THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 280, No. 7, Issue of February 18, pp. 5400–5405, 2005
Printed in U.S.A.

Alteration of Lithium Pharmacology through Manipulation of Phosphoadenosine Phosphate Metabolism*

Received for publication, July 13, 2004, and in revised form, December 3, 2004
Published, JBC Papers in Press, December 6, 2004, DOI 10.1074/jbc.M407890200

Bryan D. Spiegelberg‡, June dela Cruz, Tzuo-Hann Law, and John D. York§

From the Departments of Pharmacology & Cancer Biology and Biochemistry, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710

Bisphosphate 3′-nucleotidase (BPNT1 in mammals and Met22/Hal2 in yeast) is one of five members of a family of signaling phosphatases united through a common tertiary structure and inhibition by subtherapeutic doses of the antibipolar drug lithium. Here we report a role for 3′-nucleotidase and its substrate, 3′-phosphoadenosine 5′-phosphate (PAP), in mediating the cellular effects of lithium. Lithium-induced inhibition of growth in yeast cells may be overcome by dose-dependent heterologous expression of human BPNT1. Disruption of the yeast 3′-nucleotidase gene or treatment of cells with lithium results in a >80-fold accumulation of PAP and leads to potent growth inhibition. These data indicate that the accumulation of a 3′-nucleotidase substrate, such as PAP, mediates the toxicity of lithium. To further probe this model we examined the growth inhibitory effects of lithium under conditions in which PAP biosynthetic machinery was concomitantly down-regulated. Disruption of *met3* or *met14* genes (ATP sulfurylase or phosphosulfate kinase), transcriptional down-regulation of *MET3* through methionine addition, or administration of chlorate, a widely used cell-permeable sulfurylase inhibitor, function to reduce lithium-induced intracellular PAP accumulation and lithium toxicity; all of these effects were reversed by heterologous expression of human sulfurylase and kinase. Collectively, our data support a role for 3′-nucleotidase activity and PAP metabolism in aspects of lithium's mechanism of action and provide a platform for development of novel pharmacological modulators aimed at improving therapies for the treatment of bipolar disorder.

Lithium has been widely used for over 50 years to treat bipolar disorder (manic depressive disease). Despite its success, the mechanisms by which lithium exerts therapeutic and toxic effects remain unclear. Insights into lithium pharmacology have come with the identification of a signaling phosphatase family whose members are inhibited potently by lithium at subtherapeutic concentrations. Family members have relatively weak overall sequence similarities (<25%) but are unified by a conserved core three-dimensional structure and the conserved pattern motif $D(X)_nEE(X)_nDP(I/L)D(S/G/A)T(X)_n$-$WD(X)_{11}GG$ (1). This motif has been used to identify novel family members, for example human and mouse bisphosphate 3′-nucleotidase 1 (BPNT1),[1] through the scanning of emerging genomic databases and "reverse" biochemical characterization of the gene product (2). Importantly BPNT1 is potently inhibited by lithium (2, 3) and, to our knowledge, ranks among the most sensitive lithium targets described in the literature to date, including the glycogen synthase kinase-3 isoforms (4, 5). There are five distinct branches in the complete family of human and mouse signaling phosphatases (Fig. 1*A*), including inositol monophosphatase (IMP1 and IMP2), inositol polyphosphate 1-phosphatase (INPP1), fructose 1,6-bisphosphatase (FBP1 and FBP2), BPNT1, and a novel gene product (GenBank™ accession number AY032885) designated LPM (lithium-inhibited Phosphomonoesterase). Overall, it is our hypothesis that the cellular and clinical effects of lithium, both toxic and therapeutic, may be mediated through inhibition of one or more of these family members.

Several studies provide evidence that link this family of signaling phosphatases to lithium's action in cells and organisms. Measurements of inositol levels in lithium-treated rat brain (6) and biochemical characterizations of IMP and INPP1 (7, 8) provided initial evidence connecting inositol signaling to lithium and led to a controversial "inositol depletion" hypothesis (9). Zuker and colleagues provided genetic evidence in *Drosophila melanogaster* that the loss of INPP1 phenocopies lithium's alteration of neuromuscular junction physiology (10). In yeast, bisphosphate 3′nucleotidase activity (encoded by *MET22*, also known as *HAL2*) has been linked to the cellular toxicity of lithium (11–14). Met22/Hal2 is functionally orthologous to BPNT1, having 3′-nucleotidase activity toward 3′-phosphoadenosine 5′-phosphate (PAP) and 3′-phosphoadenosine 5′-phosphosulfate (PAPS), and it is a key regulator of the sulfur assimilation pathway (Fig. 1*B*).

In this paper we provide evidence that lithium's toxic effects in yeast occur through direct inhibition of 3′-nucleotidase and that down-regulation of PAP synthesis provides an avenue to relieve lithium's cellular effects. Furthermore, through our studies, we have developed a high throughput screening strategy for inhibitors of hBPNT1, which may have important uses in the development of novel therapeutics aimed at improving treatment of bipolar disorder.

* This work is supported by funds from the Howard Hughes Medical Institute and by National Institutes of Health Grant R01 HL-55672. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. of Pharmacology, Vanderbilt University, Nashville, TN 37232.

§ To whom correspondence should be addressed: Dept. of Pharmacology and Cancer Biology, Howard Hughes Medical Institute, Duke University Medical Center, DUMC 3813, Durham, NC 27710. Tel.: 919-681-6414; Fax: 919-668-0991; E-mail: yorkj@duke.edu.

[1] The abbreviations used are: BPNT, bisphosphate 3′-nucleotidase; APS, adenosine 5′-phosphosulfate; CSM, complete synthetic medium; dex, dextrose; GST, glutathione *S*-transferase; IMP, inositol monophosphatase; INPP1, inositol polyphosphate 1-phosphatase; PAP, 3′-phosphoadenosine 5′-phosphate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PAPSS, PAPS synthetase; hPAPSS, human PAPSS; PST, phenol sulfotransferase; ura, uracil.

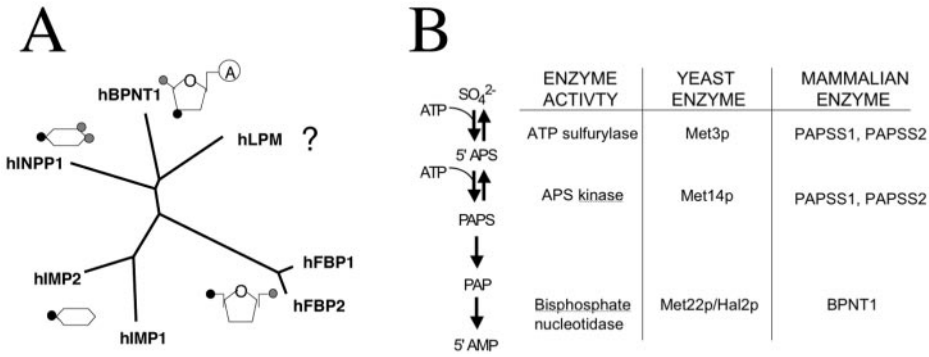

FIG. 1. **The lithium-inhibited signaling phosphatase family and PAP metabolism pathways.** *A*, a dendrogram depicting the lithium-sensitive family reveals five branches (*h*, human). Substrate models are shown with scissile phosphate colored *black*. Fructose 1,6-bisphosphatases hydrolyze the D-1 phosphate from fructose 1,6-bisphosphate. IMPs hydrolyze all monophosphorylated inositols except inositol 2-phosphate. INPP1 hydrolyzes the D-1 phosphate from inositol 1,4-bisphosphate and inositol 1,3,4-trisphosphate. BPNT1 hydrolyzes the 3′-phosphate from 3′, 5′-bisphosphorylated nucleotides (PAP and PAPS) and exhibits weak hydrolysis of INPP1 substrates, albeit 1000-fold less efficiently (2). The substrate or substrates for lithium-inhibited phosphomonoesterase or LPM are unknown. LPM (GenBank™ accession number AY032885) is incorrectly annotated as an isoform of IMP (IMP3); no published experimental evidence demonstrating IMP activity exists. *B*, PAP is generated following the utilization of the sulfate moiety of PAPS. In yeast, PAPS synthesis requires two enzymes, the ATP sulfurylase Met3 and the APS kinase Met14. In mammals, a single dual functional gene product, PAPPS, encodes both activities as autonomous domains on a single polypeptide. BPNT1 and Met22 appear to be functionally orthologous (2, 3).

MATERIALS AND METHODS

Overexpression of Human BPNT1 in 3′-Nucleotidase-deficient Yeast—A highly drug-sensitive strain of yeast, YRP1 MATa (15) (the generous gift of Dr. Jeremy Thorner, University of California at Berkeley), was used as a starting background to delete the yeast 3′-nucleotidase *MET22/HAL2*. A gene replacement strategy was employed as described (2), generating *met22*::*KAN*^{MX4}. The entire coding region of hBPNT1 was cloned into a copper-inducible yeast expression plasmid pRS316CUP1 to generate pRS316CUP1-HA-hBPNT1, and this plasmid was transformed into the *met22*-deficient yeast. A single colony of *met22*::*KAN*^{MX4} pRS316CUP1-HA-hBPNT1 was used to inoculate 2 ml of complete synthetic media lacking uracil, supplemented with 2% dextrose (CSM/dex/ura$^-$), and incubated overnight at 30 °C. Forty microliters of this culture was used to inoculate 2 ml of CSM/dex/ura$^-$ lacking methionine supplemented with an appropriate concentration of $CuSO_4$ and grown to a density of 4×10^7 cells/ml (A_{600} of 1.3). Cells were harvested by high-velocity reciprocal shaking and glass beads ("bead beating"), and lysates were analyzed by Western blot using affinity purified anti-BPNT1 antibodies as described (2).

Lithium-induced Growth Inhibition—Wild-type yeast cells (W303 background) were grown in CSM/dex overnight, and 40 μl of them were inoculated into 2 ml of CSM/dex/met$^-$ (lacking methionine) or CSM/dex (20 μg/ml methionine) supplemented with various concentrations of LiCl. In the case of *met3* and *met14* mutants (W303 background), cells were grown in CSM/dex; however, *met3* pPAPSS2 or *met14* pPAPSS2 was grown in CSM with 2% galactose, which was required to induce the *GAL* promoter-driven expression of the human PAPS synthetase isoform 2 (hPAPSS2) gene product. Note that cells grown in galactose are highly sensitive to lithium as reported by Masuda *et al.* (16). In some experiments, CSM/dex was supplemented with 25 mM sodium chlorate ($NaClO_3$). Cultures were harvested at equivalent incubation times, and the optical density was determined with a 600-nm wavelength.

For experiments in the *met22*::*KAN*^{MX4} pRS316CUP1-HA-hBPNT1, strains were first grown in CSM/dex/ura$^-$, washed extensively in CSM/dex/ura$^-$/met$^-$, and cells were diluted to 3×10^4 cells/ml in CSM/dex/ura$^-$/met$^-$ supplemented with various concentrations of $CuSO_4$ and LiCl. In this case, cells were grown in a volume of 175 μl in 96-well round bottom polystyrene plates in an aerated, humidified chamber for 4 days at 30 °C. Plates were read using a Molecular Devices SpectraMax 340PC384 microplate reader at a 600-nm wavelength (A_{600}). Note that the YPR1 strain of yeast is more sensitive than W303 to lithium-induced growth inhibition (IC_{50} of 3 mM LiCl *versus* 50 mM) because of mutations in transporters and membrane lipid biosynthetic genes (15).

Cloning and Analysis of PAPS Synthetases—The fidelity of amplification of each PCR-generated insert was confirmed by fluorescent terminator sequencing (Howard Hughes Medical Institute, Duke University Medical Center, Biopolymer Facility). hPAPSS2 was subcloned by PCR amplification from expressed sequence tag plasmid AL540583. The open reading frames for yeast *MET3* and *MET14* were PCR-amplified from genomic DNA of *Saccharomyces cerevisiae* strain W303. The amplified products were inserted into pCR2.1 using the TA cloning kit (Invitrogen). The inserts were transferred by conventional molecular biology techniques to a galactose-inducible yeast expression vector. The resulting plasmids and control vector were transformed into *met3*Δ, *met14*Δ, or wild-type BY4742 yeast strains obtained through the Saccharomyces Genome Deletion Project (17). Investigation of methionine auxotrophy was performed by serial dilution spot assays on solid media.

Measurement of Intracellular PAP Concentrations—The concentration of PAP in lysates was determined by competition for binding of radiolabeled PAP to the enzyme phenol sulfotransferase (PST), which uses PAP as a cofactor (18). The open reading frame of IMAGE Consortium Clone expressed sequence tag 1924316 (mouse PST; accession number AI316417) was amplified by PCR, ligated into the pCR2.1, and subcloned into pGEX4T-1 (Amersham Biosciences) to create a bacterial expression vector in which PST was downstream of glutathione *S*-transferase (GST-PST). GST-PST was expressed and purified essentially as described by the manufacturer of the glutathione-Sepharose resin (Amersham Biosciences). Residual bacterial PAP and PAPS was removed from GST-PST on the glutathione column with recombinant human BPNT1 (typically 1 μg).

For the assay of intracellular PAP concentrations, appropriate yeast strains were grown in CSM/ura$^-$ to mid-log phase. The cells were centrifuged and washed extensively in sterile water. The cells were resuspended in CSM/ura$^-$/met$^-$ containing methionine (20 μg/ml), lithium, or chlorate as appropriate and then incubated with shaking at 30 °C. At various time points, 2 ml of culture was removed, centrifuged, washed, and resuspended in 1 ml of water. A 250-μl aliquot was then disrupted by bead beating, and the amount of soluble protein in this extract was determined using a Bradford dye binding assay (Bio-Rad) with bovine serum albumin as a standard. The remainder of the washed cells were pelleted and resuspended in 250 μl of 2 N $HClO_4$. This was incubated at 4 °C for 20 min, after which 250 μl of 1.8 N KOH and 0.4 N $KHCO_3$ were added to neutralize and precipitate the $HClO_4$. The extract was centrifuged at 4 °C to remove the debris, and the supernatant was lyophilized using a rotary evaporator. The dried extracts were frozen at −80 °C for no more than 1 week prior to use. Extracts were reconstituted in 100 μl of 200 mM HEPES, pH 7.5, and again centrifuged to remove debris.

The GST-PST assay was performed by mixing 10 μl of appropriately diluted sample in K-lysis buffer (50 mM HEPES, pH 7.0, and 50 mM KCl) with 10 μl of radiolabeled ^{32}P-5′-end-labeled PAP (~5,000 cpm, prepared as described in Ref. 2). Eighty microliters of a reaction mix containing 100 mM HEPES, pH 7.0, 5 mM dithiothreitol, 0.2 mg/ml bovine serum albumin, and 1 μg of GST-PST were added to start the binding reaction. Following a 1-h incubation at room temperature, 20 μl of a 50:50 slurry of glutathione-Sepharose in K-lysis buffer was added. The tubes were incubated with gentle mixing for 10 min at room temperature. After centrifugation, the supernatant containing free PAP and one 200-μl wash were removed to a scintillation vial. Radioactivity was converted to percentage of isotope bound. A standard curve was generated for each assay by plotting the percentage bound *versus* the logarithm of the known PAP concentration. This standard curve was

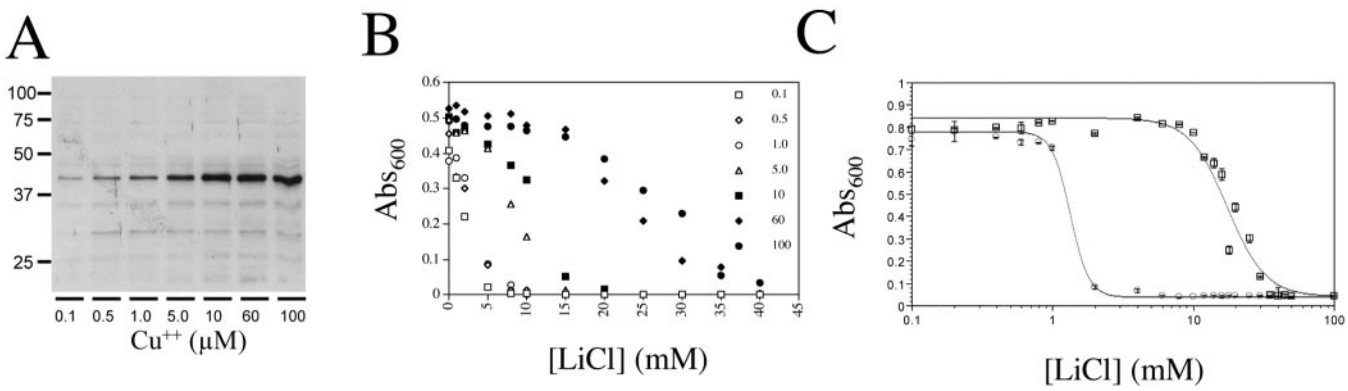

FIG. 2. **Titration of human BPNT1 expression in *met22*-deficient yeast alters lithium toxicity.** *MET22* was deleted in a YPR1 drug-sensitized yeast strain (15) to generate YPR1 *met22*::*KAN*^{MX} and transformed with a plasmid harboring the coding region of human BPNT1 under the control of a copper-inducible promoter (YPR1 *met22*::*KAN*^{MX} plus pCu-hBPNT1). Yeast cells were grown in synthetic minimal media lacking methionine at indicated copper concentrations (shown in μM). *A*, analysis of hBPNT1 at various copper concentrations using an affinity-purified anti-BPNT1 antibody indicates gradually increasing hBPNT1 expression as a function of copper (dose-dependent expression). Total protein loaded in each lane is identical. *B*, lithium inhibition of growth of YPR1 *met22*::*KAN*^{MX} plus pCu-hBPNT1 at indicated copper concentrations (shown on *right side* of graph in μM). *C*, lithium-toxicity curves at 0.2 *versus* 50 μM copper indicate IC_{50} values of 1.5 and 20 mM, respectively.

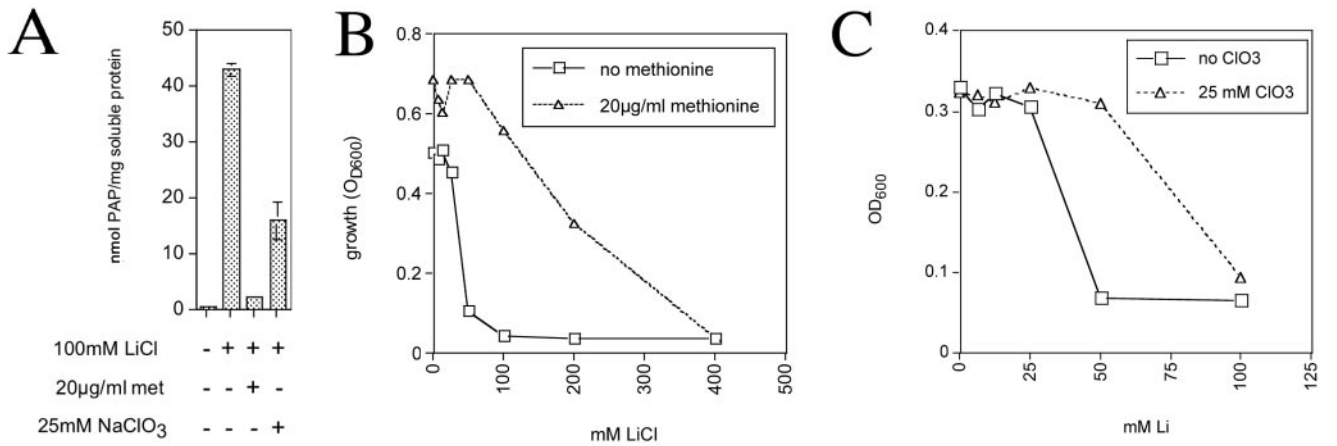

FIG. 3. **Pharmacologic modulation of PAP synthesis alters lithium-mediated effects.** Wild-type yeast were grown to mid-log phase, washed extensively, and resuspended in synthetic minimal media lacking methionine and containing noted inclusions. *A*, PAP was measured using the ligand binding assay described under "Materials and Methods." Intracellular PAP concentrations were normalized to the amount of soluble protein extracted from the cultures. *B*, yeast were inoculated at 5×10^4 cells per milliliter into synthetic media with or without methionine supplemented with the indicated concentration of LiCl. Growth was assayed by spectrophotometry following 48 h of growth at 30 °C. The IC_{50} for lithium in met$^-$ media was ~40 mM, whereas the IC_{50} in met$^+$ media was ~160 mM. *C*, yeast strains were inoculated as above in synthetic media lacking methionine with or without chlorate. The inhibitory constant for lithium in met$^-$ media was ~40 mM, whereas the IC_{50} in met$^-$ media supplemented with 25 mM chlorate was ~80 mM.

used to back-calculate the PAP concentration in the unknown samples. PAP measurements were shown to be specific by treatment of parallel samples with excess amounts of recombinant Met22 nucleotidase to hydrolyze the PAP, which eliminated >95% of the signal.

RESULTS AND DISCUSSION

3′-Nucleotidase Modulates Lithium-mediated Toxicity—Inhibition of bisphosphate 3′-nucleotidase activity in yeast has been implicated as an integral element of lithium's toxicity and cellular effects (13, 14). We and others have suggested that the effects of lithium in mammalian systems (toxic and possibly therapeutic) may be mediated through the inhibition of BPNT1 (1–3). To further probe this model, we reasoned that alteration of the levels of 3′-nucleotidase levels in cells should modulate lithium's growth-inhibitory effects in a dose-dependent manner. To this end, we deleted the endogenous copy of 3′-nucleotidase from a drug-sensitive strain of yeast to yield YRP1 *met22*::*KAN*^{mx}. We then heterologously expressed human BPNT1 under the inducible control of the CUP1 promoter such that increasing doses of copper in the growth media would result in increased BPNT1 transcription. To monitor BPNT1 expression levels, we performed immunoblot analysis of extracts prepared from cells grown at copper concentrations ranging from 0.1 to 100 μM (Fig. 2*A*). As is readily apparent, a >10-fold range of hBPNT1 expression levels was observed under these conditions.

We then tested the relative sensitivity of these cells to lithium-induced growth inhibition, reasoning that the gain of 3′-nucleotidase activity should confer resistance to lithium. To accomplish this, we monitored the growth of strains in media supplemented with copper ranging between 0.1 and 100 μM and/or 0–40 mM lithium chloride (Fig. 2*B*). At the lowest level of BPNT1 expression (0.1 μM copper), 50% inhibition of growth occurred at 2.5 mM lithium. Cells expressing increasing levels of BPNT1 were gradually more resistant to lithium toxicity in an exquisitely dose-dependent manner. The differential in IC_{50} values for lithium was >20-fold at 0.2 and 50 μM copper concentrations (Fig. 2*C*). As a control, cells were also treated with 40 mM sodium chloride, and no alterations in growth were observed at any copper concentration (data not shown).

Cellular Levels of PAP Correlate with Lithium Toxicity—If 3′-nucleotidase inhibition results in the accumulation of PAP in cells and this, in turn, exerts toxic effects, then concomitant

down-regulation of PAP synthesis may function to reverse these effects. To demonstrate the concept and specificity of this model, we utilized pharmacological and genetic approaches in budding yeast. A PAP anabolic pathway consists of enzymatic sulfurylation of ATP to create adenosine 5′-phosphosulfate (APS), which is subsequently phosphorylated to create PAPS, the immediate precursor of PAP (Fig. 1*B*). In yeast, the first step is catalyzed by the ATP sulfurylase Met3, and the second step is catalyzed by the APS kinase Met14 (Fig. 1*B*). Manipulation of either protein should therefore reduce cellular PAP synthesis.

As a first step, we measured levels of PAP under a variety of pharmacological and genetic perturbations. To accurately measure alterations in the cellular PAP levels, a sensitive method to quantify PAP was developed. Following acid lysis to halt enzymatic PAP degradation, the PAP concentrations in neutralized lysates were determined using competition with radiolabeled PAP for binding to mouse phenol sulfotransferase expressed as a fusion to GST (GST-PST). The amount of radioactivity bound to GST-PST was determined by immobilizing the protein to glutathione-Sepharose resin. To internally validate the method, we determined the dissociation constant of PAP with GST-PST. The observed K_d was ~35 nM (data not shown), consistent with the apparent K_d found for the nonfusion recombinant rat PST (18). Note that PAPS is acid-labile, and the cells to be analyzed are lysed in a strong acid solution; therefore, this technique measures the sum of intracellular PAP and PAPS. However, because of its instability, the intracellular concentration of PAPS is thought to be relatively minor compared with the intracellular concentration of PAP (19).

The GST-PST binding assay was used to determine relative intracellular PAP concentrations in yeast strains grown under various conditions. The PAP concentration determined in the binding assay was normalized to the total soluble protein in parallel extracts and verified by reporting the signal that was sensitive to exhaustive *in vitro* degradation by purified recombinant Met22 (typically >95% of the signal in our assay was PAP).

The PAP concentration in yeast cells grown in the absence of lithium was below the detectable limits of the assay (~0.5 nmol of PAP per milligram of soluble protein) (Fig. 3*A*). However, when the wild-type cells were treated for 8 h with 100 mM LiCl in the absence of methionine, the PAP concentration increased to 42.9 nmol/mg, an increase of at least 85.8-fold (Fig. 3*A*), which is consistent with the increase found in previous analyses (13). Moreover, when methionine (20 μg/ml) was included in the media, the concentration of PAP increased to only 2.3 nmol/mg in the presence of lithium (Fig. 3*A*). This suppression of lithium-induced PAP accumulation by methionine supplementation is consistent with data that suggest that methionine suppresses PAP synthesis through transcriptional down-regulation of the *MET3* promoter (20), decreases PAPS production (19), and moderates the effects of lithium (13, 14).

To verify that this effect is due to the loss of ATP sulfurylase activity, we next measured lithium-induced PAP accumulation in the presence of chlorate, a widely used cell-permeable inhibitor of mammalian and yeast ATP sulfurylase activity (21, 22). Chlorate has been used in mammalian cells to specifically decrease levels of PAPS in order to block downstream sulfurylation of biomolecules (for examples, see Refs. 23–25). In our system, chlorate supplementation reduced PAP levels 65% to ~15 nmol/mg in the presence of lithium.

Given the effects of chlorate and methionine on PAP metabolism, we next analyzed their effects on lithium-mediated growth inhibition (Fig. 3, *B* and *C*). Wild-type cells grown in synthetic minimal media lacking methionine supplemented

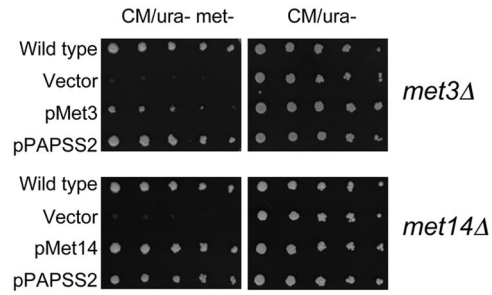

FIG. 4. **Yeast and mammalian PAPS synthetase activities complement the methionine auxotrophy of *met3*Δ and *met14*Δ strains.** Strains in which the *MET3* or *MET14* open reading frames had been deleted with a G418 resistance cassette were obtained from the *Saccharomyces* Genome Project. The deletions were complemented with yeast Met3 or Met14 or human PAPSS2 by expression on a 2 μ plasmid with a galactose-inducible promoter. Following growth to mid-log phase in CSM/ura$^-$ containing 2% galactose, cells were washed and diluted to 1×10^4, 2×10^3, 400, and 80 cells per microliter. One microliter of each dilution was plated on CSM/ura$^-$/met$^-$ or CSM/ura$^-$ containing 2% galactose. The plates were imaged following growth at 30 °C for 2 days.

with increasing amounts of lithium were growth inhibited such that 50% growth (IC_{50}) was achieved at ~40 mM lithium chloride. The addition of methionine resulted in a significant reduction in lithium toxicity, and the IC_{50} was ~160 mM (Fig. 3*B*). Similarly, the addition of 25 mM chlorate resulted in a reduction of lithium-induced growth inhibition with an IC_{50} value of ~80 mM (Fig. 3*C*). The effect of chlorate is especially significant, because methionine does not transcriptionally down-regulate mammalian PAPS synthetase enzymes as it does for Met3 in budding yeast (26). Collectively, these data provide strong evidence that the pharmacological down-regulation of PAP synthesis may be a fruitful strategy for modulating the cellular effects of lithium.

Genetic Reduction in PAP Biosynthesis Reduces the Toxic Effects of Lithium—The sulfur assimilation pathway that leads to the production of PAP is an evolutionarily conserved system, so it is reasonable that lithium's effects on PAP accumulation in yeast could be mirrored in certain tissues of other organisms. We showed previously that the expression of mammalian BPNT1 was able to complement the defect due to genetic deletion of the yeast bisphosphate nucleotidase Met22 (2). To further demonstrate the similarity between yeast and mammalian nucleotide synthesis machinery, we tested the ability of human PAPS synthesis enzymes to functionally replace those of yeast. In mammals, the ATP sulfurylase and APS kinase activities of Met3 and Met14 are expressed on a single dual-functional enzyme called PAPS synthetase (27, 28) (Fig. 1*B*). Humans express at least two isoforms of PAPS synthetase, hPAPSS1 and hPAPSS2 (29), which show >80% amino acid sequence similarity. The amino-terminal domain of hPAPSS2 has APS kinase activity and shares similarity with *S. cerevisiae* Met14 (28), whereas the carboxyl terminus of hPAPSS2 has ATP sulfurylase activity and shares similarity with Met3, including its strict conservation of active site residues (22, 30). Because of its commercial availability, hPAPSS2 was subcloned via PCR from the IMAGE Consortium expressed sequence tag clone AL540583 into a galactose-inducible yeast expression vector and transformed into wild-type yeast or homozygous diploid *met3*Δ or *met14*Δ strains. Episomal expression of the deleted gene or heterologous expression of hPAPSS2 rescued growth on media lacking methionine in both *met3*Δ and *met14*Δ (Fig. 4), confirming that the human dual functional enzyme is sufficient to replace the two PAPS synthesis activities of yeast.

In addition to complementing the methionine auxotrophy of

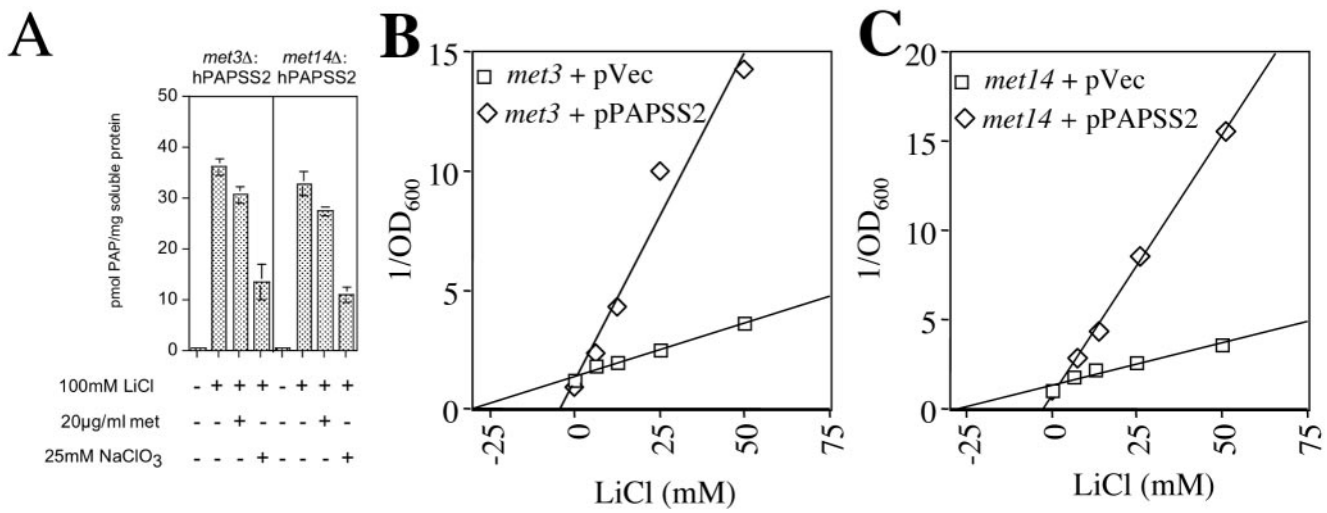

FIG. 5. **Genetic manipulation of PAP synthesis alters lithium-mediated effects.** Various strains of yeast were grown to mid-log phase, washed extensively, and resuspended in the synthetic minimal media supplemented with 2% galactose and the noted inclusions. *A*, PAP was measured using the ligand binding assay described under "Materials and Methods." Intracellular PAP concentrations were normalized to the amount of soluble protein extracted from the cultures. *B* and *C*, lithium-induced growth inhibition in *met3* (*B*) or *met14* (*C*) null cells expressing vector control (*pVec*) or dual-functional human ATP sulfurylase/APS kinase (*pPAPSS2*). Cells lacking *met3* or *met14* are nearly 10-fold more tolerant to lithium, having inhibition constants of ~25 mM as compared with 3 mM for wild-type (not shown) or null cells complemented with hPAPSS2. Note that galactose is necessary to induce GAL10 promoter and heterologous expression; however, as reported previously, galactose also renders yeast more sensitive to lithium (16). This accounts for the difference in inhibition constants observed in wild-type cells in these experiments and in those reported in Fig. 3.

the *met3* and *met14* mutant yeast, hPAPSS2 expression was also able to rescue the lithium-induced PAP accumulation. The concentrations of PAP in *met3*Δ and *met14*Δ cells transformed with an empty vector were found to be below the detectable limits of the assay and remained undetectable regardless of the concentration of methionine and lithium in the culture media (data not shown). This finding indicated that the *MET3* and *MET14* gene products work along the same linear pathway to form the major route of PAP production. Strains lacking endogenous ATP sulfurylase or APS kinase but heterologously expressing hPAPSS2 were grown and treated as described above. As shown in Fig. 5*A*, the expression of hPAPSS2 restored PAP accumulation in the presence of lithium to ~36.2 nmol of PAP per milligram of protein in *met3*Δ and 32.8 nmol/mg in *met14*Δ, which were near wild-type levels. Furthermore, the lithium toxicity in the complemented strains was restored to near that of the wild-type strain, having inhibitory values of ~2.5 mM (Fig. 5, *B* and *C*) as compared with >25 mM for *met3* or *met14* null strains. Taken together, these data suggest that a mammalian PAPS synthetase is functional in a heterologous system and that the substrates or products of the enzyme, rather than non-catalytic protein components, are sufficient for the biological functions of these proteins. Furthermore we demonstrate that synthesis of PAP or PAPS is necessary for the full potency of lithium toxicity and provide genetic proof that PAP down-regulation results in lithium resistance.

Lithium's Mechanism of Action in Human Patients and Its Therapeutic Ramifications—Studies of lithium's biological effects in model organisms have the potential to improve the treatment of bipolar disorder. Here we provide new evidence further implicating 3′-nucleotidase activity as a relevant target of lithium, contributing to its cellular toxicity in budding yeast. Our data support a mechanism in which lithium directly inhibits 3′-nucleotidase and exerts its biological effects through the accumulation of PAP (or possibly PAPS), resulting in the perturbation of cellular processes. PAP has been shown through biological and biochemical studies to affect the activity of several cellular pathways, including the yeast Xrn1p (14), eukaryotic nucleoside diphosphate kinase (31), PAPS·PAP antiport systems (32), and adenylyl cyclase (33). Our work demonstrating that genetic and pharmacological manipulation of the PAP metabolism may provide a powerful tool for attenuating lithium's effects could have important applications for improving lithium therapies in bipolar patients. The reformulation of lithium therapies to include a "chlorate-like" down-regulator of PAP synthesis may reduce unwanted toxicity and allow for the widening of its current narrow therapeutic window. Clearly, any pharmacological manipulation of PAP metabolism and sulfur assimilation pathways must proceed with caution. Sulfation of biomolecules is important in the maintenance of cellular integrity, and PAPS-dependent detoxification of certain metabolites and exogenous compounds is essential for the protection of the body from the deleterious effects of normal physiology and environmental exposure. Therefore, if a PAPS synthetase inhibitor is developed as a potential lithium antidote, controlled studies are essential to assess kidney and liver function in the presence of such a compound.

In addition to a role for 3′-nucleotidase activity in mediating lithium toxicity, it is also possible that BPNT1 and the PAP metabolism are important for therapeutic effects of lithium. BPNT1 expression, although relatively low with respect to the entire brain (2), may be enriched in portions of the brain particularly important to mood disorders and, thus, is especially pertinent to lithium therapy. Alternatively, neuronal tissue expressing low levels of BPNT1 may be more sensitive to the effects of lithium, similar to what we observed in humanized yeast grown under conditions of low *versus* high BPNT1 expression. If inhibition of BPNT1 does play a therapeutic role in bipolar patients, then development of specific inhibitors of BPNT1 will be helpful for improving therapies. In this regard, the humanized BPNT1 yeast strain reported here represents an important tool useful for novel cell-based high throughput screens aimed at the identification of inhibitors of human BPNT1.

Acknowledgments—We thank Blaine Armbruster for helpful discussions and ideas and Dr. Josh Frederick for critical review of the manuscript.

REFERENCES

1. York, J. D., Ponder, J. W., and Majerus, P. W. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 5149–5153
2. Spiegelberg, B. D., Xiong, J.-P., Smith, J. J., Gu, R. F., and York, J. D. (1999) *J. Biol. Chem.* **274,** 13619–13628
3. Lopez-Coronado, J., Belles, J., Lesage, F., Serrano, R., and Rodriguez, P. (1999) *J. Biol. Chem.* **274,** 16034–16039
4. Klein, P. S., and Melton, D. A. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 8455–8459
5. Ryves, W. J., and Harwood, A. J. (2001) *Biochem. Biophys. Res. Commun.* **280,** 720–725
6. Allison, J., and Stewart, M. (1971) *Nat. New Biol.* **233,** 267–268
7. Hallcher, L., and Sherman, W. (1980) *J. Biol. Chem.* **255,** 10896–10901
8. Inhorn, R. C., and Majerus, P. W. (1987) *J. Biol. Chem.* **262,** 15946–15952
9. Berridge, M. J., Downes, C. P., and Hanley, M. R. (1989) *Cell* **59,** 411–419
10. Acharya, J. K., Labarca, P., Delgado, R., Jalink, K., and Zuker, C. S. (1998) *Neuron* **20,** 1219–1229
11. Thomas, D., Barbey, R., and Surdin-Kerjan, Y. (1992) *J. Gen. Microbiol.* **138,** 2021–2028
12. Glaser, H.-U., Thomas, D., Gaxiola, R., Montrichard, F., Surdin-Kerjan, Y., and Serrano, R. (1993) *EMBO J.* **12,** 3105–3110
13. Murguia, J. R., Belles, J. M., and Serrano, R. (1996) *J. Biol. Chem.* **271,** 29029–29033
14. Dichtl, B., Stevens, A., and Tollervey, D. (1998) *EMBO J.* **16,** 7184–7195
15. Gray, N. S., Wodicka, L., Thunnissen, A. M., Norman, T. C., Kwon, S., Espinoza, F. H., Morgan, D. O., Barnes, G., LeClerc, S., Meijer, L., Kim, S. H., Lockhart, D. J., and Schultz, P. G. (1998) *Science* **281,** 533–538
16. Masuda, C. A., Xavier, M. A., Mattos, K. A., Galina, A., and Montero-Lomeli, M. (2001) *J. Biol. Chem.* **276,** 37794–37801
17. Winzeler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J. D., Bussey, H., Chu, A. M., Connelly, C., Davis, K., Dietrich, F., Dow, S. W., El Bakkoury, M., Foury, F., Friend, S. H., Gentalen, E., Giaever, G., Hegemann, J. H., Jones, T., Laub, M., Liao, H., Liebundguth, N., Lockhart, D. J., Lucau-Danila, A., Lussier, M., M'Rabet, N., Menard, P., Mittmann, M., Pai, C., Rebischung, C., Revuelta, J. L., Riles, L., Roberts, C. J., Ross-MacDonald, P., Scherens, B., Snyder, M., Sookhai-Mahadeo, S., Storms, R. K., Véronneau, S., Voet, M., Volckaert, G., Ward, T. R., Wysocki, R., Yen, G. S., Yu, K., Zimmermann, K., Philippsen, P., Johnston, M., and Davis, R. W. (1999) *Science* **285,** 901–906
18. Yang, Y.-S., Marshall, A. D., McPhie, P., Guo, W.-X. A., Xie, X., Chen, X., and Jakoby, W. B. (1996) *Protein Expression Purif.* **8,** 423–429
19. Jakubowski, H., and Goldman, E. (1993) *J. Bacteriol.* **175,** 5469–5476
20. Martin, R. L., Daley, L. A., Lovric, Z., Wailes, L. M., Renosto, F., and Segel, I. H. (1989) *J. Biol. Chem.* **264,** 11768–11775
21. Baeuerle, P. A., and Huttner, W. B. (1986) *Biochem. Biophys. Res. Commun.* **141,** 870–877
22. Ullrich, T. C., and Huber, R. (2001) *J. Mol. Biol.* **313,** 1117–1125
23. Girard, J., Baekkevold, E., and Amalric, F. (1998) *FASEB J.* **12,** 603–612
24. Chang, C., and Kane, K. (1998) *J. Immunol.* **160,** 4367–4374
25. Schriever, C., Breithardt, G., and Schmidt, A. (1997) *Biol. Chem.* **378,** 701–706
26. Kim, H. J., Madhu, C., Cho, J. H., and Klaassen, C. D. (1995) *Drug Metab. Dispos.* **23,** 840–845
27. Li, H., Deyrup, A., Mensch, J. R., Domowicz, M., Konstantidis, A. K., and Schwartz, N. B. (1995) *J. Biol. Chem.* **270,** 29453–29459
28. Venkatachalam, K. V., Akita, H., and Strott, C. A. (1998) *J. Biol. Chem.* **273,** 19311–19320
29. Franzon, V. L., Gibson, M. A., Hatzinikolas, G., Woollatt, E., Sutherland, G. R., and Cleary, E. G. (1999) *Int. J. Biochem. Cell Biol.* **31,** 613–626
30. Ullrich, T. C., Blaesse, M., and Huber, R. (2001) *EMBO J.* **20,** 316–329
31. Schneider, B., Xu, Y. W., Janin, J., Veron, M., and Deville-Bonne, D. (1998) *J. Biol. Chem.* **273,** 28773–28778
32. Ozeran, J. D., Westley, J., and Schwartz, N. B. (1996) *Biochemistry* **35,** 3685–3694
33. Johnson, R. A., Yeung, S.-M. H., Stubner, D., Bushfield, M., and Shoshani, I. (1989) *Mol. Pharmacol.* **35,** 681–688

Alteration of Lithium Pharmacology through Manipulation of Phosphoadenosine Phosphate Metabolism

Bryan D. Spiegelberg, June dela Cruz, Tzuo-Hann Law and John D. York

J. Biol. Chem. 2005, 280:5400-5405.
doi: 10.1074/jbc.M407890200 originally published online December 6, 2004
