershon, 1973), increase (Fadda *et al*, 1980; Hesketh *et al*, 1978), or have no effect (Bliss and Ailion, 1970; Ho *et al*, 1970) on brain dopamine turnover. Data on lithium's effects on brain dopaminergic D_2-like receptor density also are conflicting (Dziedzicka-Wasylewska and Wedzony, 1996; Reches *et al*, 1982; Staunton *et al*, 1982b; Wajda *et al*, 1983), as are data on its ability to modify haloperidol-induced supersensitivity (Carvey *et al*, 1990; Gallager *et al*, 1978; Pert *et al*, 1978; Pittman *et al*, 1984; Reches *et al*, 1982; Staunton *et al*, 1982a).

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Received 7 July 2004; revised 10 November 2004; accepted 7 December 2004
Online publication: 16 December 2004 at http://www.acnp.org/citations/NPP12160404031 5/default.pdf

Agonist binding to cholinergic $M_{1,3,5}$ (but not M_2 or M_4) receptors or D_2-like receptors can be coupled via a G-protein to the activation of phospholipase A_2 (PLA_2) (Axelrod, 1995; Bayon *et al*, 1997; Nilsson *et al*, 1998; Bymaster *et al*, 1999; Vial and Piomelli, 1995), so as to release the second messenger, arachidonic acid (AA, 20:4 n:6), from the stereospecifically numbered (*sn*)-2 position of membrane phospholipids. Nonesterified AA and its bioactive eicosanoid metabolites can influence many physiological processes, including membrane excitability, gene transcription, apoptosis, sleep, and behavior (Fitzpatrick and Soberman, 2001; Shimizu and Wolfe, 1990).

Of the nonesterified AA released by PLA_2 activation, studies in unanesthetized rats show that a fraction is rapidly reincorporated into brain phospholipids, whereas the remainder is lost by metabolism to eicosanoids, cannabinoids, β-oxidation, or other pathways (DeGeorge *et al*, 1991; Rapoport, 2001, 2003). The quantity lost is replaced rapidly by diet-derived nonesterified AA in plasma, since AA cannot be synthesized *de novo* in mammalian tissue and its precursor, linoleic acid (18:2 n-6), is not significantly elongated to AA in the adult brain (DeMar *et al*, 2004; Holman, 1986). Replacement is proportional to PLA_2 activation and can be imaged by injecting radiolabeled AA intravenously and measuring regional brain AA incorporation coefficients k^* (brain radioactivity/integrated plasma radioactivity) using quantitative autoradiography. k^* is independent of changes in cerebral blood flow and reflects only the activation process (Chang *et al*, 1997; DeGeorge *et al*, 1991; Rapoport, 2001, 2003; Robinson *et al*, 1992).

Using the fatty acid method, we reported that chronic LiCl feeding to rats, producing a therapeutically relevant brain Li^+ concentration, potentiated brain k^* responses to arecoline (Basselin *et al*, 2003b; Bayon *et al*, 1997; Bosetti *et al*, 2002b; DeGeorge *et al*, 1991; Jones *et al*, 1996). Such potentiation agreed with the hypothesis that lithium might correct a cholinergic–dopaminergic imbalance in bipolar disorder by upregulating cholinergic $M_{1,3,5}$ signaling via PLA_2. In the present study, we determined whether LiCl feeding would downregulate D_2-like receptor signaling via PLA_2, which would further support the imbalance hypothesis.

We measured k^* for AA in 61 brain regions of unanesthetized rats administered i.v. saline (controls) or the D_2-like receptor agonist, quinpirole, at 0.25 mg/kg and 1.0 mg/kg i.v. (Bach *et al*, 1980; Malmberg and Mohell, 1995; Seeman and Van Tol, 1994). We chose both doses, which have been used previously in behavioral studies (Bordi and Meller, 1989; Horvitz *et al*, 2001; Koene *et al*, 1993), to examine the dose–response relation to PLA_2 activation. Quinpirole, at 1 mg/kg i.v., is reported to increase k^* for AA in 24 of 29 brain regions having D_2-like receptors. The increases can be blocked by prior administration of the preferential D_2-like antagonist (+)-butaclamol (Bristow *et al*, 1998), or enhanced in rat brain regions ipsilateral to a chronic unilateral lesion of the substantia nigra, in proportion to their elevated D_2-like receptor density (Cory-Slechta *et al*, 1996; Eilam and Szechtman, 1989; Hayakawa *et al*, 2001). Quinpirole at the two doses chosen also can provoke orofacial activity and locomotion (Bordi and Meller, 1989; Horvitz *et al*, 2001; Koene *et al*, 1993), so we measured these as well.

Briefly, we found that 6 weeks of LiCl feeding completely blocked the quinpirole-induced increments in k^* for AA that were evident in control diet-fed rats, and altered, but did not block the stereotypic behavioral responses to quinpirole. Part of this work has been reported in abstract form (Basselin *et al*, 2003a).

MATERIALS AND METHODS

Animals and Diets

Experiments were conducted following the 'Guide for the Care and Use of Laboratory Animals' (National Institute of Health Publication No. 86-23) and were approved by the Animal Care and Use Committee of the National Institute of Child Health and Development (NICHD). Male Fischer CDF (F-344)/CrlBR rats (2 months old) (Charles River Laboratories, Wilmington, MA), weighing 180–200 g, were housed in an animal facility in which temperature, humidity, and light cycle were regulated. One group of rats was fed *ad lib* Purina Rat Chow (Harlan Telkad, Madison, WI) containing 1.70 g LiCl per kg in pelleted form for 4 weeks, followed by chow containing 2.55 g LiCl per kg for 2 weeks (Basselin *et al*, 2003b). This feeding regimen produces 'therapeutically equivalent' plasma and brain lithium levels of about 0.7 mM (Bosetti *et al*, 2002b; Chang *et al*, 1996). Control rats were fed lithium-free Purina rat chow under parallel conditions. Water and NaCl solution (0.45 M) were available *ad libitum* to both groups.

Drugs

Rats were injected intravenously with 0.9% NaCl, or with 0.25 or 1.0 mg/kg quinpirole hydrochloride (Sigma Chemical Co., Saint Louis, MO) in 0.30 ml 0.9% NaCl. [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, which contained 50 mg/ml of bovine serum albumin, as described elsewhere (Hayakawa *et al*, 2001). To confirm tracer purity, gas chromatography was performed after converting fatty acids to their methyl esters using 1 ml 1% sulfuric acid in anhydrous methanol (Makrides *et al*, 1994).

Surgical Procedures and Tracer Infusion

After 6 weeks on a control or a LiCl diet, a rat was anesthetized with halothane (2–3%) through a facemask connected to a vacuum scavenging system. Polyethylene catheters (PE 50) filled with 3% heparinized 0.9% NaCl were surgically inserted into the right femoral artery and vein. The wound was infiltrated with 1% lidocaine and closed with surgical clips. The rat was wrapped loosely, with its upper body remaining free, in a fast-setting plaster cast that was then taped to a wooden block, and allowed to recover from anesthesia for 3–4 h prior to isotope infusion. Body temperature was maintained at 37°C using a feedback heating element and rectal thermometer. Blood pressure and heart rate also were measured. Orofacial activity—sniffing, rigidity, and head turning (Bordi and Meller, 1989; Koene *et al*, 1993)—was monitored in a nonblinded

manner from the beginning of drug infusion to the end of the experiment.

One min after i.v. saline or quinpirole, the unanesthetized rat was infused for 5 min through the femoral vein with 2 ml [^{14}C]AA (170 μCi/kg), at a rate of 400 μl/min, using an infusion pump (Harvard Apparatus Model 22, Natick, MA). Timed arterial blood samples were collected during infusion and to the time of death at 20 min. At 20 min, when less than 1% of peak radioactivity remained in the plasma (DeGeorge *et al*, 1989; Zhou *et al*, 2002), the rat was killed with an overdose (50 mg/kg i.v.) of sodium pentobarbital (Richmond Veterinary Supply, Richmond, VA) and decapitated. The brain was removed, quickly frozen in 2-methylbutane at −40°C, and stored at −80°C for autoradiography.

Chemical Analysis of Plasma

Arterial plasma samples (30 μl) were extracted with 3 ml $CHCl_3$: MeOH (2 : 1, v/v) and 1.5 ml 0.1 M KCl, following a modified Folch technique (Folch *et al*, 1957). Aliquots of 100 μl of the lower organic phase were used to determine unesterified labeled AA concentrations by liquid scintillation counting. Percent efficiency of ^{14}C counting was 88%.

Quantitative Autoradiography

Brains frozen at −20°C in embedding medium were cut in 20-μm-thick serial coronal sections in a cryostat (Hacker Instruments, Fairfield, NJ). The sections were picked up on glass coverslips, dried on a hot plate at 55°C for at least 5 min, then placed sequentially in an X-ray cassette together with calibrated [^{14}C]methylmethacrylate standards (Amersham, Arlington Heights, IL). Sections and standards were exposed for 6 weeks to autoradiographic film (EMC1, Eastman Kodak Company, Rochester, NY), which was then developed in a dark room. Specific regions were identified by comparing the autoradiographs with an atlas and with published autoradiographs of rat brain (Hayakawa *et al*, 2001; Paxinos and Watson, 1987), and were sampled in both hemispheres. The average of three bilateral measurements for each region from a given brain was used to calculate the regional radioactivity (nCi/g) by digital quantitative densitometry (public domain NIH Image analysis program (version 1.62)). Regional incorporation coefficients of AA from plasma into brain phospholipids, k^* (ml/s/g brain), were calculated as

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

where c^*_{brain} (20 min) nCi/g equals brain radioactivity at the time of death, c^*_{plasma} nCi/ml equals plasma AA radioactivity determined by scintillation counting, and t equals time after beginning [^{14}C]AA infusion.

Statistical Analysis

One-way ANOVA, with Tukey–Kramer's *post hoc* test with correction for five comparisons, was performed in each of the 61 brain regions, to compare quinpirole and i.v. saline responses between control diet and LiCl-fed rats, as well as between i.v. saline responses in LiCl-fed rats and control diet rats. Other comparisons were not considered relevant. Corrections for multiple comparisons across regions were not made because the purpose of the study was to identify the regions that were involved in individual drug and group-diet effects. Additionally, unpaired *t*-tests were used to compare the durations of behavioral activity and calm periods between the two diet groups. The GraphPad Prism version 3.0a for Macintosh (GraphPad Software, San Diego, CA, http://www.graphpad.com) was used for these comparisons. Data are reported as means ± SD, with statistical significance taken as $p \leqslant 0.05$.

RESULTS

Physiological Parameters and Behavioral Responses

LiCl-fed rats gained weight more slowly than did control rats, as has been reported (Pittman *et al*, 1984). At the time of surgery, the LiCl-fed rats weighed about 20% less than the control diet-fed group (254 ± 21 g, $n = 26$ *vs* 308 ± 11 g, $n = 24$, $p < 0.0001$).

Quinpirole, 0.25 or 1.0 mg/kg i.v., provoked behavioral cycles, each consisting of an 'activity' period (repetitive sniffing, mouth, and head-turning) followed by a 'calm' period, whereas i.v. saline had no effect on activity (data not shown). The quinpirole-induced cycles were repeated during the 20-min period following the drug, and the lengths of each component were averaged during this time. As illustrated in Table 1, there was no significant difference between LiCl-fed and control rats in the mean duration of the 'activity' period at either quinpirole dose, whereas the 'calm' period was prolonged in the LiCl-fed compared with control diet rats at both doses.

LiCl Effect on Baseline Values of k^*

Figure 1 presents autoradiographs from the following: (A) a rat fed a control diet and given saline i.v., (B) a rat fed a control diet and given quinpirole 1 mg/kg i.v., (C) a rat fed a LiCl diet and given saline, (D) a rat fed a LiCl diet and given quinpirole 1 mg/kg. Values for regional incorporation coefficients k^* for AA are color-coded in the autoradiographs. The figure shows that quinpirole compared with saline increased k^* in certain brain regions in the control diet-fed (A and B) but not in the LiCl-fed (C and D) rats.

Table 1 Effect of Quinpirole on Orofacial Activity in Unanesthetized Rats Fed a Control or a LiCl Diet

	Control diet		LiCl diet	
	Quinpirole dose			
	0.25 mg/kg (9)[a]	1.0 mg/kg (9)	0.25 mg/kg (10)	1.0 mg/kg (10)
Duration (s)				
Orofacial activity	12 ± 4[b]	15 ± 3	15 ± 3	17 ± 4
Calm period	60 ± 11	35 ± 4	110 ± 15***	80 ± 7***

[a]Number of animals.
[b]Mean ± SD.
***Differs from respective control mean by unpaired *t*-test, $p < 0.001$.

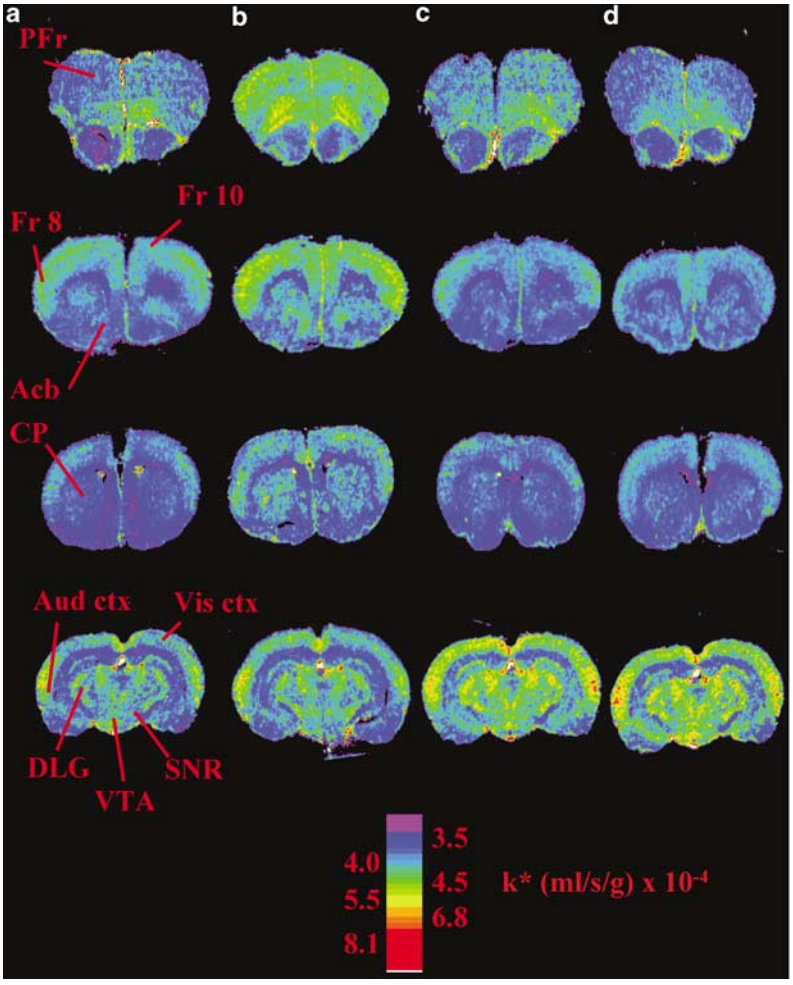

Figure 1 Effects of quinpirole on regional brain incorporation coefficients k^* of arachidonic acid in autoradiographs of coronal brain sections obtained 20 min after infusion of [1-^{14}C]arachidonate (170 μCi/kg, i.v.): (a) a control diet rat; (b) a control diet rat plus quinpirole 1 mg/kg; (c) a LiCl diet rat; (d) a LiCl diet rat plus quinpirole 1 mg/kg. Acb, accumbens nucleus; Aud ctx, auditory cortex; CP, caudate–putamen; DLG, dorsal lateral geniculate; Fr, frontal cortex; PFr, prefrontal cortex; SNR, substantia nigra pars reticulata; Vis ctx, visual cortex; VTA, ventral tegmental area.

This pattern for all rats is summarized in Table 2. Comparison between data columns 4 and 1 in Table 2 shows that LiCl feeding significantly elevated mean baseline values of k^* (k^* following i.v. saline) in 15 of the 61 brain regions examined, compared with their respective values in control diet-fed rats. Significant baseline increases were evident in central auditory regions—auditory cortex (27–34%), habenular nucleus lateral (30%), dorsal geniculate nucleus (38%), geniculate medial (34%), and colliculus inferior (17%)—and in central visual regions—visual cortex (27–33%), habenular nucleus medial (33%), and colliculus superior (22%) (Brodal, 1981; Matsumura *et al*, 1992). Increases were also evident in the cerebellar flocculus (27%), subthalamic nucleus (44%), interpeduncular nucleus (35%), and substantia nigra (24–36%).

Quinpirole Effects on k^* for AA

Table 2 (data columns 3 and 1) shows that in control diet rats quinpirole, at 1 mg/kg compared with saline, significantly increased k^* in 18 of the 61 brain regions examined. Increases were observed in the prefrontal cortex (50%), frontal cortex (26–31%), primary olfactory cortex (32%), olfactory tubercle (26%), islands of Calleja (26%), accumbens nucleus (29%), caudate–putamen (40%), substantia nigra (33%), and ventral tegmental area (29%). Quinpirole, at 0.25 mg/kg, significantly increased k^* only in ventral, lateral, and medial areas of the caudate–putamen (data columns 1 and 2). In contrast to its excitatory effects in control diet-fed rats, quinpirole at 0.25 or 1.0 mg/kg, compared with saline, did not increase k^* significantly in any region in the LiCl-fed rats (comparisons between data columns 4 and 5, and data columns 4 and 6).

DISCUSSION

Administration of 1 mg/kg i.v. quinpirole to control diet-fed rats increased mean k^* for AA significantly in 17 of the 61 (28%) brain regions examined (Table 2), many of which are rich in D_2-like (D_2, D_3, and D_4) receptors (Cooper *et al*, 1996; Cory-Slechta *et al*, 1996; Defagot *et al*, 1997; Eilam and Szechtman, 1989; Gehlert, 1993; Levant and DeSouza, 1993; Levey *et al*, 1993; Shafer and Levant, 1998; Vincent *et al*, 1995). A lower mean percent increase was reported in our prior study with the same quinpirole dose (Hayakawa *et al*,

Table 2 Effect of Quinpirole[a] on Regional Incorporation Coefficients *k** of [1-^{14}C]Arachidonic Acid[b] in LiCl-Fed Rats

	Control diet			Lithium diet		
Brain region	**Saline ($n=6$)**	**QUINP 0.25 ($n=9$)**	**QUINP 1.0 ($n=9$)**	**Saline ($n=6$)**	**QUINP 0.25 ($n=10$)**	**QUINP 1.0 ($n=10$)**
Telencephalon						
Prefrontal cortex layer I	4.07±0.25	4.60±0.64	6.05±0.89***	4.36±0.26	4.45±0.67	4.23±0.67
Prefrontal cortex layer IV	4.21±0.28	4.89±0.74	6.37±1.17***	4.37±0.26	4.39±0.67	4.02±0.62
Primary olfactory cortex	3.93±0.34	4.49±0.57	5.20±1.02**	4.09±0.21	4.16±0.77	3.89±0.56
Frontal cortex (10)						
Layer I	4.17±0.34	4.61±0.65	5.24±0.36**	4.17±0.23	4.43±0.91	4.08±0.48
Layer IV	4.31±0.27	4.74±1.00	5.63±0.25**	4.28±0.23	4.65±0.90	4.18±0.69
Frontal cortex (8)						
Layer I	4.11±0.44	4.46±0.71	5.45±0.99*	4.30±0.35	4.40±0.81	4.18±0.63
Layer IV	4.56±0.29	4.95±1.04	5.75±0.79*	4.56±0.44	4.67±0.72	4.30±0.66
Pyriform cortex	3.41±0.42	3.46±0.78	3.86±0.47	3.37±0.27	3.98±0.68	3.66±0.35
Anterior cingulate cortex	4.87±0.56	5.23±0.42	5.31±0.50	4.68±0.30	5.08±0.55	4.55±0.69
Motor cortex						
Layer I	4.49±0.70	4.66±0.93	4.78±0.55	4.17±0.25	4.51±0.65	3.97±0.46
Layer II–III	4.77±0.90	4.73±0.93	5.09±0.40	4.35±0.26	4.65±0.56	4.03±0.52
Layer IV	4.90±0.66	5.03±0.57	5.23±0.46	4.56±0.28	4.92±0.66	4.28±0.57
Layer V	3.79±0.55	4.02±0.83	4.34±0.46	3.60±0.21	3.91±0.39	3.75±0.42
Layer VI	3.63±0.52	3.81±0.60	4.14±0.48	3.46±0.20	3.76±0.43	3.39±0.48
Somatosensory cortex						
Layer I	4.38±0.65	4.67±0.96	5.00±0.73	4.12±0.40	4.63±0.67	4.13±0.62
Layer II–III	4.75±0.65	4.94±0.92	5.04±0.72	4.44±0.35	4.59±0.52	4.11±0.39
Layer IV	4.75±0.41	5.33±0.87	5.26±0.59	4.72±0.25	5.10±0.69	4.39±0.57
Layer V	4.62±0.61	4.95±1.07	5.07±0.64	4.71±1.11	4.55±0.64	4.11±0.47
Layer VI	4.16±0.29	4.78±0.96	5.07±0.64	4.15±0.62	4.49±0.63	4.14±0.64
Auditory cortex						
Layer I	4.55±0.31	4.65±0.97	4.61±0.56	5.79±0.37*	4.97±0.64	5.34±0.60
Layer IV	4.57±0.36	5.18±0.58	4.98±0.52	6.12±0.59**	5.37±0.48	6.10±0.69
Layer VI	4.44±0.18	4.90±0.71	4.49±0.65	5.19±0.54	4.97±0.42	4.22±0.37
Visual cortex						
Layer I	4.57±0.48	4.47±0.77	4.05±0.51	5.91±0.77*	4.91±0.47	5.43±0.83
Layer IV	4.63±0.20	4.66±0.90	4.14±0.65	5.89±0.26*	5.09±0.48	5.31±0.55
Layer VI	4.22±0.20	4.58±0.96	4.17±0.52	5.63±0.42*	4.83±0.44	5.52±0.88
Olfactory tubercle	4.16±0.29	4.39±0.82	5.26±0.44*	4.51±0.46	4.29±0.75	4.01±0.65
Islands of Callaja	4.12±0.45	4.19±0.56	5.20±0.41*	4.33±0.57	3.90±0.82	3.70±0.49
Accumbens nu	4.25±0.34	4.38±0.75	5.50±0.70*	4.16±0.21	4.44±0.99	3.70±0.83
Ventral pallidum	3.44±0.37	3.93±0.62	3.93±0.75	3.48±0.32	3.62±0.66	3.51±0.69
Globus pallidus	3.35±0.30	3.76±0.63	3.69±0.39	3.39±0.30	3.91±0.66	4.03±0.50
Entopeduncular nu	3.49±0.38	3.63±0.80	3.79±0.35	3.70±0.59	3.68±0.57	3.17±0.59
Caudate putamen						
Dorsal	3.92±0.20	4.43±0.71	4.95±0.38**	4.01±0.21	4.42±0.58	4.09±0.56
Ventral	3.19±0.24	4.41±0.74*	4.79±0.40**	4.02±0.22	4.37±0.57	4.11±0.54

Table 2 Continued

Brain region	Control diet			Lithium diet		
	Saline ($n=6$)	**QUINP 0.25 ($n=9$)**	**QUINP 1.0 ($n=9$)**	**Saline ($n=6$)**	**QUINP 0.25 ($n=10$)**	**QUINP 1.0 ($n=10$)**
Lateral	3.16±0.23	4.38±0.65*	4.84±0.47**	4.02±0.20	4.39±0.55	4.31±0.60
Medial	3.14±0.24	4.48±0.85*	4.87±0.44**	3.94±0.12	4.34±0.55	4.26±0.63
Hippocampus						
CA1	3.06±0.52	3.18±0.61	3.31±0.53	3.72±0.70	3.19±0.49	3.87±0.67
CA2	3.23±0.59	3.29±0.58	3.22±0.67	3.87±0.61	3.28±0.46	3.05±0.69
CA3	3.60±0.35	3.38±0.64	3.36±0.71	4.09±0.64	3.44±0.56	3.24±0.73
Diencephalon						
Habenular nucleus lateral	5.08±0.42	5.30±0.80	5.78±0.74	6.61±0.34**	7.33±0.57	6.69±0.75
Habenular nucleus medial	5.15±0.20	5.28±0.86	5.81±0.58	6.87±0.54**	7.06±0.54	6.78±0.63
Dorsal lat. geniculate nu	4.92±0.65	4.82±0.47	5.29±0.54	6.79±0.52**	6.82±0.93	6.34±0.49
Geniculate medial	5.11±0.65	4.97±0.42	5.53±0.42	6.85±0.42**	6.92±0.69	6.69±0.57
Thalamus						
Ventroposterior lat. nu	4.51±0.53	4.79±0.64	4.39±0.89	4.68± 0.88	4.15±0.68	4.17±0.75
Ventroposterior med. nu	4.48±0.42	4.64±0.97	4.40±0.89	4.78±0.86	4.13±0.64	3.99±0.83
Ventrolateral	4.64±0.44	4.57±0.93	4.56±0.73	4.70±1.15	4.53±0.63	4.17±0.66
Ventromedial	4.15±0.49	4.79±0.86	4.52±0.79	4.94±1.07	4.54±0.60	4.24±0.72
Parafascicular nu	4.60±0.43	4.76±0.37	4.05±0.48	4.75±0.80	4.27±0.69	4.61±0.71
Subthalamic nu	4.56±0.52	4.54±0.76	5.08±0.79	6.55±0.70***	5.64±0.78	6.18±0.56
Mesencephalon						
Interpeduncular nu	5.93±0.52	5.64±0.90	6.79±0.51	7.98±0.60***	7.20±0.43	7.12±0.55
Substantia nigra						
Pars reticulata	4.36±0.48	5.07±0.46	5.79±0.45*	5.40±0.57*	5.70±0.41	5.18±0.71
Pars compacta	4.33±0.81	5.02±1.01	5.75±0.91*	5.90±0.47***	5.55±0.41	5.28±0.75
Ventral tegmental area	4.24±0.14	4.53±0.67	5.46±0.42*	4.17±0.54	4.22±0.83	4.20±0.52
Colliculus superior	4.63±0.29	4.56±0.93	5.16±0.83	5.65±0.44	5.35±0.81	5.29±0.54
Colliculus inferior	6.59±0.21	6.66±0.69	6.62±0.78	7.72±0.50*	7.03±0.48	7.70±0.45
Deep layers of sup. colliculus	5.14±0.49	4.52±0.70	5.15±0.77	5.61± 0.45	5.10±0.70	5.15±0.54
Pedunculopontine tegmental nu	3.92±0.30	4.26±0.75	3.66±0.35	4.09±0.50	4.44±0.55	4.26±1.00
Rhombencephalon						
Flocculus	5.60±0.45	5.40±0.79	5.63±0.74	7.12±0.46***	6.93±0.96	6.95±0.41
Cerebellar gray matter	4.84±0.74	5.19±0.71	4.71±0.75	5.24±0.97	4.89±0.50	5.25±0.16
Molecular layer cerebellar gray matter	5.24±0.68	5.70±0.58	5.64±0.35	6.11±0.45	5.50±0.44	5.66±0.47
White matter						
Corpus callosum	3.04±0.41	3.12±0.50	3.70±0.51	2.93±0.12	3.07±0.64	2.90±0.90
Cerebellar white matter	2.76±0.64	3.07±0.68	3.13±0.63	3.09±0.66	3.44±0.60	2.81±0.34

[a]Quinpirole administration: QUINP 0.25 mg/kg and 1.0 mg/kg i.v.
[b]$k^* = (\text{ml/s/g}) \times 10^{-4}$.
Each value is a mean±SD.
*$p<0.05$; **$p<0.01$; ***$p<0.001$ mean different from control saline mean.
No mean k^* in response to 0.25 mg/kg or 1.0 mg/kg quinpirole in the LiCl-fed rats differed significantly from k^* in response to i.v. saline LiCl-fed rats.
nu, nucleus; lat, lateral; med, medial.

2001), but in that study only 29 brain regions largely having D_2-like receptors were analyzed, and they were analyzed in the hemisphere contralateral to a chronic unilateral lesion of the nucleus basalis. Such contralateral regions can have disturbed dopaminergic signaling (Lawler *et al*, 1995).

Quinpirole 1 mg/kg significantly increased k^* by 49–51% in the prefrontal cortex, which is known to receive dopamine fibers from the ventral tegmental area (Brozoski *et al*, 1979) and to have a high density of D_4 receptors (Ariano *et al*, 1997; Mrzljak *et al*, 1996). An increment in k^* in the Islands of Callaja may represent selective activation of D_3 receptors, since this region lacks D_2 receptors (Levant and DeSouza, 1993; Levey *et al*, 1993; Shafer and Levant, 1998). Quinpirole at 0.25 mg/kg significantly increased k^* only in caudate–putamen areas, by 38–43%, consistent with a dose-response relation of D_2-like receptor mediated activation of PLA_2. The caudate–putamen has a high density of D_2-like receptors and receives strong dopaminergic input from the substantia nigra (Levant and DeSouza, 1993). In contrast, in the rats fed LiCl, quinpirole at the two doses did not provoke a statistically significant change in k^* in any of the 61 brain regions.

In the control diet-fed rats, quinpirole at 0.25 and 1.0 mg/kg provoked repeated cycles of orofacial activity followed by a calm period. Such cycles have been ascribed to activation of D_2-like receptors in the striatum and nucleus accumbens (Bordi and Meller, 1989; Koene *et al*, 1993). That the cycles were altered but were not blocked by LiCl feeding (Table 1) suggests that they are mediated by D_2-coupled signaling pathways involving effector enzymes other than or in addition to PLA_2 (Arnt *et al*, 1987; Cooper *et al*, 1996; Delfs and Kelley, 1990; Senogles, 2000).

Chronic lithium's inhibition of quinpirole-induced elevations in k^* for AA contrasts with its ability to potentiate k^* responses in rats given the cholinergic muscarinic agonist arecoline (Basselin *et al*, 2003b), and with its ability to reduce the convulsant threshold in rats to arecoline and other cholinomimetics (Evans *et al*, 1990; Jope, 1993; Lerer, 1985; Morrisett *et al*, 1987). As k^* responses to arecoline and quinpirole represent PLA_2 activation coupled to $M_{1,3,5}$ and D_2-like receptors, respectively (Bayon *et al*, 1997; DeGeorge *et al*, 1991; Hayakawa *et al*, 2001; Nilsson *et al*, 1998; Rapoport, 2001; Vial and Piomelli, 1995), the arecoline and quinpirole data suggest that lithium affects $M_{1,3,5}$ and D_2-like receptor-mediated activation of PLA_2 in opposite ways. In contrast, LiCl has a more complex effect on k^* responses in rats to DOI, an agonist of 5-$HT_{2A/2C}$ receptors that also can be coupled to PLA_2 (Basselin *et al*, 2005; Felder *et al*, 1990; Qu *et al*, 2003).

The hypothesis that bipolar disorder symptoms arise from excess dopaminergic compared with reduced cholinergic signaling is based, in part, on reports that cholinomimetics as well as inhibitors of dopamine signaling can be therapeutic in bipolar disorder (see Introduction) (Bunney and Garland-Bunney, 1987; Bymaster and Felder, 2002; Janowsky and Overstreet, 1995; Post *et al*, 1980). Nevertheless, a hyper-dopaminergic state has not been demonstrated directly in bipolar disorder, and it is not clear if brain dopamine receptor density is altered in the disease (Bowden *et al*, 1997; Ebert *et al*, 1996; Wong *et al*, 1997b). Reports do suggest that the dopamine reuptake transporter is altered (Greenwood *et al*, 2001) and that a D_2 receptor polymorphism can contribute to the disease (Massat *et al*, 2002).

If lithium's therapeutic efficacy in bipolar disorder depends partly on its normalizing a cholinergic–dopaminergic neurotransmission imbalance, our results suggest that the imbalance involves signaling via PLA_2 rather than via other receptor-coupled effector enzymes (eg adenylate cyclase, phospholipase C (PLC)) (Cooper *et al*, 1996). Supporting this interpretation are observations in rats that lithium feeding reduces arecoline-initiated hydrolysis of phosphatidylinositol-4,5-bisphosphate by PLC in the brain, that lithium is a pro-convulsant with regard to arecoline and other cholinomimetics (Casebolt and Jope, 1989; Ormandy *et al*, 1991; Song and Jope, 1992), and that it does not inhibit dopamine-sensitive accumulation of cyclic AMP in the guinea pig brain (Reches *et al*, 1978).

In our study (Table 2), chronic LiCl blocked the quinpirole-evoked elevation of k^* for AA in the prefrontal cortex and caudate–putamen, regions having D_2-like receptors. In bipolar disorder patients, glucose metabolism has been reported to be reduced in the prefrontal cortex, particularly in unipolar and bipolar depression (Baxter *et al*, 1985; Drevets *et al*, 1997; Ketter *et al*, 2001). Additionally, D_2 receptor density has been reported to be elevated in the caudate–putamen of bipolar disorder patients (Pearlson *et al*, 1995; Wong *et al*, 1997a) but not of nonpsychotic manic patients (Yatham *et al*, 2002). Our finding that chronic lithium downregulated D_2-like receptor-mediated neurotransmission via AA in the caudate–putamen (k^* was elevated even at 0.25 mg/kg i.v. quinpirole) suggests that lithium may be therapeutic in bipolar patients by counteracting signaling effects of increased D_2 receptor density in the caudate–putamen.

Extrapyramidal motor symptoms of Parkinson disease are considered to reflect reduced dopaminergic compared with increased cholinergic neurotransmission (Brooks *et al*, 1995; Katzenschlager *et al*, 2003; LeWitt and Nyholm, 2004; Sovner and DiMascio, 1978). As LiCl feeding to rats potentiates $M_{1,3,5}$ receptor-signaling via AA (Basselin *et al*, 2003b) but suppresses D_2-like receptor-signaling via AA (Table 2), it is not surprising that lithium in humans frequently causes a tremor and, less frequently, severe extrapyramidal symptoms resembling those of Parkinson disease (Ghadirian *et al*, 1996; Rondot and Bathien, 1979; Sansone and Ziegler, 1985), and that lithium can exacerbate extrapyramidal symptoms in Parkinson patients (Dalen and Steg, 1973).

There are three major PLA_2 enzyme classes in the brain, a Ca^{2+}-dependent cytosolic PLA_2 selective for AA, a Ca^{2+}-dependent secretory PLA_2, and a Ca^{2+}-independent PLA_2 that is more selective for docosahexaenoic acid (22:6 n–3) than for AA (Dennis, 1994; Strokin *et al*, 2003). D_2-like receptors can be coupled specifically to $cPLA_2$ (Nilsson *et al*, 1998; Vial and Piomelli, 1995), which is localized at postsynaptic neuronal sites (Basavarajappa *et al*, 1998; Ong *et al*, 1999; Pardue *et al*, 2003; Tu and Bazan, 2003) as well as at astrocytes (Stephenson *et al*, 1996). Quinpirole-evoked increases in k^* for AA are likely mediated at the neuronal receptors (Cory-Slechta *et al*, 1996; Eilam and Szechtman, 1989; Reus *et al*, 2001), as the increments occur in regions with high neuronal D_2-like receptor densities (Cooper *et al*, 1996; Gerfen *et al*, 1995; Hayakawa *et al*, 2001;

Wedzony *et al*, 2000) and are potentiated where neuronal D_2-like receptor density is elevated in a rat model of Parkinson disease (Hayakawa *et al*, 2001). Additionally, quinpirole is reported to reduce regional glucose metabolism, measured with 2-deoxy-D-glucose, in the caudate/putamen, lateral habenular, and motor cortex of rats (Carpenter *et al*, 2003, Basselin *et al*, unpublished observations; Engber *et al*, 1990). Decreased glucose metabolism is considered to represent reduced firing of axon terminals derived from neuronal cell bodies (Sokoloff, 1999).

Chronic LiCl feeding to rats is reported to transcriptionally downregulate brain $cPLA_2$ expression, without affecting $sPLA_2$ or $iPLA_2$ expression (Bosetti *et al*, 2002a; Rintala *et al*, 1999; Weerasinghe *et al*, 2004). Thus, the blocking by lithium of k^* responses to quinpirole may be due to reduced expression of the $cPLA_2$ coupled to D_2 receptors (Vial and Piomelli, 1995). Lithium also has been reported to reduce brain levels of the inhibitory Gα protein subunits—$G\alpha i_1$ and $G\alpha i_2$—that couple D_2-like receptors to PLA_2 or PLC (Carli *et al*, 1994; Di Marzo *et al*, 1993; Sidhu and Niznik, 2000; Winitz *et al*, 1994), and to reduce GTP binding to these subunits (Colin *et al*, 1991; Jakobsen and Wiborg, 1998; Li *et al*, 1991; Wang and Friedman, 1999). These effects also may contribute to its ability to block quinpirole k^* responses.

In contrast, the $G\alpha_q$ subunits of G-proteins that can couple $M_{1,3,5}$ and serotonergic 5-HT receptors to PLC or PLA_2 (Chen *et al*, 1999; Cooper *et al*, 1996; Roth *et al*, 1998; Sidhu and Niznik, 2000) are not reported to be reduced by chronic lithium (Dwivedi and Pandey, 1997; Wang and Friedman, 1999). This may explain why lithium does not reduce k^* responses to the muscarinic receptor agonist arecoline (it even increases them), nor to the $5\text{-}HT_{2A/2C}$ receptor agonist, DOI, in most brain regions (responses are blocked by lithium in visual and auditory areas in which baseline values of k^* are elevated) (Basselin *et al*, 2005, 2003b).

In humans, lithium has been reported to augment the amplitudes of the P1/N1 components of auditory evoked responses and of the 65-P95 and P95-N125 components of visual evoked responses (Fenwick and Robertson, 1983; Hegerl *et al*, 1990; Ulrich *et al*, 1990). The finding that LiCl feeding upregulated baseline values of k^* for AA in central auditory and visual areas of rat brain (see Results), which agrees with our previous observations (Basselin *et al*, 2005, 2003b), may be related to these clinical effects and to lithium's ability to reduce visual and auditory hallucinations in bipolar disorder patients (Goodnick and Meltzer, 1984; Potash *et al*, 2001).

In a rat model of neuroinflammation, elevated baseline values for k^* correlate with increased activities of $cPLA_2$ and $sPLA_2$ but not $iPLA_2$, as well as increased turnover of AA within brain phospholipids (Lee *et al*, 2004; Rosenberger *et al*, 2004). These correlations suggest that the elevated baseline values for k^* in brain visual and auditory regions in LiCl fed rats (Table 2) also reflect increased PLA_2 activity and increased AA turnover, possibly due to increased retinal and cochlear inputs, respectively, to these regions (Jung and Reme, 1994; Pfeilschifter *et al*, 1988).

In summary, chronic LiCl feeding prevents regional increases in brain incorporation coefficients k^* for AA that can be provoked in control diet-fed rats by the D_2-like receptor-agonist, quinpirole. The increases likely represent augmented quinpirole-induced PLA_2-mediated release of AA from membrane phospholipids. Their inhibition suggests that lithium's efficacy in bipolar disorder is related to its ability to downregulate the D_2 receptor-mediated activation of PLA_2. This suggestion might be tested in human subjects by means of positron emission (Esposito *et al*, 2003; Giovacchini *et al*, 2002).

ACKNOWLEDGEMENTS

We thank Dr Richard Bazinet for his helpful comments on this paper.

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