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Effect of chronic lithium treatment on $D_{2/3}$ autoreceptor regulation of dopaminergic function in the rat

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Abstract

Dysregulation of mesolimbic dopamine (DA) neurotransmission has been implicated in bipolar disorder. DA release in the nucleus accumbens is reduced in rats treated chronically with the mood stabiliser lithium, and this effect is maintained for 3 days after withdrawal from the lithium treatment. We tested whether this decrease in DA release is due to an increase in $D_{2/3}$ autoreceptor sensitivity. In vivo microdialysis studies showed that in the shell of the nucleus accumbens, dialysate DA was decreased following chronic lithium treatment and 3 days after withdrawal from lithium treatment. The elevation of dialysate DA induced by local blockade of the terminal $D_{2/3}$ receptor was reduced in both lithium treated and lithium withdrawn groups. In vitro electrophysiology studies showed that chronic lithium treatment (and lithium withdrawal) did not alter either basal firing rate of DA neurones in the ventral tegmental area, or somatodendritic $D_{2/3}$ autoreceptor-mediated inhibition of firing. D_2 mRNA expression in the ventral tegmental area was unchanged by lithium treatment and lithium withdrawal. Our data suggest that the decrease in dopamine release in the nucleus accumbens induced by chronic lithium treatment is not the result of increased terminal or somatodendritic autoreceptor sensitivity or decreased firing rate of DA neurones.
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Keywords: Dopamine; Lithium; Nucleus accumbens; Ventral tegmental area; Microdialysis; Electrophysiology; In situ hybridization histochemistry

1. Introduction

Bipolar affective disorder is a common psychiatric disorder with a lifetime incidence of at least 1% (Lopez and Murray, 1998). Lithium treatment is an effective prophylaxis for bipolar disorder reducing relapse and the incidence, severity and duration of recurring cycles of affective episodes. However, it has been proposed that abrupt withdrawal from lithium treatment can lead to rebound mania (Goodwin, 1994).

The neurobiological basis of bipolar disorder remains unclear. However it has been reported that alterations in dopaminergic (DA) neurotransmission can precipitate manic states in euthymic bipolar patients and in healthy controls (Silverstone, 1985). Furthermore, inhibition of DA neurotransmission e.g. by administration of D_2 receptor antagonists, or by inhibition of dopamine synthesis ameliorates manic symptoms (McTavish et al., 2001; Yatham et al., 2002). More recently evidence of positive associations between bipolar disorder and genetic polymorphisms within a number of components of DA signalling has supported the hypothesis that dysregulated DA transmission plays a key role in the neurobiology of the disease (Greenwood et al., 2001; Greenwood et al., 2006; Massat et al., 2002; Severino et al., 2005).

Although the clinical benefit of lithium in bipolar disorder is well established, the biological mechanism underlying its action remains unclear. This said, lithium is reported to produce a variety of adaptive neurobiological effects which may contribute to its therapeutic efficacy. Amongst these effects is the ability of lithium, on chronic administration, to attenuate DA neurotransmission and signalling (Basselin et al., 2005; Beaulieu et al., 2004; Ferrie et al., 2005; Ferrie et al., 2006; Gambarana et al., 1999; Guitart and Nestler, 1992; Lesch et al., 1991; Montezinho et al., 2006). One of the major DA pathways in the brain is the so-called mesolimbic pathway linking the

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ventral tegmental area (VTA) with the shell of the nucleus accumbens (NAcc). Activity in this pathway is thought to be important in the regulation of mood and dysfunction of the pathway has been implicated in bipolar disorder (Koob and Swerdlow, 1988). We have previously shown that DA release in the NAcc is reduced in rats treated chronically with lithium and that this effect is maintained for at least 3 days after withdrawal from the lithium treatment (Ferrie et al., 2006).

Whilst the evidence for decreased DA release during chronic lithium treatment is strong, the mechanistic basis of this effect is as yet unclear. We hypothesised that the lithium-induced decrease in DA release is the result of a decrease in the firing rate of VTA DA neurones which innervate the NAcc and/or an increase in the sensitivity of somatodendritic or terminal $D_{2/3}$ autoreceptors. We investigated this hypothesis using in vivo microdialysis, in vitro extracellular electrophysiology, and in situ hybridization histochemistry, in rats treated chronically with lithium.

2. Materials and methods

2.1. Experimental animals and treatments

All procedures conformed to the UK Home Office requirements of the Animal (Scientific Procedures) Act 1986. Male Hooded Lister rats (Charles Rivers) (80–100 g) were housed in groups of four in controlled conditions of light (12 h light/dark cycle, lights on 7am), temperature (21±2 °C) and humidity (~40%). Control animals were given control diet (TD 79092 Teklad, USA) and maintained on this diet for 28 days. The lithium treated animals were given lithium diet containing 1.696 g/kg LiCl (TD 80295, Teklad USA) and maintained on this diet for 28 days. Lithium withdrawn animals were given lithium diet for 25 days then switched to control diet for the remaining 3 days of the 28 day treatment period. All groups were given free access to water and 0.9% saline. Trunk blood was taken from all treatment groups. Plasma samples (~1 ml) were collected and stored at −20 °C until analysed for lithium using a flame photometer (Instrumental Laboratory Flame photometer 943).

2.2. In vivo microdialysis

2.2.1. Surgical procedures

Rats were anaesthetised (anaesthesia initiated between 8am and 9am) with urethane (1.2 g/kg) and placed in a stereotaxic frame. An incision was made exposing the skull surface, and a trephine hole drilled in the skull. A microdialysis probe (1.5 mm window of Hospal membrane) was implanted into the shell of the NAcc (RC: +2.70, ML: −1.20, DV; −7.0 mm relative to bregma and dura, (Paxinos and Watson, 1986)) and perfused at 2 μl/min with aCSF (composition: NaCl 140 mM, KCl 3 mM, $CaCl_2$ 2.4 mM, $MgCl_2$ 1 mM, Na_2HPO_4 1.20 mM, NaH_2PO_4 0.27 mM, Glucose 7.2 mM, pH 7.4 in H_2O) containing the DA uptake inhibitor bupropion (10 μM). Dialysates were collected every 20 min. Once DA levels had been stable for 1 h (2–3 h after probe implantation) the perfusion medium was switched to one containing the $D_{2/3}$ receptor antagonist sulpiride (100 μM) in addition to bupropion. Dialysates were collected for a further 3 h.

Anaesthesia was maintained throughout the course of the experiment and the body temperature of the rat was maintained at 36–37 °C with a homeostatic heating pad and rectal temperature probe. At the end of the experiment, the brain was removed for verification of probe placement and trunk blood was collected for analysis of lithium levels.

2.2.2. Measurement of DA by HPLC–ED

High performance liquid chromatography (HPLC) with electrochemical detection (ED) was used to analyse DA content in dialysates. The mobile phase consisted of: 83 mM NaH_2PO_4, 15% methanol, 0.23 mM octane sulfonic acid sodium salt, 0.84 mM EDTA in aqueous solution, pH 4.0. DA was separated on a C18 column (Varian 4.6 mm × 100 mm Microsorb 100-3) and detected using a Coulochem II detector with the guard cell, E1, and E2 potentials set at −300 mV, +400 mV, and +450 mV, respectively.

2.3. Electrophysiology

Animals were decapitated, the brain was immediately removed and submerged in oxygenated (95%/5% O_2/CO_2) freezing sucrose slush (composition (mM): sucrose: 200, HEPES 10, $MgSO_4$ 7, NaH_2PO_4 1.2, KCl 2.5, $NaHCO_3$ 25, $CaCl_2$ 0.5, D-glucose 10, pH 7.4) for 5 min. The caudal portion of the brain was mounted on a block and submerged in oxygenated sucrose slush in the chamber of a vibrating microtome (Vibratome 1000, Vibratome, St. Louis, USA). Coronal slices of the midbrain (350–400 μm), containing the VTA (bregma −5.2 and −6.3 mm, Paxinos and Watson, 1986), were cut and placed in an interface perfusion chamber and perfused at 0.5 ml/min with oxygenated artificial cerebrospinal fluid (aCSF) (composition (mM): NaCl 123, $MgSO_4$ 1.2, NaH_2PO_4 1.25, KCl 3.75, $NaHCO_3$ 22, $CaCl_2$ 2.5, D-glucose 10, pH 7.4) warmed to 36 °C.

Extracellular recordings were made from neurones in the VTA using glass microelectrodes (1.5 mm OD, Clarke Electromedical, Reading, UK) filled with 2 M NaCl (1–3 MΩ in vitro impedance). Signals were amplified (×1000) with an AC differential preamplifier, notch filtered at 50 Hz, and fed to a PC via a computer interface (1401 or micro1401, CED, Cambridge, UK) at a sampling rate of 10 KHz. The signal was also fed to a pulse discriminator and the TTL pulses generated were fed to the PC (sampling rate 100 Hz). Data were collected using Spike2 software (CED, Cambridge, UK). Between 1 and 6 neurones were recorded from the VTA in each animal. Neurones were identified as DAergic on the basis of their location and electrophysiological characteristics (action potential shape and duration and firing rate and pattern) (see Results). Following a period of recording of basal firing activity, DA (30, 100, 300 μM) was applied via the perfusion medium in 2 min pulses at intervals of at least 4 min. If maximal inhibition was achieved with 30 or 100 μM DA then the higher concentration(s) of DA were not applied.

2.4. In situ hybridization histochemistry

Rats were killed by decapitation and the brains removed and snap frozen. Coronal sections (12 μm) containing the VTA were

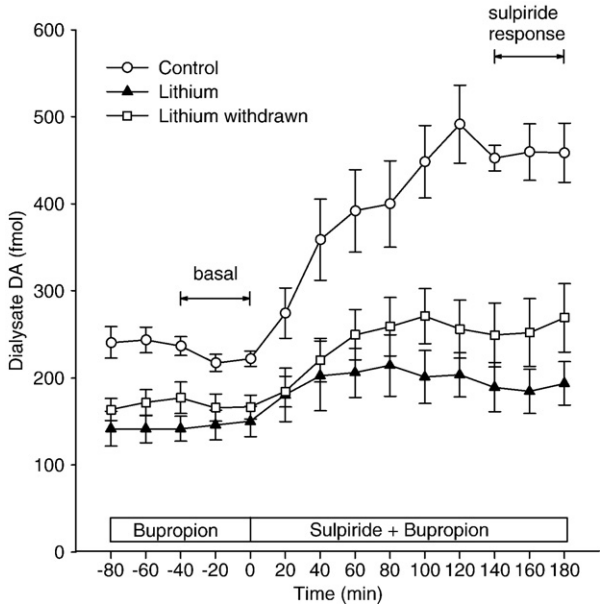

Fig. 1. Effect of lithium and lithium withdrawal on extracellular DA in the NAcc at baseline and in response to local perfusion of the $D_{2/3}$ receptor antagonist sulpiride (100 μM). Measurements were made in the continuous presence of the DA reuptake inhibitor bupropion (10 μM). Values are mean±S.E.M., $n=6–8$ per group.

cut on a cryostat and thaw mounted onto gelatinised slides and stored at −70 °C. The sections were then fixed and pre-treated for in situ hybridization in a single batch using the established protocol (McQuade et al., 2004). A 36 base oligonucleotide probe sequence complimentary to bases 785 to 820 (5′ GTG TTG ACC CGC TTC CGG CAC TTC CGG AGG ACG ATG 3′) of the rat D_2 receptor (X17458) was 3′-tail labelled using [^{35}S] dATP (Perkin Elmer, USA) with terminal deoxynucleotide transferase (Roche). The labelled oligonucleotide probe was added to each section (3×10^5 cpm/section) in hybridization buffer (50% formamide, 4×standard saline citrate (SSC), 10% dextran sulphate, 5×Denhardts, 200 μg/ml salmon sperm DNA, 100 μg/ml polyA, 25 mM sodium phosphate, 1 mM sodium pyrophosphate and 5% dithiothreitol (Pei et al., 1995)). The sections were incubated overnight (32 °C) in sealed boxes containing 50% formamide in 4×SSC. Sections were then washed (3×20 min) in 1 SSC at 58 °C and twice (60 min) at room temperature. After air drying, the sections were exposed to Biomax Hyperfilm™ (Amersham) for 14 days before automatic development.

The relative abundance of D_2 mRNA in the VTA was determined by densitometric quantification of triplicate sections using NIH Scion image software. Density values were calibrated to [^{14}C] standards, and converted to nCi/g tissue.

2.5. Data analysis and statistics

2.5.1. Electrophysiological studies

Basal firing rate was determined for the recording period before addition of DA. Responses to DA were measured as the number of spikes in a 120 s epoch centred on the maximum change in firing rate. For DA the response is reported and analysed as a percentage of the basal firing rate (in the 120s immediately before addition of the DA). The data from neurones from a single animal were averaged before statistical analysis. One-way ANOVA was used to assess the difference between the three treatment groups with respect to the basal firing rate and two-way ANOVA was used to assess between group differences in response to the application of DA.

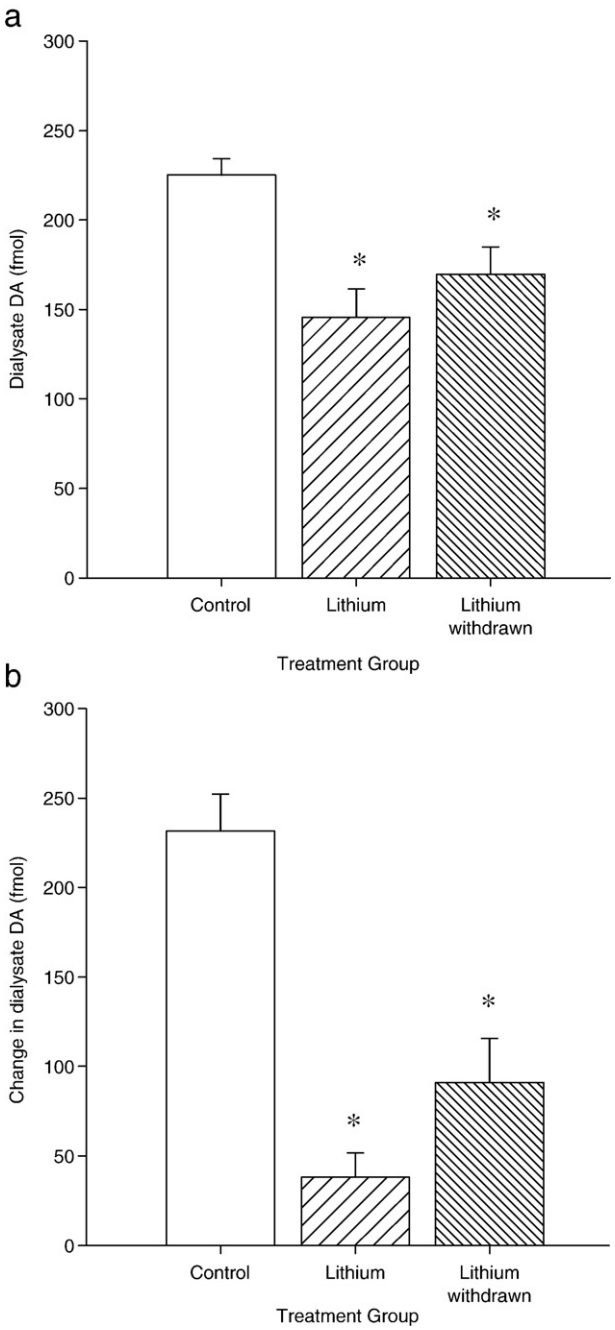

Fig. 2. Effect of lithium and lithium withdrawal on DA levels in the NAcc. Part (a) shows DA in the presence of bupropion (10 μM) calculated from the average of 3 baseline samples. Part (b) shows the response to local perfusion of sulpiride (100 μM) calculated from the average of the last 3 samples minus the average baseline. Values are mean±S.E.M., $n=8$ per group. *$p<0.01$ vs control group.

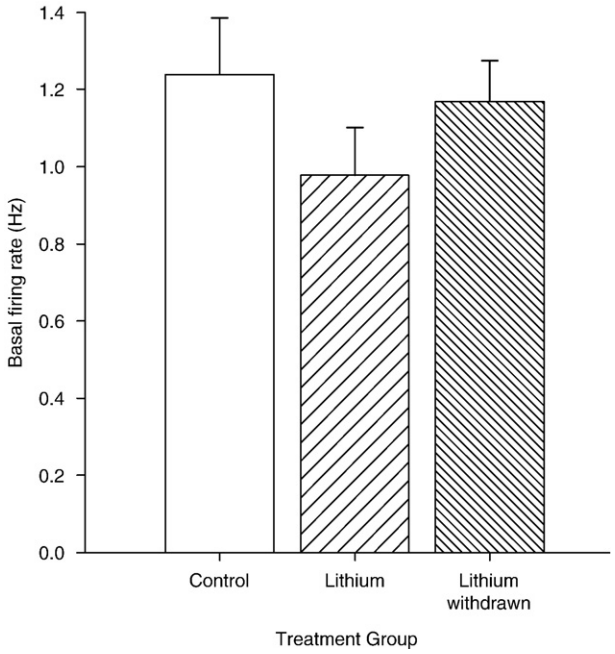

Fig. 3. Effect of lithium and lithium withdrawal on the basal firing rate of DAergic neurones in the VTA in vitro. Recordings were made from up to 6 VTA neurones in each animal and the basal firing rates averaged. Values are mean ± S.E.M., $n = 8–14$ rats per group.

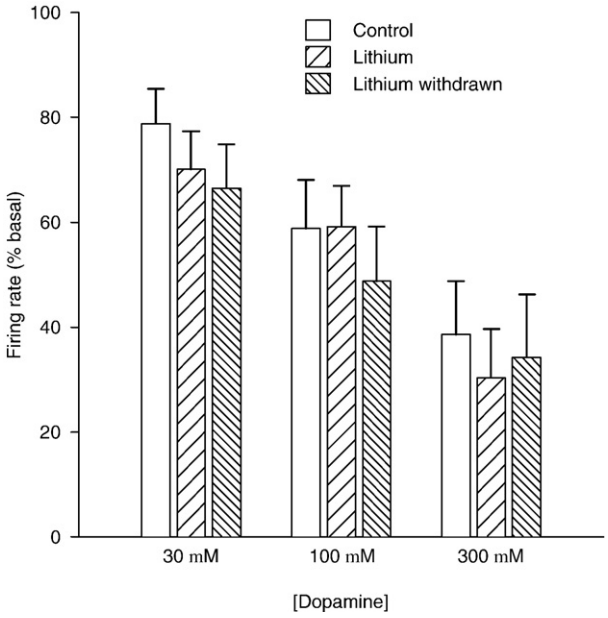

Fig. 5. Effect of lithium and lithium withdrawal on DA-induced inhibition of DAergic neurones in the VTA. DA (30, 100, 300 μM) was applied via the superfusion medium and the inhibitory response recorded. Recordings were made from up to 6 VTA neurones from each animal and responses averaged. Values are mean ± S.E.M., $n = 8–14$ rats per group.

2.5.2. Microdialysis studies

DA levels were calculated as the average of the 3 samples (1 h) before sulpiride perfusion. The response to sulpiride was calculated as the average of the last 3 samples of sulpiride perfusion (1 h) relative to the presulpiride level. Both the pre-sulpiride DA levels and the responses to sulpiride were analysed using one-way ANOVA and post hoc LSD test.

2.5.3. In situ hybridization

The data from the 3 sections from each brain were averaged before statistical analysis. One-way ANOVA was then used to compare control, lithium and lithium withdrawn D_2 mRNA expression in the VTA.

3. Results

3.1. Plasma lithium concentrations

Following the 28 day treatment period, the average plasma lithium concentration in the lithium treatment group was 0.73 ± 0.03 mmol/l ($n = 24$) with individual values ranging between 0.56 and 1.00 mmol/l. Mean plasma lithium

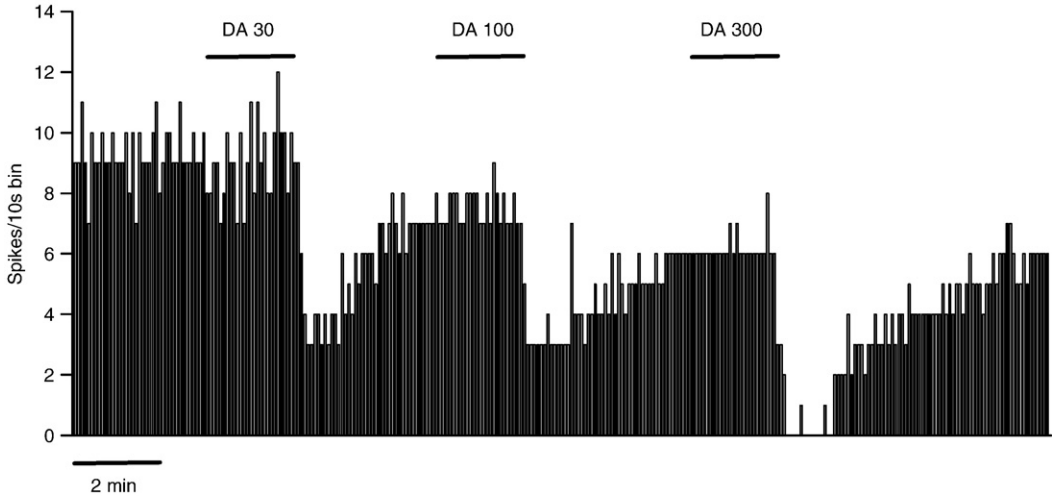

Fig. 4. Example of a rate meter recording from an individual DAergic neurone in the VTA showing inhibitory responses to DA (30, 100, 300 μM).

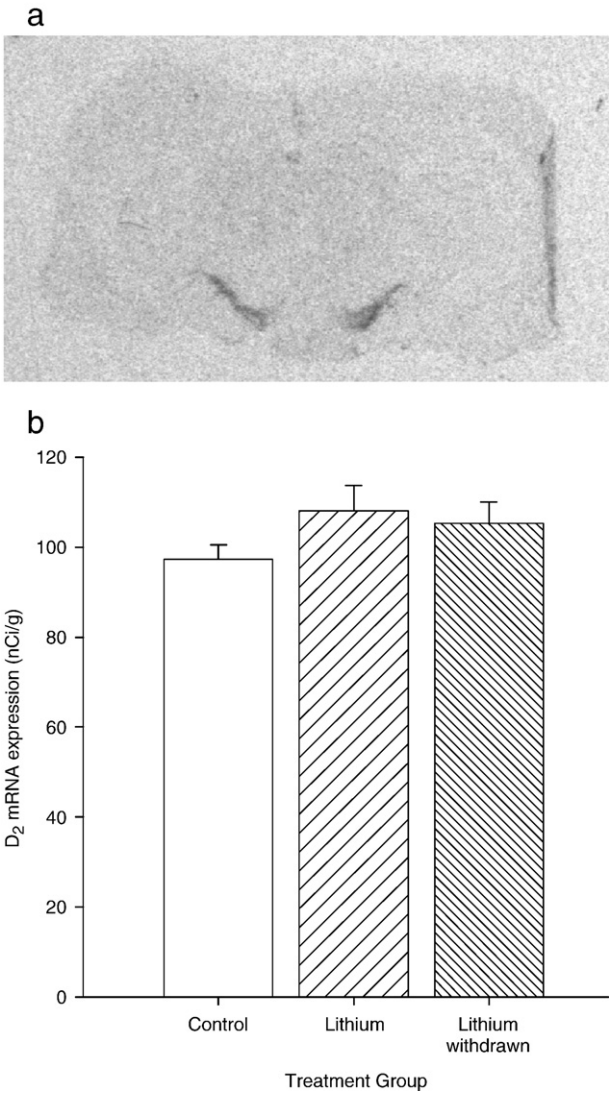

Fig. 6. Effect of and lithium withdrawal on D_2 receptor mRNA expression in the VTA. Panel (a) shows the distribution of D_2 mRNA in a coronal slice of the midbrain. Panel (b) shows the optical density of the signal in the VTA. Measures were taken from 3 sections per animal and the data averaged. Values are mean± S.E.M., $n=8$ per group.

concentration in control animals was (0.01±0.00 mmol/l) ($n=32$) with individual values ranging between 0.00 and 0.07 mmol/l. Mean plasma lithium concentration in lithium withdrawn animals was (0.03±0.00 mmol/l) ($n=24$) with individual values ranging between 0.00 and 0.07 mmol/l.

3.2. Effect of chronic lithium and lithium withdrawal on extracellular DA in NAcc

Fig. 1 shows the full time-course of the microdialysis experiment in the three treatment groups.

3.2.1. DA levels

Dialysate DA levels in the NAcc (in the presence of 10 μM bupropion) were significantly lower in lithium treated (65% of control) and lithium withdrawn rats (75% of control) compared to controls (Fig. 2a). Thus, dialysate DA levels were 225.1±9.1, 145.7±15.9, and 169.6±15.4 fmol in the control, lithium, and lithium withdrawn groups respectively. One-way ANOVA revealed a main effect of treatment ($F_{2,21}=8.7$ $p<0.02$). Post hoc testing revealed that both lithium ($p=0.001$) and lithium withdrawn ($p=0.01$) groups were different to the control group but the lithium and lithium withdrawn groups themselves did not differ.

3.2.2. Response to sulpiride

Local infusion of the $D_{2/3}$ receptor antagonist sulpiride (100 μM) increased DA levels in all 3 treatment groups (Fig. 1). However in the lithium treated group and the lithium withdrawn group this response to sulpiride was attenuated compared to the response in the control group. Thus, mean dialysate DA levels over the last hour of perfusion were: 456.8±21.9, 188.9±26.0 and 256.6±37.7 fmol in control, lithium treated and lithium withdrawn rats respectively. This represented an increase to 204%, 127% and 154% of presulpiride levels in control, lithium treated and lithium withdrawn rats respectively. One-way analysis of variance of the change in DA with post hoc LSD test revealed a significant difference between control and both lithium ($p<0.001$), and lithium withdrawn groups ($p=0.003$) (see Fig. 2b). The same pattern of significant differences was seen when the response was considered as a % change from presulpiride levels.

3.3. Effect of chronic lithium and lithium withdrawal on firing activity of DA neurones in the VTA

DA neurones were identified on the basis of their location in the VTA and their basal electrophysiological characteristics (Grace and Onn, 1989). The neurones included in the analysis were spontaneously active with slow and regular firing rate with biphasic or triphasic action potentials of long duration. The basal firing rate of the 86 presumed DAergic neurones included in the study ranged from 0.33–2.72 Hz. For these 86 neurones the action potential duration (measured from the start of the depolarization to the trough of the hyperpolarization) ranged from 1.25–3.39 ms.

3.3.1. Basal firing rate

The mean (per animal) basal firing rate did not differ significantly between treatment groups (control: 1.24±0.15 Hz ($n=12$), lithium: 0.98 ± 0.12 ($n=8$), lithium withdrawn: 1.19±0.11 ($n=8$) (Fig. 3).

3.3.2. Sensitivity to exogenous DA

The majority of neurones tested with DA (30–300 μM) displayed a concentration-dependent inhibitory response (for example see Fig. 4). However, the degree of inhibition between individual neurones varied markedly and some neurones failed to respond to even the highest concentration of DA. The neurones which were insensitive to DA did not differ from the DA sensitive neurones with respect to basal firing rate or AP duration. Responses of individual neurones from each animal were averaged (Fig. 5). Analysis by two-way ANOVA showed a highly significant effect of DA concentration ($F_{2,50}=52.0$,

$p<0.0001$) but no significant effect of treatment and no significant interaction between treatment and DA concentration.

3.4. Effect of chronic lithium and lithium withdrawal on D_2 receptor mRNA expression in the VTA

D_2 mRNA was clearly detectable in the region of the VTA and substantia nigra (Fig. 6a). Quantitative analysis of the level of expression of D_2 mRNA revealed no difference between any of the treatment groups (one-way ANOVA $p=0.99$) (Fig. 6b).

4. Discussion

Here we have investigated the effect of chronic lithium treatment and withdrawal from lithium treatment on terminal DA release in the shell of the NAcc in the rat. We also examined the effects of these treatments on the principal factors regulating terminal DA release. We hypothesised that the lithium-induced decrease in DA release occurs as a result of a decrease in the firing rate of DA neurones and/or an increase in the sensitivity of autoreceptors located on cell bodies or terminals of these DA neurones. Consistent with our previous data we found that, in the shell of the NAcc, DA release measured in vivo was decreased following chronic lithium treatment and that this effect was maintained after 3 days withdrawal. The elevation of dialysate DA induced by local infusion of the $D_{2/3}$ receptor antagonist sulpiride was reduced in both lithium treated and lithium withdrawn groups. Chronic lithium treatment and withdrawal from lithium failed to alter the spontaneous in vitro firing of VTA DAergic neurones. The inhibition of firing of VTA DA neurones induced by perfusion of DA was also unaltered in lithium treated and lithium withdrawn animals. Finally we found the expression of D_2 mRNA in the VTA to be unchanged by lithium treatment and lithium withdrawal.

4.1. Lithium treatment

Our present finding that DA levels (in the presence of bupropion) in the NAcc shell were significantly lower in lithium treated rats is consistent findings from one of our earlier studies (Ferrie et al., 2005), however this said, in another study we observed no difference in DA levels (Ferrie et al., 2006). It is important to note that in all three studies we measured extracellular DA in the presence of the uptake inhibitor bupropion. One possible explanation for the discrepancies is the different concentrations of bupropion used in the three studies (ranging from 1–30 μM). Bupropion, whilst having high affinity for the DA transporter, is relatively nonselective and at higher concentrations has affinity for the noradrenaline transporter and also for nicotinic receptors (Slemmer et al., 2000). Given recent reports of nicotinic receptor regulation of DA release it is possible that at higher concentrations, bupropion's effect on nicotinic receptors may influence its effect on DA levels (Sidhpura et al., 2007). In the present study a concentration of bupropion of 10 μM was chosen on the basis recent experience that this concentration produces most consistent dialysate DA levels. It should also be noted that in the present study urethane was used as the anaesthetic, whilst previously chloral hydrate had been used. The difference in anaesthesia may also contribute to inconsistencies between the three studies.

The main aim of this present study was to determine the mechanisms underlying the lithium-induced decrease in DA levels. Given that extracellular levels of a transmitter are determined by the opposing processes of release and reuptake, in the presence of a reuptake blocker the lithium-induced changes in dialysate levels may be assumed to reflect a change in transmitter release.

One important regulator of terminal DA release in the NAcc is the terminal autoreceptor, activation of which negatively regulates transmitter release. Here we probed the sensitivity of the terminal autoreceptor by local perfusion of a $D_{2/3}$ receptor antagonist, sulpiride. In the control animals, sulpiride perfusion caused a large increase in DA release consistent with a high degree of autoreceptor tone in the NAcc. However, this sulpiride-induced increase was markedly attenuated in the lithium treated group. This finding suggests firstly, that the lower DA levels produce less tonic activation of the autoreceptor and secondly, that the decreased DA release cannot be explained by an *increase* in terminal autoreceptor sensitivity or function.

The NAcc is innervated by DA neurones which have their cell bodies in the VTA (Fallon and Moore, 1978). We used electrophysiology to examine the firing activity of VTA DAergic neurones. Putative DAergic neurones in the VTA were selected on the basis of electrophysiological criteria (Grace and Onn, 1989). They also fulfilled the additional criterion of having an AP (start to trough) duration longer than 1.1 ms which has recently been shown to distinguish VTA DAergic neurones recorded in vivo from non-DAergic with similar electrophysiological characteristics (Ungless et al., 2004). We found no difference in the basal firing rate of presumed DAergic neurones between the treatment groups. We also examined the sensitivity of the presumed DAergic neurones to autoinhibition. In common with many other studies (Kiyatkin and Rebec, 1998; Yim and Mogenson, 1980), we found a wide variation in DA sensitivity in the presumed DAergic neurones recorded. We found no effect of lithium treatment on sensitivity of VTA DAergic neurones to DA. Evidence suggests that both D_2 and D_3 receptor subtypes are present on VTA neurones and may function as autoreceptors (Diaz et al., 2000; Levant, 1997). In our study we used the endogenous agonist DA which would be expected to activate both receptor subtypes hence we conclude there is no difference in either D_2 or D_3 sensitivity following lithium treatment.

In our in situ hybridization study we found no change in the density of D_2 receptor mRNA in the VTA following lithium treatment. D_2 receptor mRNA in the VTA codes for both somatodendritic and terminal D_2 receptor populations. This lack of effect of lithium on the expression of D_2 mRNA is fully consistent with both our microdialysis and electrophysiology studies where we found no functional change in terminal and somatodendritic D_2 receptors, respectively. Taken together our data are also consistent with a recent study showing no effect of chronic lithium treatment on D_2 sensitivity in the NAcc and VTA using a 2-deoxy-D-glucose model (Basselin et al., 2006).

However other studies have reported decreased D_2 receptor immunoreactivity and also decreased D_2 receptor signalling via PLA_2 in both these regions after lithium administration regimes similar to our own (Basselin et al., 2005; Montezinho et al., 2006).

4.2. Lithium withdrawal

As well as investigating the effect of lithium on regulation of DA release, we also investigated how any neurobiological effects of lithium responded to withdrawal from the drug. Interestingly we found that, consistent with our previous finding, the attenuated DA release was maintained 3 days after withdrawal from lithium treatment (Ferrie et al., 2006). Following 3 days withdrawal plasma lithium levels were negligible. Although one study has suggested that the wash-out of lithium from the brain is much slower than from the plasma (Bosetti et al., 2002), in a separate group of animals we measured brain levels of lithium 3 days after withdrawal and found undetectable or very low levels (unpublished) confirming clearance of the drug from both plasma and brain is all but complete after 3 days withdrawal. This suggests that this effect of lithium is adaptive and persists for a period of time after removal of the lithium ion and furthermore indicate that it is unlikely that DA hyperfunctionality occurs after withdrawal from lithium treatment, at least within the time-frame captured in this study.

4.3. Conclusion

In conclusion, we have shown (here and previously) that chronic lithium treatment decreases DA release in the NAcc. In the present study we explored a number of putative mechanisms. Our dialysis study indicated that the decrease in DA release could not be accounted for by an increase in the sensitivity of terminal $D_{2/3}$ autoreceptors. Electrophysiological evidence indicated that the decrease in release is unlikely to be due to a decrease in basal firing rate, or an increase in somatodendritic $D_{2/3}$ function. Whilst, it is possible that the effect of lithium is mediated by a decrease in DA synthesis, we have previously found that the expression of mRNA coding for tyrosine hydroxylase, in the VTA is not affected by chronic lithium treatment (Ferrie et al., 2006). Further studies to examine the effects of lithium on other intracellular factors (e.g. VMAT2), terminal and somatodendritic heteroceptors as well as in vivo functional studies of afferent regulation, may contribute to further understanding the neurobiological mechanisms underlying the effects of lithium on DA release.

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