

# The effect of prolonged administration of lithium on the level of dopamine D<sub>2</sub> receptor mRNA in the rat striatum and nucleus accumbens

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**Abstract.** In the present study the alterations in the levels of mRNA coding for dopamine receptor D<sub>2</sub>, were determined in the rat striatum (STR) and the nucleus accumbens septi (NAS), in dependence on duration of lithium administration. The levels of mRNA coding for D<sub>2</sub> receptor (determined using an *in situ* hybridization technique) were determined after 1, 7 and 14 days of lithium administration (LiCl, 6 mEq/kg, p.o.), at 3 and 24 h after the last dose of the drug. This treatment resulted in the increase in the levels of mRNA coding for dopamine receptor D<sub>2</sub> in all brain regions examined and the effect depended on the time after lithium administration. However the effect was most pronounced in the shell region of NAS, 24 h after the 14-day treatment.

**Key words:** lithium, mRNA D<sub>2</sub>, striatum, nucleus accumbens, rat

## INTRODUCTION

Lithium salts are among the most effective drugs in the prevention and treatment of manic-depressive episodes (Post 1986). The substantial number of clinical and pharmacological investigations suggest that overactivity of dopaminergic pathway may, at least in part, mediate human mania (Schildkraut 1965, Bunney et al. 1971, Gerner et al. 1976, Garver and Davis 1979). Behavioural experiments indeed suggest that lithium renders less active dopaminergic system. For example it has been reported that rats maintained on a lithium diet for three weeks exhibited less spontaneous activity during habituation to a new environment than their controls did (Bloom et al. 1983). In addition, acute and chronic lithium treatment have been repeatedly reported to reduce the locomotor stimulation induced by amphetamine (Berggren 1985) as well as the responses to apomorphine in animals rendered supersensitive by chronic haloperidol treatment (Bloom et al. 1983, Bunney and Garland 1983).

When the issue of the effects of prolonged lithium treatment on the activity of dopaminergic system was addressed *via* the parameters characteristic for DA receptors, the outcome of numerous studies neither had indicated any clear answer. Original finding that chronic lithium administration interferes with the development of DA postsynaptic supersensitivity was reported by Pert et al. (1987) but it could not be confirmed by subsequent studies (Reches et al. 1982, Staunton et al. 1982, Bloom et al. 1983, Pittman et al. 1984). Also in our laboratory we were not able to see any changes in the binding parameters of [<sup>3</sup>H]SCH23390 or [<sup>3</sup>H]spiperone to the membranes of striatum or limbic forebrain of rats treated with LiCl for 21 days (Dziedzicka-Wasylewska and Klimek, unpublished results).

Since clinical effect of lithium is generally observed only after the prolonged treatment, apparently the biochemical changes requiring such prolonged administration of the drug suggest alterations at the genomic level. Until recently, however, little has been known about transcriptional and posttranscriptional factors regulated by chronic

drug treatment, although long-term changes in neuronal synaptic function are known to be dependent upon selective regulation of gene expression. Therefore the present study was designed in order to obtain information whether repeated administration of LiCl modifies the biosynthesis of postsynaptic dopamine D<sub>2</sub> receptors in the rat striatum (STR) and the nucleus accumbens septi (NAS) core and shell.

## METHODS

### Lithium administration

Male Wistar rats (weight 180-200 g) were administered LiCl (6 mEq/kg) by means of intragastric intubation, once daily, for the period of 1, 7 or 14 days. Animals were sacrificed 3 or 24 h after the indicated dose of lithium. The trunk blood was collected when the animals were sacrificed and the serum was separated. The lithium content of the serum was determined by atomic absorption spectrometry (Pybus and Bowers 1970).

On the repeated administration of lithium the levels in serum varied as a function of time after each number of doses. At 3 h following the single as well as repeated administration of lithium the mean serum level rose to  $0.75 \pm 0.02$  mmol/l. This level diminished to  $0.35 \pm 0.02$  mmol/l by 24 h after each dose.

### *In situ* hybridization

For the *in situ* hybridization study the brains of the rats were rapidly removed at 3 or 24 h after the indicated dose of LiCl and frozen on dry ice. Coronal sections (12 µm thick) were made on cryostat through the NAS and STR. The sections were thaw-mounted onto chrome alum pretreated slides, post-fixed in 4% paraformaldehyde for 10 min and processed for *in situ* hybridization as described previously (Dziedzicka-Wasylewska and Rogoż 1995). Briefly, a 48-mer synthetic oligonucleotide mixture (New England Nuclear) complementary to the rat dopamine D<sub>2</sub> receptor was labelled using [<sup>35</sup>S]dATP (1200 Ci/mmol, New England Nuclear) to obtain a specific activity of about  $4 \times 10^5$  cpm/µl.

The sections were hybridized with the labelled oligonucleotide for 20 h at 37°C in a humidified incubator. After washing at 40°C, the sections were dried in a cool-air stream and exposed to a film (Hyperfilm MP, Amersham) for 20 days at -70°C. The specificity of in situ hybridization was assessed by pretreatment of some tissue sections with RNAase A (20 µg/ml) for 40 min at 30°C, which completely eliminated the hybridization signal with the cDNA probe. Moreover, when the hybridization was carried out in the presence of a 100-fold excess of the unlabelled probe, the signal also disappeared.

Optical density measurements were made from the autoradiograms corresponding to the sections of the NAS and STR, using an image analysing system (Java: Jandel, Corte Madera CA, USA). The average optical density values were calculated after subtraction of the film background density. The mean optical density values were obtained by averaging out the measurements from autoradiograms of the sections obtained from 5-6 animals per group.

### Statistical analysis

The results were statistically assessed by one-way analysis of variance (ANOVA), and inter-

group differences were analysed by Duncan's multiple-range test.

## RESULTS

The typical autoradiogram showing the dopamine D<sub>2</sub> receptor mRNA is presented in the Fig. 1.

The effect of lithium on the level of mRNA coding for dopaminergic D<sub>2</sub> receptor in the rat STR and NAS core and shell is presented in Table I. Single dose of lithium already produced an increase in the levels of mRNA coding for D<sub>2</sub> receptor but the effect was statistically significant only in the core region of the NAS. Further administration of lithium up to 7 or 14 days resulted in the statistically significant increase in the levels of mRNA coding for D<sub>2</sub> receptor in all brain regions examined and the effect was the strongest in the shell region of the NAS.

The increase in the levels of mRNA coding for D<sub>2</sub> receptors strongly depended on time after the last dose of the drug; i.e. the effect were the most pronounced at 24 h after the administration of lithium.

## DISCUSSION

In the present study we determined the time course of changes in the levels of mRNA coding for

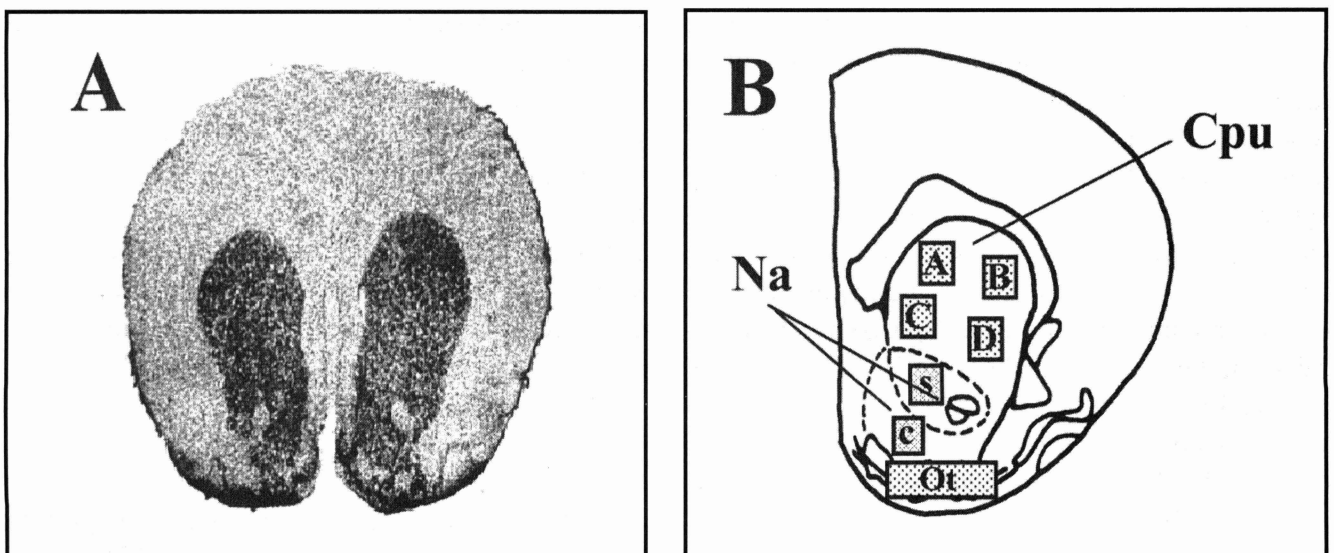


Fig. 1. A, example photomicrograph of brain section hybridised with probe against D<sub>2</sub> receptor mRNA. B, diagrammatic representation of the areas used in optical density analysis of specific brain regions.

TABLE I

The effect of lithium administration on the level of mRNA (optical density arbitrary units) coding for dopamine D<sub>2</sub> receptors in the striatum (STR) and the nucleus accumbens (NAS) shell and core of the rat

Treatment (days)	STR	NAS core	NAS shell
	11.02±1.3	8.52±1.0	13.40±1.5
1 day, 3 h	11.22±1.3	10.52±1.2*	15.40±1.9
7 days, 3 h	12.33±1.42	10.15±1.25	16.35±2.1*
7 days 24 h	12.55±1.35*	11.35±1.4*	17.99±1.9*
14 days 3 h	13.95±1.28*	12.23±1.36*	17.01±2.2*
14 days 24 h	14.45±1.52*	12.95±1.40*	20.12±2.4*

LiCl (6 mEq/kg, p.o.) was administered as a single dose or once daily for 7 or 14 days. The rat brains were removed at 3 or 24 h after the last administration of the drug. The mean optical density values were obtained by averaging out the measurements from autoradiograms of the sections obtained from 5-6 animals. ANOVA followed by Duncan's test; \**P*<0.01 vs. the control level.

dopamine D<sub>2</sub> receptors in the rat striatum (STR) and the nucleus accumbens (NAS) shell and core upon the prolonged administration of lithium. Since the literature concerning the effect of lithium on the dopaminergic system is full of discrepancies which might result from different experimental paradigms, it seemed interesting to see whether any alterations develop in dependence on various periods of drug administration as well as on time after the last dose of the drug.

The increase in the levels of mRNA coding for dopaminergic D<sub>2</sub> receptor in both examined brain structures was observed in the present study upon the repeated administration of lithium, and the effect was also more pronounced in the NAS. Although the mechanism of signal transduction from synaptic cleft to the nuclear compartments responsible for regulation of synthesis of D<sub>2</sub> receptors are not known at present, it is tempting to postulate the enhancement of biosynthesis of postsynaptic dopaminergic D<sub>2</sub> receptor as the adaptive change to the weakened DA release from terminals, the effect which we have shown recently (Dziedzicka-Wasylewska et al. 1995). A neurochemical transsynaptic mechanism which would enhance dopaminergic compensation involves an increase in postsynaptic DA receptors. It has been already shown (Coirini et al. 1990, Srivastava et al. 1990, Angulo et al. 1991,

Bernard et al. 1991, Chen et al. 1991, 1993, Jaber et al. 1992, 1994) that dopaminergic transmission indeed regulates the level of mRNA coding for D<sub>2</sub> DA receptor, by exerting tonic inhibition on dopamine D<sub>2</sub> mRNA. Unfortunately up to the present it was not possible to show unequivocally any effects of lithium, at "therapeutic" doses, on the parameters characteristic for ligand binding to DA receptors. It must be stressed, however, that in majority of studies the radioligands of antagonistic nature towards the DA receptors have been used, such as [<sup>3</sup>H]spiperone or [<sup>3</sup>H]raclopride. DA D<sub>2</sub> (as well as D<sub>1</sub>) receptor exists in two different conformational states of the same protein which can be revealed by agonist competition curves, but not by using antagonistic ligands, which have equal affinity for the two states of the receptor so that any alterations in the high- and low-affinity sites of D<sub>2</sub> receptor elicited by chronic lithium treatment may not be revealed by the examination of antagonistic binding. However, as has been recently shown by Carli et al. (1994), the affinity of the DA receptors for DA was lower after lithium treatment. The changes in the affinities were proposed to be due rather to the conformational change at the G protein level. The increase in the biosynthesis of D<sub>2</sub> receptors observed in the present study the most probably is the adaptive compensatory mechanism and may

well result from the complex events taking place in the dopaminergic synaptic cleft following prolonged lithium administration, i.e.: attenuated DA release from terminals and diminished coupling efficacy between the DA receptors and the guanine nucleotide regulatory protein (possibly due to the changes in gene expression for various G proteins following treatment with lithium (Li et al. 1991) but also to the alterations in the organization of neuronal plasmatic membranes, as has been shown by Lopez-Corcuera et al. (1988) - the events which are postulated as the mechanism responsible for anti-manic action of lithium. On the other hand, the changes observed at the level of mRNA coding for D<sub>2</sub> receptor are at present interpreted as an adaptive change to the weaken dopamine release. However, further studies, possibly using radioligands of agonistic nature towards D<sub>2</sub> dopaminergic receptors, might reveal new aspects of the role of D<sub>2</sub> receptor itself in the mechanism of therapeutic action of lithium.

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