Published in final edited form as:

Brain Res. 2011 October 28; 1420: 29–36. doi:10.1016/j.brainres.2011.08.054.

Acute lithium administration selectively lowers tyrosine levels in serum and brain

Hewlet G. McFarlane^{a,*}, John Steele^a, Keenan Vinion^a, Rodolfo Bongiovanni^b, Manda Double^b, and George E. Jaskiw^{b,c}

John Steele: john.steele@mssm.edu; Keenan Vinion: vinionk@gmail.com; Rodolfo Bongiovanni: rodolfo.bongiovanni@va.gov; Manda Double: manda.double@va.gov; George E. Jaskiw: george.jaskiw@va.gov

^aDepartment of Psychology, Kenyon College, Gambier, OH, USA

^bPsychiatry Service, Louis Stokes Cleveland Veterans Affairs Medical Center, Cleveland, OH, USA

^cDepartment of Psychiatry, Case Western Reserve University, Cleveland, OH, USA

Abstract

Lithium exerts anti-dopaminergic behavioral effects. We examined whether some of these might be mediated by changes in brain levels of tyrosine (TYR), the precursor to dopamine. Lithium chloride (LiCl₂) 3.0 mEq/kg IP acutely lowered serum TYR and the ratio of serum TYR to other large neutral amino acids (LNAA); it also selectively lowered striatum TYR levels as measured in tissue or in vivo. While LiCl₂ 3.0 mEq/kg IP also augmented haloperidol (0.19 mg/kg SC)-induced catalepsy, this lithium effect was not attenuated by administration of TYR 100 mg/kg IP. We conclude that lithium acutely and selectively lowers brain TYR by lowering serum levels of tyrosine relative to the LNAA that compete with it for transport across the blood–brain barrier. However, the lowering of TYR does not appear to significantly contribute to the ability of lithium to potentiate haloperidol-mediated catalepsy.

Keywords

Striatum; Dopamine; Tyrosine; Haloperidol; Catalepsy; Schizophrenia

1. Introduction

Even though lithium (Li) has been a mainstay in the treatment of bipolar mania for over 50 years (Smith et al., 2007), its therapeutic mechanisms remain to be fully defined. Part of the challenge is that Li exerts numerous effects in the central nervous system (Gould et al., 2004; McQuillin et al., 2007). These include anti-dopaminergic properties, evident in both pre-clinical (Beaulieu et al., 2004; Berggren, 1985; Berggren et al., 1980; O'Donnell and Gould, 2007; Otero Losada and Rubio, 1985) and clinical research studies (Sharma et al., 1989; van Kammen et al., 1985). Interestingly, some data have suggested that Li can lower brain tyrosine (TYR) availability (Berggren, 1985; Berggren et al., 1980). While a primary

* Corresponding author at: Department of Psychology, Kenyon College, Gambier, OH 43022, USA. Fax: +1 740 427 5237. mcfarlane@kenyon.edu (H.G. McFarlane).

lowering of brain TYR can itself attenuate dopamine (DA)-mediated neurotransmission (Fernstrom and Fernstrom, 2007; Jaskiw and Bongiovanni, 2004; Jaskiw et al., 2008; Milner and Wurtman, 1986); the potential relationship between these Li effects has not been clarified.

One group reported that acutely administered Li lowered serum and brain levels of TYR as well as an index of DA synthesis (Berggren, 1985; Berggren et al., 1980); in those studies, however, Li was administered in conjunction with NSD-1015, a drug that affects brain TYR levels per se (Bongiovanni et al., 2008; Westerink and Wirix, 1983). In addition, possible Li effects on other large neutral amino acids (LNAAs) were not examined; the latter is important mechanistically, insofar as competition between serum LNAAs for blood–brain barrier transport is thought to be the major determinant of influx into and brain levels of LNAAs (Fernstrom and Faller, 1978; Kanai et al., 1998; Pardridge, 1998).

For these reasons, we examined the acute effect of systemically administered Li on LNAA levels in serum, striatal tissue and striatal microdialysate. Our primary hypothesis was that Li would lower brain TYR levels. Our secondary hypothesis was that it would do so by lowering the serum ratio of TYR relative to the other LNAAs (TYR/ Σ LNAA). We had previously found that TYR depletion induced by a tyrosine- and phenylalanine-deficient mixture of neutral amino acids NAA(–) augmented haloperidol (HAL)-induced catalepsy and that this augmentation was reversed by administration of TYR (Jaskiw and Bongiovanni, 2004). Accordingly, our third hypothesis was that Li would potentiate HAL-induced catalepsy and that this could be reversed by administration of TYR.

2. Results

2.1. Lithium lowers serum and brain tissue TYR levels

In the first experiment, we examined serum and striatal tissue levels over the course of 3 h after administration of vehicle or LiCl₂ 3 mEq/kg IP. Basal serum levels (μ M) were: TYR 94.5 ± 4.1 (mean $\pm$ SEM), phenylalanine 98.35 ± 4.65 , tryptophan 126.32 ± 5.21 , leucine 199.15 ± 10.29 , isoleucine 100.42 ± 4.08 , valine 300.2 ± 15.4 . The overall ANOVA for serum TYR levels showed significant treatment ($F(1,49)=20.54$, $p<0.0001$), time ($F(6,49)=13.17$, $p<0.0001$) and treatment $\times$ time ($F(6,49)=4.3$, $p<0.002$) effects. Serum TYR levels in Li-treated animals were significantly lower than in controls ($p<0.01$) 60–90 min after Li administration (Fig. 1A). The ANOVAs for the individual other LNAAs or Σ LNAA (the sum of LNAAs other than TYR) did not reach statistical significance. In contrast, the ANOVA for the serum ratio TYR/ Σ LNAA showed a significant effect of treatment ($F(1,28)=6.10$, $p<0.02$), time ($F(6,28)=3.79$, $p<0.007$) and treatment $\times$ time ($F(6,28)=3.30$, $p<0.01$); significant differences were evident at 60–90 min (Fig. 1B).

Basal brain levels (nmol/mg protein) were: TYR 1.39 ± 0.10 , phenylalanine 0.85 ± 0.04 , tryptophan 0.30 ± 0.01 , leucine 0.91 ± 0.09 , isoleucine 0.44 ± 0.03 , valine 0.94 ± 0.06 . ANOVA of brain TYR levels showed a significant effect of treatment ($F(1,42)=21.53$, $p<0.0001$) but not of time ($F(6,42)=0.60$, $p=0.7$). The treatment $\times$ time interaction did not reach significance ($F(6,42)=2.07$, $p<0.08$). Brain TYR levels were significantly lower in Li treated rats 90 ($p<0.05$) and 120 min ($p<0.01$) after administration (Fig. 1C). ANOVAs for individual other

LNAAs were not significant (data not shown). A linear regression of striatum TYR tissue levels vs. serum TYR/ Σ LNAAs across all animals yielded a weak and non-significant relationship ($r=0.2$, $p>0.1$).

2.2. Lithium lowers striatal TYR levels in vivo

In the second study, we examined striatal LNAAs levels in vivo. Basal levels in microdialysate were (in μM): TYR 2.90 ± 0.49 , valine 11.01 ± 1.88 , isoleucine 5.64 ± 1.13 , leucine 8.43 ± 1.77 , phenylalanine 5.19 ± 1.45 , 1.93 ± 0.28 (mean $\pm$ SEM). A two-way ANOVA showed a significant effect of treatment ($F(1,85)=4.1$, $p<0.05$) but not of time ($F(11,85)=1.2$, $p>0.3$) or treatment $\times$ time ($F(11,80)=0.21$, $p=0.99$) (Fig. 2). The differences at individual time points between the two groups did not reach significance. None of the two-way ANOVAs for individual competing LNAAs showed significant effects (data not shown). A 2-way ANOVA of TYR/ Σ LNAAs did not show any significant effects (treatment, ($F(1,97)=1.76$, $p=0.18$), time ($F(10,97)=0.26$, $p=0.98$), treatment $\times$ time ($F(10,97)=0.35$, $p=0.96$)).

2.3. Lithium augmentation of haloperidol-induced catalepsy not reversed by TYR

The third study examined lithium effects on HAL-induced catalepsy (Fig. 3A, B). Animals which received HAL only (0.19 mg/kg SC) had a cumulative catalepsy time significantly different from all other groups (Figs. 3B). HAL-induced catalepsy was augmented by pretreatment with LiCl_2 (3 MEq/kg IP). This augmentation was not affected further by subsequent administration of TYR (100 mg/kg SC).

To determine how these different treatments affected TYR levels, brain tissue was harvested from a second animal cohort. Groups of animals ($n=6$) were treated with vehicle (saline), LiCl_2 chloride (3 mg/kg IP) or selected combinations of these treatments and HAL (0.19 mg/kg SC)+TYR (100 mg/kg SC). For comparison, some groups also were treated with NAA(-), a mixture of neutral amino acids known to lower brain TYR levels (Jaskiw et al., 2005; McTavish et al., 1999). The treatment schedule was the same as that for evaluation of catalepsy. Brain tissue was harvested at the time point (time=150 min) when the first cohort had started catalepsy testing.

TYR levels were not significantly different in Li-treated rats compared to controls (Table 1). In rats treated with a combination of Li+HAL+TYR, brain TYR levels were elevated to approximately 200% of control levels. NAA(-) lowered TYR levels to about 50% of controls and this was not affected by pretreatment with Li (Table 1). A linear regression of TYR tissue levels vs. serum TYR/ Σ LNAAs for control rats and those treated with NAA(-) yielded significant relationships for all regions (striatum $r=0.92$, nucleus accumbens $r=0.93$, ventral hippocampus $r=0.87$, all $p<0.0001$) except the medial prefrontal cortex ($r=0.52$, $p<0.08$).

3. Discussion

3.1. Main findings

Li acutely and selectively lowered serum TYR, the serum ratio TYR/ Σ LNAAs as well as TYR levels in tissue and microdialysate from striatum. Thus our primary and secondary hypotheses were supported. Li also potentiated HAL-induced catalepsy but this effect of Li was not reversed by a systemic dose of TYR that doubled brain tyrosine levels.

3.2. Lithium effect on serum amino acid levels

High dose Li (7 mEq/kg IP) was previously reported to lower serum TYR (Berggren, 1985) in animals pretreated with NSD-1015 (Berggren, 1985), a potent inhibitor of hepatic tyrosine aminotransferase (Dyck, 1987). Whether Li affected other LNAAs had not been known. We now report that a moderate dose of lithium selectively lowers both serum TYR levels and the serum ratio TYR/ Σ LNAAs (Figs. 1A, B).

Acute exposure to Li is not known to affect transport of LNAAs across cell membranes (Bader et al., 1978). However, acute Li administration acutely elevates serum corticosterone (Vatal and Aiyar, 1983). Systemic corticosterone administration rapidly stimulates hepatic tyrosine aminotransferase, the main determinant of peripheral TYR metabolism (Reik et al., 1994; Shirwany et al., 1986) and lowers serum and brain TYR levels (Pao and Dickerson, 1975). Thus, acute Li administration likely lowers serum TYR levels through a corticosterone-mediated enhancement of hepatic TYR metabolism.

3.3. Lithium effect on brain TYR levels

While lithium (2–7 mEq/kg IP) had been previously reported to acutely lower striatal tissue TYR levels, those studies were conducted in animals pretreated with doses of NSD-1015 (Berggren, 1985; Berggren et al., 1980) that elevate brain TYR levels (Bongiovanni et al., 2008; Carlsson et al., 1972). Our data demonstrate that LiCl₂ (3 MEq/kg IP) selectively lowers brain TYR levels in otherwise untreated animals. Striatal tissue TYR levels reach a nadir of 45% of control levels 2 h after Li administration (Fig. 1C). Levels of other LNAAs are not affected.

In brain microdialysate an overall effect of Li was statistically evident even though there were no individual time points at which TYR levels in the Li-treated group were significantly below those in the vehicle-treated animals (Fig. 2). This is likely a result of a modest Li effect and greater experimental variance in microdialysate levels. In our experience, postmortem brain tissue assays are more sensitive than in vivo indices to small changes in LNAAs levels.

A single dose of Li is not known to affect synaptosomal uptake of LNAAs (Mandell and Knapp, 1977). In addition, brain tyrosine aminotransferase is insensitive to corticosteroids (Chesnokov et al., 1990) and hence would not be affected by lithium-induced elevations of corticosterone (Vatal and Aiyar, 1983). Under most conditions, competition between LNAAs for transport across the blood–brain barrier is the main determinant of their net influx into and levels in the brain (Fernstrom and Wurtman, 1972; Pardridge, 1998). The serum ratio

TYR/ Σ LNAAs is an index of this influx (Crandall and Fernstrom, 1983; Fernstrom and Faller, 1978). Since Li selectively lowered both the serum ratio TYR/ Σ LNAAs and brain tissue TYR levels, the lowering of brain TYR is most likely mediated by the lowering of serum TYR relative to competing LNAAs. The serum ratio TYR/ Σ LNAAs and brain TYR show nadirs at 60–90 min and 90–120 min after Li administration respectively (Fig. 1B), that is about 30 min apart. This suggests a delay between a change in LNAAs influx and the attainment of new brain tissue levels. Although there was no significant relationship between brain tissue TYR levels and the serum ratio TYR/ Σ LNAAs in this study, that is not surprising. Such a relationship reflects competition between LNAAs for blood–brain barrier transport when the transporter is close to or above full saturation (Crandall and Fernstrom, 1983; Fernstrom and Faller, 1978). When there is a selective decline in one or more LNAAs, competition should become less important. In contrast, the relationship between tissue TYR and the serum ratio TYR/ Σ LNAAs was generally highly significant when control rats and those treated with NAA(–) were evaluated. In that paradigm, high levels of competing amino acids were administered to lower brain TYR.

3.4. Lithium augments haloperidol-induced catalepsy

Acute administration of Li is known to attenuate locomotor exploration (Otero Losada and Rubio, 1985; Syme and Syme, 1973, 1974; Ueki et al., 1974), amphetamine-induced locomotion (Nozu and Furukawa, 1976; Segal et al., 1975) and conditioned avoidance (Berggren et al., 1980), behaviors at least in part mediated by nigrostriatal and mesolimbic DA systems (Dunnett and Robbins, 1992). As a group the observations indicate that Li antagonizes the behavioral expression of increased DA transmission in the basal ganglia. Since HAL-induced catalepsy is a classical striatal DA-mediated behavior (Sanberg, 1980; Wadenberg et al., 2001) we were surprised to find only one report examining acute behavioral interactions of Li and HAL in the rat; very high dose LiCl₂ (12 mEq/kg IP) potentiated HAL-induced catalepsy (Dessaigne et al., 1978). Our current results show that a modest dose of LiCl₂ (3 mEq/kg) also augments HAL-induced catalepsy (Fig. 3).

3.5. Tyrosine administration does not attenuate lithium effects on haloperidol-induced catalepsy

The development and maintenance of HAL-induced catalepsy is determined in part by the balance between two functionally opposing processes, direct blockade of postsynaptic DA type II (D2) receptors in the striatum (Sanberg, 1980; Wadenberg et al., 2001) and an indirect reflex increase in striatal DA efflux (Garris et al., 2003). We had previously demonstrated that a primary lowering of brain TYR by NAA(–) limits HAL-induced striatal DA levels and augments HAL-induced catalepsy (Jaskiw and Bongiovanni, 2004). The augmentation was reversed by the administration of exogenous TYR, thus supporting a DA-mediated mechanism (Jaskiw and Bongiovanni, 2004). Hence, in the current study we posited that if the ability of Li to augment HAL-induced catalepsy was mediated by its ability to lower striatal TYR levels (Fig. 1C), it too could be reversed by the administration of TYR. We used the same administration sequence and doses of HAL and TYR as previously (Jaskiw and Bongiovanni, 2004) but unexpectedly found no effect of TYR.

We considered a kinetic explanation, namely that the administered dose of TYR did not sufficiently raise striatum TYR levels. To this end we conducted an additional study and harvested brains 150 min after administration of Li, the same time at which catalepsy assessment commenced in the behavioral cohort. For comparison, we included groups treated with NAA(-). Striatum TYR levels were significantly lower in animals treated with NAA(-) or with NAA(-)+Li pretreatment (Table 1). In contrast, the lowering of striatal TYR levels after Li alone was modest and no longer reached statistical significance (Table 1). In rats pretreated with Li and HAL, TYR 100 mg/kg IP elevated striatum tissue tyrosine levels to 230% of controls (Table 1), comparable to the elevation TYR 100 mg/kg IP alone produced in naive rats (Bongiovanni et al., 2003). Since, the selected dose of TYR was more than adequate to counteract any TYR-lowering effects of Li, a kinetic explanation for the lack of TYR effects is unlikely.

Other mechanistic explanations must also be considered. In order for TYR administration to augment DA synthesis and/or efflux, the availability of TYR rather than activity of tyrosine hydroxylase must become rate limiting for DA synthesis; this normally requires activation of tyrosine hydroxylase as well and/or a lowering of tyrosine availability (Bongiovanni et al., 2005; Jaskiw and Bongiovanni, 2004; Jaskiw et al., 2001, 2005, 2008; Milner and Wurtman, 1986). HAL, for instance, potently activates tyrosine hydroxylase (Salvatore et al., 2000; Zivkovic and Guidotti, 1974) and modestly lowers TYR levels in striatal tissue (Bongiovanni et al., 2006; Westerink and Wirix, 1983). We had previously posited that superimposing a primary NAA(-)-mediated TYR depletion on a HAL-activated nigrostriatal DA system makes TYR availability rate-limiting for DA synthesis and hence explains why administered TYR attenuates the ability of NAA(-) to potentiate HAL-induced catalepsy (Jaskiw and Bongiovanni, 2004). If Li counteracted the HAL-mediated activation of tyrosine hydroxylase, then the conditions by which TYR availability becomes rate-limiting for DA synthesis might not be met.

We are unaware of any studies of the combined acute effects of Li and HAL on tyrosine hydroxylase activity. Li alone certainly does not exert any acute effects on either tyrosine hydroxylase or dopa-decarboxylase (Friedman and Gershon, 1973; Otero Losada and Rubio, 1985; Yanagihara et al., 1987). However, Li has been reported by some groups to lower brain dihydroxyphenylalanine (DOPA) accumulation, albeit after NSD1015 pretreatment (Berggren, 1985; Berggren et al., 1980). Indeed, this initially raised the possibility that Li lowered DA synthesis by lowering brain TYR availability (Berggren, 1985).

We were surprised that at the first time point of catalepsy testing (time=60 min) the group rats treated with HAL+Li+ TYR was comparable to the Li alone group, but by 90 min displayed catalepsy comparable to rats from the HAL+Li group (Fig. 3A). Inter-group differences at these single time points were not significant. We cannot preclude the possibility that administration of TYR has a very transient effect on Li-potential of HAL catalepsy. What we can conclude is that lithium's TYR-lowering action contributes little to the overall potentiation of HAL-mediated catalepsy. Li has been shown to attenuate several other DA-mediated behaviors by affecting the DA-AKT/GSK3 signaling cascade (Beaulieu et al., 2004). Since HAL-induced catalepsy is mediated by antagonism at D2 receptors (Wadenberg et al., 2001) which in turn regulate brain AKT/GSK3 signaling (Beaulieu et al.,

2007), the latter is likely the dominant mechanism by which Li potentiates HAL-induced catalepsy.

3.6. Summary and conclusions

We conclude that acute systemic administration of Li selectively but transiently lowers TYR levels in serum and in brain in the rat. However, this does not contribute significantly to the ability of Li to affect haloperidol-induced catalepsy, a classical striatal-DA mediated behavior. This may be of relevance to bipolar disorder, where TYR transport across cell membranes is disturbed (Persson et al., 2009). A primary TYR depletion alone lowers striatal DA levels and acutely attenuates ratings of bipolar mania, (McTavish et al., 2001; Scarna et al., 2003). The anti-manic effects of Li are likely mediated through a different mechanism (Beaulieu et al., 2009).

4. Experimental procedure

4.1. Animals

Male Sprague–Dawley rats (225–250 g) were housed 2 to a plastic cage (30×30×36 cm) and maintained on a standard 12 h on/off light cycle with food and water ad libitum in an AAALAC accredited facility. Procedures were approved by Animal Care Committee and conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals. Each rat was used only once, either for a tissue study or for microdialysis.

For serum and tissue studies, animals received an injection of LiCl₂ or vehicle (normal saline) and were decapitated without analgesia or anesthesia at various time points afterwards. Trunk blood was collected and the striatum dissected over wet ice as previously described (Bongiovanni et al., 2003).

4.2. Surgery and microdialysis

A locking intracerebral guide cannula with stylet was stereotactically implanted during anesthesia as described previously (MD-2251; www.Bioanalytical.com). The cannula was lowered onto the skull surface above the striatum (AP+1.2, ML±3.2, V 7.5) (Paxinos and Watson, 1982). One guide cannula was implanted in each rat and the side of cannulation (R vs. L) was balanced between rats. Animals were allowed to recover for 24–48 h. On the evening before the start of microdialysis, the rats were gently restrained, the stylet was removed from the guide cannula and the probe 4.5 mm (MD-2204; www.Bioanalytical.com) implanted. The probe was connected to polyethylene tubing (0.965 OD, 0.58 ID in mm, length 2 m) to a swivel and perfusion pump. The following morning, the dialysis probe was perfused at 1.0 µl/min for routine sample analysis. The normal perfusate consisted of Dulbecco phosphate buffered saline containing (in mM) 122 NaCl, 3.0 KCl, 1.2 MgCl₂, 0.4 KH₂PO₄, 25 Na₂HPO₄, 1.2 CaCl₂ and 10 glucose (final pH 7.4). Samples were collected every 30 min, frozen and maintained at –80 °C until assayed for LNAAs. The brain was removed, stored at –40 °C and then sectioned for probe placement verification. If the probe extended outside of the targeted region, the data were discarded.

4.3. Catalepsy

For this study, 8–12 rats were randomly selected for each treatment. Rats were transported and allowed to habituate to the testing area for 18 h. The testing apparatus consisted of a wooden platform with a horizontal metal bar (1.2 cm in diameter) mounted 10 cm above the horizontal (Jaskiw and Bongiovanni, 2004). Catalepsy was measured as the time from the placement of both of the animal's forepaws on the bar until at least one forepaw was removed; it was assessed for a maximal duration of 300 s at 30-min intervals. At each interval, catalepsy was not counted unless the rat maintained both paws on the bar for a minimum of 6 s. Catalepsy testing began 30 min after HAL injection and continued for 4 h.

4.4. Drugs

For the HAL stock solution (5 mg/ml), 50 mg HAL (Sigma Chemical) was dissolved in 500 μ l of 0.1 M tartaric acid, diluted with 5 ml of distilled water, adjusted to a pH of 6.2 with 1.0 M NaOH and then brought to a final volume of 10 ml with distilled water. A solution of HAL 0.19 was made fresh daily by diluting the stock solution. TYR 100 mg/ml (as the free base) was prepared by dissolving TYR methylester (Sigma Chemical) in water and adjusting to pH 6.2 with 1 N NaOH. A combined TYR+HAL solution was prepared by adding 190 μ l of stock HAL and 640 mg TYR methylester to 3 ml distilled water, partially neutralizing with 50 μ l of 1 N NaOH (pH=5.8) and diluting to a 5 ml final volume.

NAA(–) (1.8 g/6.5 ml) was prepared as previously described (Jaskiw et al., 2008; McTavish et al., 1999); a total of 1 g/kg was administered in two equal IP doses 60 min apart. This formulation (McTavish et al., 1999) differs from earlier depleting solutions (Biggio et al., 1976; Fernstrom and Fernstrom, 1995) in that it is administered IP rather than orally and does not contain histidine, the precursor for histamine. While histamine–catecholamine interactions are known (Subramanian, 1977), there are no studies suggesting that brain histamine levels are lowered by peripheral administration of NAAs.

For catalepsy testing, saline (vehicle) or LiCl₂ chloride (3 mEq/kg) (in saline vehicle) IP was administered at time=0. At 90 min, rats received HAL 0.19 mg/kg±TYR 100 mg/kg SC. Catalepsy testing commenced at t=150 min and was repeated every 30 min until time=4 h.

4.5. Assay of LNAAs

The HPLC system consisted of a reverse phase C18 column (15×4.6 cm, 3 μ m particle size), an electrochemical detector (ECD) operated at a relative potential of 0.75 V to a Ag/AgCl reference electrode. The mobile phase consisted of 0.133 M Na₂HPO₄ and 25% methanol (v/v) adjusted to pH 6.8 with α -phosphoric acid. To detect the amino acids, a derivatizing agent was used (OPA-S; 10 mg α -phthaldehyde and 30 mg sodium sulfite diluted to 5.0 ml with 0.1 M sodium carbonate pH 10.4) for the reaction media. To a series of 0.3 ml tubes was added 10 μ l of sample, standards (0.1 to 2.5 μ g/ml), and blanks, followed by 10 μ l of internal standard (0.5 μ g/ml norvaline) and 10 μ l OPA-S, reacted for 5 min and brought to a final volume of 75 μ l with HPLC mobile phase. A 10 μ l sample was injected on-column (Bongiovanni et al., 2001).

4.6. Statistics

For each animal, microdialysis data were expressed as a percentage of baseline (average of three control samples). These values were then subjected to a two-factor (treatment×time) analysis of variance (ANOVA). For catalepsy, absolute time in seconds was collected for each animal at 30-min intervals and a cumulative catalepsy time calculated. Groups were compared by a one-way ANOVA followed by Bonferroni post-hoc tests. Statistical significance was set at $P<0.05$.

Acknowledgments

This material is based on work supported by the Kenyon College Interdisciplinary Neuroscience Program and Psychology Department: Gambier, OH (H.M., J.S., K.V.) and by the Office of Research and Development Medical Research Service, Department of Veterans Affairs, Louis Stokes Cleveland VAMC, Cleveland OH (R.B., M.D., G.J.).

Abbreviations

DA	dopamine
HAL	haloperidol
Li	lithium
LNAA	large neutral amino acid
NAA(–)	tyrosine and phenylalanine deficient neutral amino acid mixture
TYR	L-tyrosine

REFERENCES

- Bader JP, Sege R, Brown NR. Sodium concentrations affect metabolite uptake and cellular metabolism. *J. Cell. Physiol.* 1978; 95:179–188. [PubMed: 565784]
- Beaulieu JM, Sotnikova TD, Yao WD, Kockeritz L, Woodgett JR, Gainetdinov RR, Caron MG. Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade. *Proc. Natl. Acad. Sci. U.S.A.* 2004; 101:5099–5104. [PubMed: 15044694]
- Beaulieu JM, Tirotta E, Sotnikova TD, Masri B, Salahpour A, Gainetdinov RR, Borrelli E, Caron MG. Regulation of Akt signaling by D2 and D3 dopamine receptors in vivo. *J. Neurosci.* 2007; 27:881–885. [PubMed: 17251429]
- Beaulieu JM, Gainetdinov RR, Caron MG. Akt/GSK3 signaling in the action of psychotropic drugs. *Annu. Rev. Pharmacol. Toxicol.* 2009; 49:327–347. [PubMed: 18928402]
- Berggren U. The effect of acute lithium administration on brain monoamine synthesis and the precursor amino acids tyrosine and tryptophan in brain and plasma in rats. *J. Neural Transm.* 1985; 61:175–181. [PubMed: 3989522]
- Berggren U, Ahlenius S, Engel J. Effects of acute lithium administration on conditioned avoidance behavior and monoamine synthesis in rats. *J. Neural Transm.* 1980; 47:1–10. [PubMed: 6965708]
- Biggio G, Porceddu ML, Gessa GL. Decrease of homovanillic, dihydroxyphenylacetic acid and cyclic-adenosine-3',5'-monophosphate content in the rat caudate nucleus induced by the acute administration of an amino acid mixture lacking tyrosine and phenylalanine. *J. Neurochem.* 1976; 26:1253–1255. [PubMed: 180254]
- Bongiovanni R, Yamamoto BK, Jaskiw GE. Improved method for the measurement of large neutral amino acids in biological matrices. *J. Chromatogr. B Biomed. Sci. Appl.* 2001; 754:369–376. [PubMed: 11339280]

- Bongiovanni R, Yamamoto BK, Simpson C, Jaskiw GE. Pharmacokinetics of systemically administered tyrosine: a comparison of serum, brain tissue and in vivo microdialysate levels in the rat. *J. Neurochem.* 2003; 87:310–317. [PubMed: 14511108]
- Bongiovanni R, Kirkbride B, Walmire P, Jaskiw GE. Tyrosine administration does not affect desipramine-induced dopamine levels as measured in vivo in prefrontal cortex. *Brain Res.* 2005; 1054:203–206. [PubMed: 16083866]
- Bongiovanni R, Young D, Newbould E, Jaskiw GE. Increased striatal dopamine synthesis is associated with decreased tissue levels of tyrosine. *Brain Res.* 2006; 1115:26–36. [PubMed: 16934236]
- Bongiovanni R, Newbould E, Jaskiw GE. Tyrosine depletion lowers dopamine synthesis and desipramine-induced prefrontal cortex catecholamine levels. *Brain Res.* 2008; 1190:39–48. [PubMed: 18082673]
- Carlsson A, Davis JN, Kehr W, Lindqvist M, Atack CV. Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo using an inhibitor of the aromatic amino acid decarboxylase. *Naunyn Schmiedeberg's Arch. Pharmacol.* 1972; 275:153–168. [PubMed: 4404948]
- Chesnokov VN, Gadzhiev VG, Leontyeva GI, Zelenin SM, Mertvetsov NP. Hydrocortisone regulation and expression of tyrosine aminotransferase gene in various tissues of rat. *Biomed. Biochim. Acta.* 1990; 49:1177–1186. [PubMed: 1982985]
- Crandall EA, Fernstrom JD. Effect of experimental diabetes on the levels of aromatic and branched-chain amino acids in rat blood and brain. *Diabetes.* 1983; 32:222–230. [PubMed: 6337901]
- Dessaigne S, Scotto AM, Guigues M. Lithium modifications of the cataleptogenic properties of various neuroleptic drugs in the rat. *C. R. Seances Soc. Biol. Fil.* 1978; 172:1173–1180. [PubMed: 40676]
- Dunnett SB, Robbins TW. The functional role of mesotelencephalic dopamine systems. *Biol. Rev. Camb. Philos. Soc.* 1992; 67:491–518. [PubMed: 1361154]
- Dyck LE. Effect of decarboxylase inhibitors on brain p-tyrosine levels. *Biochem. Pharmacol.* 1987; 36:1373–1376. [PubMed: 2885004]
- Fernstrom JD, Faller DV. Neutral amino acids in the brain: changes in response to food ingestion. *J. Neurochem.* 1978; 30:1531–1538. [PubMed: 670995]
- Fernstrom MH, Fernstrom JD. Acute tyrosine depletion reduces tyrosine hydroxylation rate in rat central nervous system. *Life Sci.* 1995; 57:97–102.
- Fernstrom JD, Fernstrom MH. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *J. Nutr.* 2007; 137:1539S–1547S. [PubMed: 17513421]
- Fernstrom JD, Wurtman RJ. Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science.* 1972; 178:414–416. [PubMed: 5077329]
- Friedman E, Gershon S. Effect of lithium on brain dopamine. *Nature.* 1973; 243:520–521. [PubMed: 4743648]
- Garris PA, Budygin EA, Phillips PE, Venton BJ, Robinson DL, Bergstrom BP, Rebec GV, Wightman RM. A role for presynaptic mechanisms in the actions of nomifensine and haloperidol. *Neuroscience.* 2003; 118:819–829. [PubMed: 12710989]
- Gould TD, Quiroz JA, Singh J, Zarate CA, Manji HK. Emerging experimental therapeutics for bipolar disorder: insights from the molecular and cellular actions of current mood stabilizers. *Mol. Psychiatry.* 2004; 9:734–755. [PubMed: 15136794]
- Jaskiw GE, Bongiovanni R. Brain tyrosine depletion attenuates haloperidol-induced striatal dopamine release in vivo and augments haloperidol-induced catalepsy in the rat. *Psychopharmacology (Berl.).* 2004; 172:100–107. [PubMed: 14586541]
- Jaskiw GE, Collins KA, Pehek EA, Yamamoto BK, Jaskiw GE, Collins KA, Pehek EA, Yamamoto BK. Tyrosine augments acute clozapine- but not haloperidol-induced dopamine release in the medial prefrontal cortex of the rat: an in vivo microdialysis study. *Neuropsychopharmacology.* 2001; 25:149–156. [PubMed: 11377928]
- Jaskiw GE, Kirkbride B, Newbould E, Young D, Durkalski V, Bongiovanni R. Clozapine-induced dopamine release in the medial prefrontal cortex is augmented by a moderate concentration of locally administered tyrosine but attenuated by high tyrosine concentrations or by tyrosine depletion. *Psychopharmacology (Berl.).* 2005; 179:713–724. [PubMed: 15682305]

- Jaskiw GE, Newbould E, Bongiovanni R. Tyrosine availability modulates potassium-induced striatal catecholamine efflux in vivo. *Brain Res.* 2008; 1209:74–84. [PubMed: 18400209]
- Kanai Y, Segawa H, Miyamoto K, Uchino H, Takeda E, Endou H. Expression cloning and characterization of a transporter for large neutral amino acids activated by the heavy chain of 4F2 antigen (CD98). *J. Biol. Chem.* 1998; 273:23629–23632. [PubMed: 9726963]
- Mandell AJ, Knapp S. Regulation of serotonin biosynthesis in brain: role of the high affinity uptake of tryptophan into serotonergic neurons. *Fed. Proc.* 1977; 36:2142–2148. [PubMed: 872948]
- McQuillin A, Rizig M, Gurling HM. A microarray gene expression study of the molecular pharmacology of lithium carbonate on mouse brain mRNA to understand the neurobiology of mood stabilization and treatment of bipolar affective disorder. *Pharmacogenet. Genomics.* 2007; 17:605–617. [PubMed: 17622937]
- McTavish SF, Cowen PJ, Sharp T. Effect of a tyrosine-free amino acid mixture on regional brain catecholamine synthesis and release. *Psychopharmacology (Berl.).* 1999; 141:182–188. [PubMed: 9952043]
- McTavish SF, McPherson MH, Harmer CJ, Clark L, Sharp T, Goodwin GM, Cowen PJ. Antidopaminergic effects of dietary tyrosine depletion in healthy subjects and patients with manic illness. *Br. J. Psychiatry.* 2001; 179:356–360. [PubMed: 11581118]
- Milner JD, Wurtman RJ. Catecholamine synthesis: physiological coupling to precursor supply. *Biochem. Pharmacol.* 1986; 35:875–881. [PubMed: 2869759]
- Nozu T, Furukawa T. Effects of lithium chloride administered in combination with methamphetamine or a reserpine-like drug on brain norepinephrine and dopamine metabolism in rats. *Nippon Yakurigaku Zasshi.* 1976; 72:715–723. [PubMed: 1051410]
- O'Donnell KC, Gould TD. The behavioral actions of lithium in rodent models: leads to develop novel therapeutics. *Neurosci. Biobehav. Rev.* 2007; 31:932–962. [PubMed: 17532044]
- Otero Losada ME, Rubio MC. Striatal dopamine and motor activity changes observed shortly after lithium administration. *Naunyn Schmiedebergs Arch. Pharmacol.* 1985; 330:169–174. [PubMed: 2865683]
- Pao SK, Dickerson JW. Effect of a low protein diet and isoenergetic amounts of a high protein diet in the weanling rat on the free amino acids of the brain. *Nutr. Metab.* 1975; 18:204–216. [PubMed: 1196550]
- Pardridge WM. Blood-brain barrier carrier-mediated transport and brain metabolism of amino acids. *Neurochem. Res.* 1998; 23:635–644. [PubMed: 9566601]
- Paxinos, G.; Watson, D. *The Rat Brain in Stereotaxic Coordinates.* New York: Academic Press; 1982.
- Persson ML, Johansson J, Vumma R, Raita J, Bjerkenstedt L, Wiesel FA, Venizelos N. Aberrant amino acid transport in fibroblasts from patients with bipolar disorder. *Neurosci. Lett.* 2009; 457:49–52. [PubMed: 19429160]
- Reik A, Stewart AF, Schutz G. Cross-talk modulation of signal transduction pathways: two mechanisms are involved in the control of tyrosine aminotransferase gene expression by phorbol esters. *Mol. Endocrinol.* 1994; 8:490–497. [PubMed: 7914348]
- Salvatore MF, Garcia-Espana A, Goldstein M, Deutch AY, Haycock JW. Stoichiometry of tyrosine hydroxylase phosphorylation in the nigrostriatal and mesolimbic systems in vivo: effects of acute haloperidol and related compounds. *J. Neurochem.* 2000; 75:225–232. [PubMed: 10854265]
- Sanberg PR. Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. *Nature.* 1980; 284:472–473. [PubMed: 7189016]
- Scarna A, Gijsman HJ, McTavish SF, Harmer CJ, Cowen PJ, Goodwin GM. Effects of a branched-chain amino acid drink in mania. *Br. J. Psychiatry.* 2003; 182:210–213. [PubMed: 12611783]
- Segal DS, Callaghan M, Mandell AJ. Alterations in behaviour and catecholamine biosynthesis induced by lithium. *Nature.* 1975; 254:58–59. [PubMed: 234597]
- Sharma R, Javaid JI, Janicak P, Faull K, Comaty J, Davis JM. Plasma and CSF HVA before and after pharmacological treatment. *Psychiatry Res.* 1989; 28:97–104. [PubMed: 2740469]
- Shirwany TA, Hubbard JR, Kalimi M. Glucocorticoid regulation of hepatic cytosolic glucocorticoid receptors in vivo and its relationship to induction of tyrosine aminotransferase. *Biochim. Biophys. Acta.* 1986; 886:162–168. [PubMed: 2869788]

- Smith LA, Cornelius V, Warnock A, Tacchi MJ, Taylor D. Pharmacological interventions for acute bipolar mania: a systematic review of randomized placebo-controlled trials. *Bipolar Disord.* 2007; 9:551–560. [PubMed: 17845269]
- Subramanian N. On the brain ascorbic acid and its importance in metabolism of biogenic amines. *Life Sci.* 1977; 20:1479–1484. [PubMed: 327180]
- Syme LA, Syme GJ. Effects of lithium chloride on the activity of rats tested alone or in pairs. *Psychopharmacologia.* 1973; 29:85–89. [PubMed: 4695209]
- Syme LA, Syme GJ. The role of sex and novelty in determining the social response to lithium chloride. *Psychopharmacologia.* 1974; 40:91–100. [PubMed: 4445448]
- Ueki S, Ogasawara T, Ogawa N. Behavioral effects of lithium chloride. *Nippon Yakurigaku Zasshi.* 1974; 70:285–304. [PubMed: 4858873]
- van Kammen DP, Docherty JP, Marder SR, Rosenblatt JE, Bunney WE Jr. Lithium attenuates the activation-euphoria but not the psychosis induced by d-amphetamine in schizophrenia. *Psychopharmacology (Berl.).* 1985; 87:111–115. [PubMed: 3933028]
- Vatal M, Aiyar AS. Some aspects of corticosterone metabolism in lithium treated rats. *Chem. Biol. Interact.* 1983; 45:277–282. [PubMed: 6309419]
- Wadenberg ML, Soliman A, VanderSpek SC, Kapur S. Dopamine D(2) receptor occupancy is a common mechanism underlying animal models of antipsychotics and their clinical effects. *Neuropsychopharmacology.* 2001; 25:633–641. [PubMed: 11682246]
- Westerink BHC, Wirix E. On the significance of tyrosine for the synthesis and catabolism of dopamine in rat brain: evaluation by HPLC with electrochemical detection. *J. Neurochem.* 1983; 40:758–764. [PubMed: 6827272]
- Yanagihara N, Yokota K, Wada A, Izumi F. Intracellular pH and catecholamine synthesis in cultured bovine adrenal medullary cells: effect of extracellular Na⁺ removal. *J. Neurochem.* 1987; 49:1740–1746. [PubMed: 2890712]
- Zivkovic B, Guidotti A. Changes of kinetic constant of striatal tyrosine hydroxylase elicited by neuroleptics that impair the function of dopamine receptors. *Brain Res.* 1974; 79:505–509. [PubMed: 4153770]

Fig. 1.

Groups of animals (n=16) were injected with saline (VEH) or LiCl₂ (Li) (3 mEq/kg IP) at time=0 and euthanized at the times shown. A — serum tyrosine (TYR), B — serum ratio TYR/ Σ LNAA, C — striatum tyrosine (per mg protein). Significant differences between groups (*p<0.05, **p<0.01).

Fig. 2. Groups of rats (n=8) were injected with saline (VEH) or LiCl₂ (Li) (3 mEq/kg IP) at the time shown. Microdialysate was collected from striatum. ANOVA showed a significant overall effect ($p<0.05$) of treatment.

Fig. 3.

Groups of rats ($n=7-12$) were injected with saline (VEH) or LiCl_2 (Li) (3 mEq/kg IP) at time=0 and 90 min later with vehicle or haloperidol (HAL) (0.19 mg/kg SC)±tyrosine (TYR) (100 mg/kg SC). Catalepsy testing started at time=150 min and was repeated every 30 min for 4 h. A — time course of catalepsy, B — cumulative catalepsy time. Significantly different from VEH/HAL, * $p<0.01$, ** $p<0.001$.

Table 1

Tissue tyrosine (TYR) levels were measured in groups of animals (n=6) which received injections at times=0, 60 and 90 min and were euthanized at 150 min. V — vehicle, HAL — haloperidol 0.19 mg/kg SC, Li — LiCl₂ 3.0 MEq/kg IP, NAA(–) tyrosine and phenylalanine deficient mixture of neutral amino acids. TYR — 100 mg/kg IP. MPFC — medial prefrontal cortex, NAS — nucleus accumbens, VHIPP — ventral hippocampus. Data are presented as % control (V/V/V striatum 1.71±0.15, MPFC 1.18±0.15, NAS 1.06±0.14, VHIPP, 1.28±0.07 nmol/mg protein).

Treatment	Tissue tyrosine levels (% control)			
	Striatum	MPFC	VHIPP	NAS
Li/V/V	79.1±21.8	73.36±20.3	78.6±21.27	84.3±22.54
Li/V/HAL+TYR	233.1±15.1 ***	216.08±20.49 ***	212.45 ±20.46 ***	179.54±16.9 ***
NAA(–)/NAA(–)/V	44.2±5.6 *	63.84±13.8 **	39.23 ±3.90 *	46.70 ±3.85 ***
Li+NAA(–)/NAA(–)/V	48.2 ±2.4 *	45.92 ±6.23 ***	39.12 ±2.93 *	45.13±3.67 ***

* p<0.05 (significantly different from control).

** p<0.01 (significantly different from control).

*** p<.001 (significantly different from control).