Effect of lithium and lithium withdrawal on potassium-evoked dopamine release and tyrosine hydroxylase expression in the rat

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Abstract
The mood stabilizer lithium is used successfully in the treatment of bipolar affective disorder. However, treatment compliance is frequently poor and sudden withdrawal from lithium therapy is associated with a significantly increased risk of rebound mania. In this study we have used rodents to identify neurobiological changes in dopamine function occurring during chronic lithium treatment and withdrawal from chronic lithium treatment. Rats were maintained for 28 d on a lithium diet or a control diet. A subgroup had their lithium diet substituted with a control diet from day 25 of the treatment period. In-vivo microdialysis was used to study both basal dopamine release and potassium-evoked dopamine released in the shell of the nucleus accumbens. In-situ hybridization histochemistry was used to study the abundance of mRNA coding for dopamine's synthetic enzyme, tyrosine hydroxylase in the ventral tegmental area. Basal dopamine levels did not differ across any of the three treatment groups. However, the potassium-evoked dopamine release was significantly attenuated in lithium and lithium-withdrawn rats compared to control rats. Tyrosine hydroxylase mRNA abundance in the ventral tegmental area did not differ between any of the three treatment groups. These data suggest that decreased dopamine release may mediate the mood stabilizing action of lithium. However, in this paradigm a rebound increase in dopamine release was not found after withdrawal from lithium treatment.

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Key words: Dopamine, in vivo microdialysis, lithium, lithium withdrawal, nucleus accumbens.

Introduction
In a growing number of patients receiving maintenance lithium therapy treatment may be interrupted owing to unacceptable side-effects, poor compliance, pregnancy or medical illness (Goodwin, 1994). However, on interruption of lithium therapy, these patients are highly susceptible to a rapid return of affective symptoms including mania (Mander and Loudon, 1988; Suppes et al., 1991). This rebound mania is not simply a re-emergence of the original disorder but appears to be a worsening of the condition with patients being at higher risk of suffering a manic episode than would be predicted by the history of the disease (Goodwin, 1994; Klein et al., 1991; Mander and Loudon, 1988; Suppes et al., 1991).

The neurobiological mechanisms underlying lithium’s therapeutic efficacy and the rebound mania associated with withdrawal are unclear. Although the neurobiological bases of bipolar disorder are not well understood, reports that manipulations of central dopamine (DA) pathways have effects on mood in bipolar patients do indicate that dysfunction in central mesolimbic DA function may have a role in this disease. For example, stimulation of DA neurotransmission induces manic symptoms in euthymic (stable) patients and inhibition of DA neurotransmission eases manic symptoms in manic patients (Anand et al., 2000; Gelenberg and Hopkins, 1996; Silverstone, 1985; Yatham et al., 2002). Preclinical studies in rodents report that chronic lithium treatment attenuates DA function suggesting that normalization of a dysfunctional DA neurotransmission may underlie lithium’s anti-manic and mood-stabilizing effects (Berggren, 1985; Dziedzicka-Wasylewska, et al., 1996;
Dziedzicka-Wasylewska and Wedzony, 1996; Ferrie et al., 2005; Gambarana et al., 1999).

Few studies have attempted to determine how the changes in DA function induced by chronic lithium treatment respond to withdrawal of the drug. Recently, we have reported that the effect (impairment) of chronic lithium on DA uptake rapidly normalizes 3 d after withdrawal from the lithium treatment (Ferrie et al., 2005). However, DA uptake is only one component of DA function. Central DA synthesis and release both appear to be decreased in rodents during chronic lithium treatment and it is unclear how these components of neurotransmitter function respond to withdrawal from the lithium treatment (Berggren, 1985; Dziedzicka-Wasylewska et al., 1996; Gambarana et al., 1999).

In the present study we have addressed this question by characterizing the effects of lithium and lithium withdrawal on DA release and DA synthesis in the rat. We have used in-vivo microdialysis to measure basal, and potassium (K⁺)-evoked, release of DA in the shell of the nucleus accumbens of rats during chronic lithium treatment or 3 d after withdrawal from lithium treatment. The shell of the nucleus accumbens is an important component of the mesolimbic DA system which has been implicated in mood disorders (Pania and Gessab, 2002). In addition we have used in-situ hybridization histochemistry to measure changes in the abundance of the mRNA coding for the DA synthetic enzyme tyrosine hydroxylase (TH) during the same treatment and withdrawal regimes.

**Methods**

**Experimental animals**

Male Lister Hooded rats (100–120 g) (Charles River UK, Margate, Kent, UK) were housed in groups of four or six and maintained on a 12-h light–dark cycle (lights on 07:00 hours) with free access to food and water (see Lithium treatment below). All procedures concerning the use of animals were strictly in accordance with the United Kingdom – Home Office Animals (Scientific Procedures) Act 1986.

**Lithium treatment**

Rats were maintained on either an isocaloritic control diet (n = 14) or a lithium-supplemented diet (1.696 LiCl g/kg; Teklad, Harlan Olac, Bicester, Oxon, UK) (n = 28) for 28 d. This treatment regime has previously been reported to produce lithium plasma levels which are within the therapeutic range without having a detrimental effect on the health of the rats (Ferrie et al., 2005). For the last 3 d of the treatment period, a group of lithium-treated rats (n = 14) were withdrawn from the lithium diet and maintained on control diet. Each treatment cage was supplied with fresh water and 0.9% saline (to minimize any ionic imbalances that may occur due to the diuretic properties of lithium). For each treatment group, seven rats were assigned for in-vivo microdialysis studies and seven rats were assigned to in-situ hybridization histochemistry studies.

**In-vivo microdialysis studies**

**Surgical procedures**

Rats were anaesthetized (anaesthesia initiated between 08:00 and 09:00 hours) with chloral hydrate (400 mg/kg plus supplementary doses of 200 mg/kg chloral hydrate as necessary) and placed in a small rodent stereotaxic frame. An incision was made exposing the skull surface, and a trephine hole (1 mm in diameter) drilled in the skull directly over the desired position of the microdialysis probes (leaving the dura mater membrane intact). Microdialysis probes were connected to a perfusion pump via polythene tubing (0.38 × 1.09 mm), and perfused at 2 µl/min with artificial cerebrospinal fluid (aCSF) containing (m M): 140 NaCl, 3 KCl, 2.4 CaCl₂, 1 MgCl₂, 1.20 Na₂HPO₄, 0.27 NaH₂PO₄, 7.2 glucose (pH 7.4) in H₂O. The perfusion medium also contained the DA uptake inhibitor bupropion at a concentration of 30 µM. The probe was then positioned in the nucleus accumbens shell (RC+2.70 mm, ML − 1.20 DV − 7.0 mm relative to bregma) according to Paxinos and Watson (1997). Once the probe was in position, quick drying dental cement (Kemdent Simplex Rapid powder; Kemdent, Swindon, Wiltshire, UK) was applied to the skull surface surrounding the probe, and the probe holder removed. Dialysates were collected every 20 min. Anaesthesia was maintained throughout the course of the experiment and the body temperature of the rat was maintained at 37–38 °C by means of a homeostatic heating pad and rectal probe. At the end of the experiment, the brain was removed for verification of probe placement and trunk blood collected for analysis of lithium levels.

**Measurement of DA by HPLC-EC**

High-performance liquid chromatography (HPLC) in combination with electrochemical detection (EC) was used to analyse DA content in dialysates with the minimum detectable concentration of DA approximately 5 fmol. The mobile phase consisted of: 83 mM

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H₂O, 1.20 Na₂HPO₄, 0.27 NaH₂PO₄, 7.2 glucose (pH 7.4) in H₂O. The perfusion medium also contained the DA uptake inhibitor bupropion at a concentration of 30 µM. The probe was then positioned in the nucleus accumbens shell (RC+2.70 mm, ML − 1.20 DV − 7.0 mm relative to bregma) according to Paxinos and Watson (1997). Once the probe was in position, quick drying dental cement (Kemdent Simplex Rapid powder; Kemdent, Swindon, Wiltshire, UK) was applied to the skull surface surrounding the probe, and the probe holder removed. Dialysates were collected every 20 min. Anaesthesia was maintained throughout the course of the experiment and the body temperature of the rat was maintained at 37–38 °C by means of a homeostatic heating pad and rectal probe. At the end of the experiment, the brain was removed for verification of probe placement and trunk blood collected for analysis of lithium levels.
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NaH₂PO₄, 15% HPLC grade methanol, 0.23 mM sodium octanyl sulphonate, 0.84 mM EDTA in aqueous solution (pH 4.0), flow rate 1.0 ml/min. DA was separated on a Varian 4.6 mm × 100 mm Microsorb 100-3 C18 column and detected on a Coulochem detector (E1 = 300 mV, E2 = 400 mV, GC = 450 mV).

Experimental protocol

Extracellular basal DA

Dialysates were collected for an initial 2 h to determine basal DA levels in the presence of the DA uptake inhibitor bupropion (30 μM).

Effect of K⁺-induced depolarization

Following this basal period the perfusion medium was changed to one containing 90 mM K⁺ (and 30 μM bupropion). After 20 min K⁺ perfusion the original perfusion medium was reinstated and samples collected for a further 60 min.

In-situ hybridization studies

Rats were killed by decapitation, trunk blood collected for lithium analysis and their brains removed and snap frozen. Brain sections (12 μm) were cut on a cryostat and thaw mounted onto gelatinized slides and stored at −70 °C. The sections were then fixed and pre-treated for in-situ hybridization in a single batch using the following protocol. Sections were initially fixed for 5 min in 4% paraformaldehyde in phosphate buffered saline (PBS). Following two PBS washes the sections were acetylated for 10 min with 0.25% acetic anhydride in 0.1 M triethanolamine buffer. The sections were dehydrated through graded ethanol washes and then defatted by a 10-min chloroform wash. The sections were then rehydrated with 100% and 95% ethanol, air-dried and stored at −20 °C prior to use.

Oligonucleotide probes

The TH probe comprised a 36 base probe complementary to bases 1380–1417 of the rat TH gene (5′-GGG AGA ACT GGG CAA ATG TGC GGT CAG CCA ACA TGG-3′).

The oligonucleotide probes were 3′-tail-labelled using [³⁵S]dATP with terminal deoxynucleotide transferase. The labelled oligonucleotide probe (approximate specific activity 10⁷ cpm/pmol) was added to each section (3 × 10⁶ cpm/section) in hybridization buffer comprising 50% formamide, 4 x standard citrate saline (SSC), 10% dextran sulphate, 5 x Denhardt’s solution, 200 μg/ml salmon sperm DNA, 100 μg/ml poly A, 25 mM sodium phosphate, 1 mM sodium pyrophosphate and 5% dithiothreitol (Pei et al., 1997). The sections were incubated overnight (32 °C) in sealed boxes containing 50% formamide in 4 x SSC, after which they were washed in 1 x SSC at 58 °C for 3 x 20 min followed by 2 x 60 min at room temperature. After air drying, the sections were exposed to bio-max hyperfilm (Amersham Biosciences, Bucks, UK) for 11 d before automatic development.

The relative abundance of TH mRNA in the ventral tegmental area (VTA) was determined by densitometric autoradiogram quantification of triplicate sections using NIH Scion image software (Scion Image, Frederick, MD, USA). Brain sections corresponded to Paxinos and Watson (1997, plate 40) (−5.20 relative to bregma for VTA sections). Density values were calibrated to [³⁴C] standards, and converted to nCi/g tissue.

Measurement of serum lithium levels

Blood samples were immediately centrifuged (15,000 rpm) for 15 min and plasma collected. Plasma samples were analysed for lithium using a flame photometer (Instrumentation Laboratory Flame Photometer 943).

Statistics

In-vivo microdialysis

Dialysate DA levels are presented as absolute values, and are expressed as means ± S.E.M. in a fmol/20 min sample. Mean values of groups were analysed by one-way ANOVA, and post-hoc Dunnett’s t test.

In-situ hybridization

The relative abundance of TH mRNA in the VTA was determined by densitometric autoradiogram quantification of triplicate sections using NIH Scion image software. Density values were calibrated to [³⁴C] standards corrected for [³⁵S] decay and converted to nCi/g tissue. mRNA levels were then analysed statistically using one-way ANOVA.

Results

Lithium levels

In lithium-treated rats plasma lithium levels were 0.62 ± 0.04 mmol/l. In lithium-withdrawn rats and control rats plasma lithium levels were negligible.
(lithium-withdrawn 0.02 ± 0.00 mmol/l, control 0.03 ± 0.00 mmol/l).

**In-vivo microdialysis studies**

**Basal DA**

Basal DA levels in the presence of 30 μM bupropion did not differ across any of the three treatment groups. Thus, in control rats basal dialysate levels were 281.7 ± 33.9 fmol/20 min. In lithium-treated rats basal DA levels were 286.0 ± 75.0 fmol/20 min and lithium-withdrawn basal DA levels were 338.5 ± 30.4 fmol/20 min (Figure 1, inset).

**K⁺-induced increase in DA**

A 20-min infusion with aCSF containing 90 mM K⁺ caused an increase in extracellular DA in all three groups of animals. However, this K⁺-evoked release was significantly attenuated in lithium and lithium-withdrawn rats compared to control rats. Thus, in control rats dialysate DA levels increased to 2262 ± 437 fmol/20 min, in lithium-treated rats dialysate DA levels increased 1203 ± 177 fmol/20 min, and in lithium-withdrawn rats dialysate DA levels increased to 1058 ± 268 fmol/20 min. ANOVA revealed a significant between-group effect (p < 0.01, F_{2,15} = 6.87) for the K⁺-evoked release. Post-hoc tests revealed that this effect was significantly different in both lithium (p < 0.05) and lithium-withdrawn (p < 0.01) rats compared to control rats, but was not different in lithium-treated compared to lithium-withdrawn rats (Figure 1).

**In-situ hybridization studies**

**Effect of lithium on the abundance of TH mRNA in the rat brain**

The mean intensity of mRNA signal revealed no difference in TH mRNA abundance in the VTA between the three treatment groups. Thus, in control animals mean signal intensity values were 50.46 ± 6.3 nCi/g. In lithium-treated animals intensity values were 47.56 ± 4.45 nCi/g and in lithium-withdrawn animals 43.74 ± 5.25 nCi/g (Figure 2).

**Discussion**

We have investigated the effects of chronic lithium treatment and withdrawal from chronic lithium treatment on indices of DA release and synthesis in rats. At the end of 28 d lithium treatment there was attenuation in the K⁺-evoked DA release. The attenuation in K⁺-evoked DA release was maintained 3 d after withdrawal from lithium treatment. We observed no change in mRNA coding for the DA synthetic enzyme TH at the end of lithium treatment or 3 d after withdrawal from the drug.

Our finding of attenuated DA release is consistent with previous reports of lithium’s effect, on chronic...
administration, of attenuating presynaptic DA function (Berggren, 1985; Dziedzicka-Wasylewska et al., 1996; Gambarama et al., 1999). We have also recently observed that the increase in extracellular DA induced by local DA reuptake inhibition is attenuated during chronic lithium treatment (Ferrie et al., 2005). Together, these data are indicative of decreased DA release during chronic lithium, and support the hypothesis that decreased DA function underlies lithium’s therapeutic mechanism of action. However, not all investigations of this type have observed decreased presynaptic DA function during chronic lithium treatment. Thus, unchanged, or indeed increased DA presynaptic function have been reported after similar lithium regimens to that used in our study (Baptista et al., 1993; Pei et al., 1995; Rastogi and Singhal, 1977; Reches et al., 1984). Furthermore, in the present study, whilst the $K^+$-evoked release was attenuated after lithium, there was no change in basal DA release. Chloral hydrate anaesthesia is reported to have an effect on the firing pattern and activity of DA cells (Hyland et al., 2002). Since basal DA release gives an indication of the inherent activity of DA neurones this may not be the best measure of DA function in the chloral hydrate-anaesthetized rat. In contrast, $K^+$-evoked release gives an indication of the DA-releasing capacity of DA neurones, and may be a better measure of DA function in this preparation. Indeed, our findings suggest that although DA release is compromised during chronic lithium, under certain conditions (e.g. at low levels of neuronal activity) DA terminals can maintain normal levels of DA release. However, when release is evoked by $K^+$ stimulation the compromised DA release is revealed.

Recently it has been reported that lithium can exert an acute effect on post-synaptic DA function by interfering with the effect of DA on an Akt/glycogen synthase kinase-3 (GSK-3) signalling cascade (Beaulieu et al., 2004). In the present study we report that lithium also has an effect on presynaptic DA function, and whilst we did not directly investigate whether this effect is due to an acute effect of lithium, we propose that this is unlikely since the effect is maintained 3 d after withdrawal from lithium – when lithium levels are negligible (see below).

As well as investigating the effect of lithium on DA release, we also attempted to determine how DA release responded to withdrawal from lithium treatment. We found that although basal DA levels did not differ between any of the three groups, the attenuation in $K^+$-evoked DA release induced by lithium was maintained (still attenuated) 3 d after withdrawal from lithium. This is not consistent with our previous finding of normalized DA release 3 d after withdrawal from lithium, but again may be due to the difference in DA function under basal and stimulated conditions (Ferrie et al., 2005). These studies do, however, reveal that even within presynaptic DA neurotransmission different components of neurotransmission can respond to lithium withdrawal in different ways and indicate that certain neuroadaptive processes associated with lithium treatment are maintained some time after withdrawal from the drug treatment. Just how long this effect is maintained is unclear, and requires further study. Indeed a constraint of the present study is the use of the 3 d withdrawal period. Given that the half-life of lithium in rats is 5–6 h and 2 d after withdrawal from treatment, lithium levels have been shown to be completely cleared from the rats, it seemed apparent that any neurobiological changes in DA function might be evident 3 d after lithium withdrawal (Wood et al., 1986). However, it is possible that any changes in DA function actually occur outside this time-point. Clinical data indicate that the 3 months following withdrawal from lithium treatment is the period of increased risk of rebound mood symptoms (Goodwin, 1994). To examine the longer term, future studies are planned which will characterize changes in DA function at greater time intervals after withdrawal from lithium treatment. Another caveat of the study is that we have examined the effects of lithium on brain physiological DA neurotransmission in rats. However, if the DA system is dysfunctional in patients with bipolar disorder (unlike in rats or normal volunteers), this may respond differently to the withdrawal of lithium (Silverstone, 1985).

In this study we used a microdialysis preparation which included the use of the DA uptake inhibitor bupropion (30 $\mu$M) in the perfusion medium. By blocking endogenous uptake of a neurotransmitter, a more accurate measure of neurotransmitter release can be achieved (Hjorth et al., 2000). We have previously used a much lower concentration of bupropion (1 $\mu$M) and revealed differences in DA transporter function during lithium treatment (Ferrie et al., 2005). Interestingly, this effect appears to be bupropion concentration-dependent since at the higher dose of bupropion used in the present study (30 $\mu$M) the previously observed differences were not evident. The rationale for using the 30 $\mu$M bupropion dose in this study was based on our experience that this concentration produced more consistent and stable levels of DA. However bupropion, as well as having affinity for the DA transporter, at high concentrations also has affinity for the norepinephrine transporter (Tatsumi et al., 1997). Inhibition of the norepinephrine
transporter has been shown to increase extracellular DA level in certain brain areas (Valentini et al., 2004). It might be speculated that this effect of lithium on DA uptake is masked by an increase in extracellular DA resulting from inhibition of the norepinephrine transporter.

As well as DA release we also monitored changes in DA synthesis by quantifying levels of mRNA coding for the DA synthetic enzyme TH. We found no change in TH mRNA levels either during lithium treatment or after lithium withdrawal. This suggests that the observed change in DA release is not mediated by a change in DA synthesis, at least at the level of mRNA. These findings are not consistent with previous reports that indices of DA synthesis were increased after lithium withdrawal but may be explained by differences in brain regions studied and/or lithium withdrawal regimes used (Ahluwalia and Singhal, 1984).

Here we have shown that, in rats, DA release is attenuated during chronic treatment with clinically relevant doses of lithium. Three days after abrupt withdrawal from lithium treatment DA release is still attenuated. From these data it can be speculated that decreased DA release may mediate the mood-stabilizing action of lithium. However, in this paradigm a rebound increase in DA release was not found after withdrawal from lithium treatment.

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Statement of Interest
None.

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