Effect of bipolar disorder on lymphocyte inositol monophosphatase mRNA levels

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Abstract

The activity of inositol monophosphatase (IMPase), the lithium (Li)-inhibitable enzyme in the phosphatidylinositol (PI) signal transduction system, has recently been found significantly lower in lymphoblastoid cell lines from bipolar (BP) patients, particularly in Li-responders. To probe for possible quick detection of the disease and prediction of the therapeutic response we repeated our study in fresh lymphocytes. Since IMPase in fresh lymphocytes is inhibited in vivo by ongoing Li treatment and its pre-Li activity cannot be evaluated, IMPase mRNA levels were measured. Relative (to β-actin) mRNA levels were quantified by reverse transcriptase (RT)-PCR in 5 drug-free and 31 drug-treated BP patients compared with 36 control subjects in fresh lymphocytes. In agreement with our findings with IMPase activity, the small group of drug-free BP patients exhibited ~4 reduction in IMPase relative mRNA levels compared to control subjects. Approximately 2-fold elevation of these levels toward control values was found for patients treated with Li and other mood stabilizers. The study further suggests the possible importance of IMPase in the aetiology of BP disorder and in the mediation of the therapeutic efficacy of Li. It may be that chronic inhibition of IMPase activity by Li results in up-regulation of its gene at the transcriptional level.

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Introduction

Lithium (Li) at therapeutically relevant concentrations is an uncompetitive inhibitor of IMPase (Hallcher and Sherman, 1980). Hallcher and Sherman (1980) and Berridge and his associates (Berridge, 1984; Berridge et al., 1989) proposed that the clinical efficacy of the action of Li is derived through a depletion of free inositol following IMPase inhibition. Some investigators (Banks et al., 1990; Manji et al., 1995; Jope et al., 1996) suggest that PI-signalling pathways may be involved in the pathophysiology of bipolar (BP) disorder. We have recently found (Shamir et al., 1998) significantly lower IMPase activity in lymphoblastoid cell lines from BP patients, particularly in Li-responders, compared with control subjects. The growth of these cell lines in culture requires extensive laboratory work and cannot be done as a real-time rapid diagnostic test. We therefore planned to repeat our study in fresh lymphocytes. However, IMPase in fresh lymphocytes is inhibited in vivo by ongoing Li treatment and its pre-Li activity cannot be evaluated. Therefore, we studied IMPase relative (to β-actin) mRNA levels of the gene (IMPA chromosome 8) coding for the predominant brain form of this enzyme (Sjoholt et al., 1997; Yoshikawa et al., 1997). The mRNA levels were measured in fresh lymphocytes of heterogeneous groups of BP patients and controls, including a limited number of drug-free BP patients.

Methods

Subjects

Thirty-six non-hospitalized BP patients (22 male, 14 female; average age ± s.d. = 43.8 ± 13.0 yr; range 21–70), that included 15 Ashkenazi Jews, 19 Sephardic Jews and 2 mixed individuals, were diagnosed according to DSM-IV criteria, and recruited from the Lithium Clinic of the Beer Sheva Mental Health Center. Eleven patients were treated with Li only whereas 20 were treated with Li and other drugs (such as carbamazepine, valproate, haloperidol, chlorpromazine, thioridazine, mocllobemide, clonazepam and fluoxetine) or other drugs only. Five patients were drug-free. All patients were euthymic. Thirty-six control subjects (18 male, 18 female; average
age ± s.d. = 55.7 ± 15.7 yr; range 19–86), that included 21 Ashkenazi Jews, 14 Sephardic Jews and 1 Arab were recruited from the Beer Sheva area and had no history of psychiatric illness. The control subjects included volunteers (n = 11) recruited from staff and students at the Soroka Hospital and hospitalized non-psychiatric patients in the Department of Internal Medicine (n = 25). Heparinized blood (10–20 ml) was withdrawn by venepuncture. Informed consent was obtained from all patients.

**Biochemical**

Isolation of lymphocytes, isolation of RNA from lymphocytes, and quantitative reverse transcriptase polymerase chain reaction (RT-PCR) have been previously described in detail by our laboratory (Ebstein et al., 1996). The method we employed for the quantification of relative mRNA levels is modified from the procedure first suggested by Horikoshi and his colleagues (1992). In brief, lymphocytes were isolated from whole fresh blood by density gradient centrifugation over Ficol–Paque (Pharmacia). RNA was purified using Trizol reagent (Gibco). Purity and concentration of the RNA were determined spectrophotometrically (GeneQuant, Pharmacia). Cells were cultured in RPMI medium containing 35 mg/l inositol.

First-strand cDNA synthesis was performed using IMP-LOW as a primer (the sequence is given below) and SuperScript II reverse transcriptase (Gibco, Gent). The 20 µl reaction mixture contained first-strand buffer (Gibco), 1 µg total RNA, 10 pmol of the reverse primer, 10 U RNasin, 200 U reverse transcriptase, 0.5 mM dNTPs. After heating the RNA and primer to 95 °C for 2 min and cooling immediately on ice for at least 5 min, the remaining reagents were added and the reaction incubated at 37 °C for 1 h. Reverse transcription is terminated by heating to 65 °C for 10 min. β-actin and IMPase first-strand cDNA synthesis was carried out in separate reactions. The β-actin mixture was diluted 1:800 and the IMPase mixture was diluted 1:50, and 4 µl from the first-strand synthesis from each reaction mixture was used in separate PCR reactions. In the absence of the reverse transcriptase enzyme no amplification of product was observed in the PCR reaction excluding DNA contamination in the RNA preparation as a source of template in the PCR reaction.

The PCR reaction mixture (50 µl final volume) consisted of the following components: 0.5 U Taq polymerase (MBI), either 5 pmol forward and back primers (β-actin) or 2 pmol forward and back (IMPase), and the provided Taq buffer containing 2.0 mM MgCl₂, 5 µg BSA, 50 mM KCl, 10 mM Tris (pH 8.8), 0.08% Nonidet P40, 200 µM dATP, dGTP, dTTP and 20 µM dCTP. A total of 1–2 µCi [α-32P]dCTP was added to the reaction mixture to monitor the course of the reaction and to quantify the subsequent PCR products. The PCR conditions were: 94 °C for 1 min (denaturation); 55 °C for 1 min (annealing); 72 °C for 2 min (extension) for either 24 (IMPase) or 18 (β-actin) amplification cycles. A final extension was carried out at 70 °C for 5 min. Either a Hybaid or Perkin–Elmer thermal cycler were used to carry out the PCR. Aliquots of the reaction mixture (5 µl each of the β-actin and IMPase PCR mix for a total of 10 µl + 2 µl loading buffer) were then electrophoresed on a 6% acrylamide gel (1 mm) in a vertical gel apparatus (Hoeffer) at 150 V constant voltage for approx. 2.5 h. β-actin and IMPase from the same subject were loaded on the same lane. A [γ-32P]ATP labelled molecular weight ladder was run simultaneously to subsequently ascertain the size of the amplified fragments. Following electrophoresis, the gel was dried (BioRad gel dryer) and then exposed to X-ray film (Kodak X-OMAT AR) for appropriate periods. The autoradiogram was analysed by scanning with a laser scanner (Hewlett–Packard) and the intensity of the bands quantified using densitometry software (Quantiscan, Biosoft).

The IMPase primers (below) were designed using the computer program oligo based on the published cDNA structure of IMPase obtained from Genbank.

1. IMP-UP: 5′-TCCATCTCACGTTCAT-3′
2. IMP-LOW: 5′-CATCTTGCCTTCCACACA-3′.

This primer pair was predicted from the cDNA sequence to amplify a 205 bp fragment (222–427 bp) which was verified by comparison to a known DNA ladder marker on both agarose and acrylamide gels. The amplified DNA product was sequenced (Biological Services, Weizman Institute) and was identical to the cDNA published sequence.

Human β-actin served as the control standard. The primers for β-actin amplification were:

1. Upper primer: 5′-GAGAAAGATGCACCATGTG-3′
2. Lower primer: 5′-ACTCCATGC CCAGGAAGAAGG-3′.

The amount of IMPase mRNA present in lymphocytes was determined by the method of Horikoshi and colleagues (1992) who developed a novel approach to competitive quantitative PCR. The key features of this method are: (i) the relative, rather than the absolute, levels of gene expression are determined by comparing the ratio of PCR products generated by amplification of the target DNA segment and an endogenous internal standard gene in separate reactions, and (ii) linear amplification regions are determined simply by serial dilution of the cDNA.
Effect of bipolar disorder on lymphocyte IMPase mRNA levels

Figure 1. Relationship between starting amount of cDNA and the amount of the PCR product formed. Band intensity is expressed in arbitrary units. (a) β-Actin – the initial dilution of the reverse-transcriptase product was 1:800. (b) IMPase – the initial dilution of the reverse-transcriptase product was 1:50.

Figure 2. Reproducibility of IMPase mRNA levels: \( r = 0.84; n = 59; p < 0.001 \).

(first-strand) synthesis reaction sample, without the need for quantifying input RNA. As shown in Figure 1, the linear range of amplification of cDNA was established for IMPase and β-actin by a series of dilutions of the cDNA first-strand reverse-transcriptase reaction mixture. For β-actin the linearity range was between 1 and 4 µl (Figure 1a) and for IMPase, 1–4 µl (Figure 1b). Each measurement of relative IMPase mRNA was carried out in duplicate. Experiments in our laboratory demonstrate good reproducibility for these determinations (Figure 2).

Statistical analysis

All tests were carried out using SPSS PC version 8.

Results

IMPase relative mRNA levels were significantly lower in the drug-free \((n = 5)\) BP patients (Figure 3, independent samples \(t\) test: \(t = 2.13, p = 0.04\), two-tail, determined for unequal variances) compared to the control subjects \((n = 36)\). No significant effect of age or sex was observed on lymphocyte IMPase relative mRNA levels in either the patient or control groups. There was a nearly 2-fold increase in IMPase relative mRNA levels in the drug-treated (Li only, Li and other drugs and other drugs only) patient cohort \((n = 31)\) compared to the drug-free patient group (Figure 3; \(t = 1.74, p = 0.049\), one-tail). For patients receiving solely Li there was a correlation between Li plasma concentrations and IMPase relative mRNA level \((r = 0.54\) Pearson correlation, \(p = 0.042\) one-tail, \(n = 11)\). Since the control group comprised two subgroups (hospitalized and non-hospitalized) we also examined, by one-way ANOVA, differences in IMPase
mRNA between three groups: non-hospitalized control subjects, drug-free and drug-treated BP patients ($F = 2.81, p = 0.07, \text{d.f.} = 2, 46$). Post-hoc (L.S.D.) analysis showed a difference between drug-treated BP patients vs. non-hospitalized control subjects ($p = 0.03$) and between drug-free BP patients ($n = 5$) vs. non-hospitalized control subjects ($p = 0.08$).

**Discussion**

A previous preliminary study in a small group of post-mortem BP patient brains found no reduction in IMPase activity, but drug treatment and agonal state were not controlled (Agam and Shimon, In Press). However, in lymphoblastoid cell lines devoid of differing environmental and humoral variables, a recent study (Shamir et al., 1998) showed significantly lower IMPase activity in BP patients, particularly in Li-responders. In the present study, there was a corroborating reduction in lymphocyte IMPase relative mRNA levels in drug-free BP patients. Considering the well-established in vitro inhibition of IMPase by therapeutic Li concentrations, our results appear counterintuitive. If Li is an inhibitor of IMPase, and Li is a mood-stabilizing drug, one would expect elevated IMPase levels in drug-free BP patients. Regulation of IMPase activity is likely to be more complicated and might be subject to transcriptional modulation. In BP Li responders with low enzymatic activity, it is possible that chronic inhibition of IMPase activity by Li therapy leads to transcriptional up-regulation of mRNA levels and normalized enzyme activity. A similar phenomenon of acute inhibition but chronic up-regulation exerted by Li has recently been described for glutamate uptake in presynaptic nerve endings (Dixon and Hokin, 1998). Our previous results (Shamir et al., 1998) and the present results suggest that low IMPase transcription and translation are possibly trait markers of BP disorder.

Chronic Li for 3 wk elevated rat brain IMPase activity in one early report (Renshaw et al., 1986) although it has not been found by others (Honchar et al., 1989). In our recent study (Shamir et al., 1998) in vitro incubation of 59 cell lines for 5 d with 1 mM Li significantly up-regulated IMPase mRNA levels by 40%. In the present study, patients treated with mood stabilizers, including Li, showed a trend for increased IMPase mRNA levels. This raises the possibility that a common denominator in the mechanism of action of mood-stabilizing drugs in BP disorder is regulation of IMPase transcription.

A second IMPase gene has recently been described on chromosome 18 (IMP.18p) with ~50% homologous sequence for the chromosome 8 IMPase (IMPA) gene (Sjoholt et al., 1997; Yoshikawa et al., 1997). This second gene is close to a region which has recently been implicated in linkage studies to BP disorder (Berrettini et al., 1994). Our results suggest that the chromosome 8 gene coding for the predominant brain form of IMPase is a possible candidate to play a role in the aetiology of BP disorder and in the mechanism of action of mood-stabilizing drugs at the levels of transcription and translation.

The results from the current study of fresh lymphocytes and our previous report in which we examined lymphoblastoid cell lines from 77 BP patients and 29 control subjects (Shamir et al., 1998) suggest the hypothesis that IMPase transcription and translation are disturbed in BP disorder. However, these results are tentative and need to be validated in independent samples of larger size and the specificity of these findings examined across other diagnostic groups such as schizophrenia.

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**References**


Effect of bipolar disorder on lymphocyte IMPase mRNA levels


