Chapter 12

Mammalian Inositol 3-phosphate Synthase: Its Role in the Biosynthesis of Brain Inositol and its Clinical Use as a Psychoactive Agent

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Abbreviations used: EMSA, electrophoretic mobility shift analysis; IMPase 1, inositol monophosphatase 1; inositol synthase, *Myo*-inositol 3-phosphate synthase; NCBI, National Center for Biotechnology Information; PCR, polymerase chain reaction; Rb, retinoblastoma protein; *tss*, transcriptional start site; IP_3 , Inositol trisphosphate; DAG, diacyl glycerol; FISH, fluorescent *in situ* hybridization.

1. INTRODUCTION

myo-inositol,[1] a carbocyclic sugar that is abundant in brain and other mammalian tissues, mediates cell signal transduction in response to a variety of hormones, neurotransmitters and growth factors and participates in osmoregulation (Agranoff and Fisher, 2001; Berridge and Irvine, 1989; Fisher *et al*., 2002; Thurston *et al*., 1989; Parthasarathy and Eisenberg, 1986). The *myo*-inositol structure represents a unique paradigm for the illustration of chirality and optical and geometrical stereoisomerisms (Parthasarathy and Eisenberg, 1991). Among the nine possible isomers of inositol (*myo, neo, scyllo, epi, D-chiro, L-chiro, cis,*

[1]In this chapter, inositol indicates the *myo*-isomer.

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muco and allo-inositols) the *myo*-isomer is most abundant and biologically active molecule in nature. *myo*-inositol is a major biologically active cyclitol having a single axial hydroxyl group at carbon-2 leading to only one plane of symmetry (Parthasarathy and Eisenberg, 1986). It is the substrate for the synthesis of cell membrane inositol phospholipids linked to calcium-mobilizing receptors in mammalian brain.

myo-inositol exists in mammalian tissues in several bound and free forms: (i) as the primary cell membrane inositol phospholipid, phosphatidylinositol (PI), which by stepwise phosphorylation gives rise to mono-, bis- and tris-

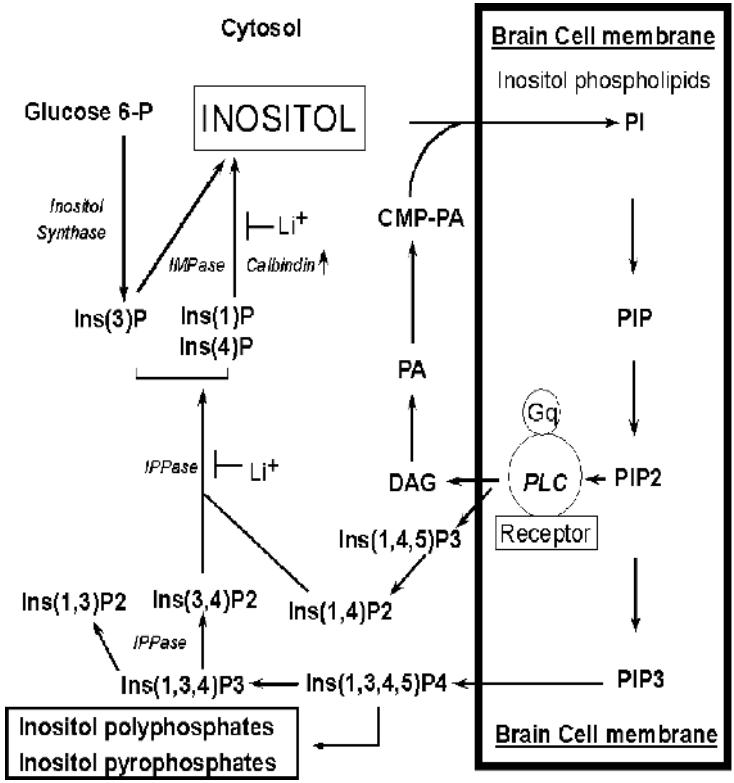

Figure 1. Schematic representation of the brain inositol signaling system. The quantities of IMPase isoenzymes and IPPase are increased by chronic lithium treatment occurring at either the gene or protein levels. Inositol in this diagram indicates the *myo*-inositol isomer. Calbindin − calcium binding protein; DAG- diacyl glycerol; Gq-GTP binding protein; IMPase 1− inositol mono phosphatase 1; IPPase- inositol polyphosphate 1-phosphatase; Ins(1)P, Ins(3)P, Ins(4)P-inositol monophosphates; Ins(1,3)P_2 − inositol 1,3-bisphosphate; Ins(1,4)*P*2 − inositol 1,4-bisphosphate; Ins(3,4)*P*2- inositol 3,4-bisphosphate; Ins (1,4,5)*P*3 – inositol 1,4,5-trisphosphate; Ins(1,3,4)*P*3 – inositol 1,3,4-trisphosphate; Li^+-lithium; PA – phosphatidic acid; PI- phosphatidyl inositol; PIP- phosphatidyl inositol 4-phosphate; PIP_2- phosphatidyl inositol 4,5-bisphosphate; PIP_3- phosphatidyl inositol 3,4,5 trisphosphate; PLC – phospholipase-C, VPA-valproate.

phospho- inositides (PIP, PIP_2 and $PIP_{3;}$ Parthasarathy *et al*., 1993); (ii) as water soluble inositol mono-, bis-, tris- and poly-phosphates; (iii) as the glycosyl-phosphatidylinositol (GPI) anchors of membrane proteins and enzymes, and (iv) as free inositol which is the precursor for the brain inositol signal system (Agranoff and Fisher, 2001; Majerus *et al*., 1999; Parthasarathy *et al*., 1994). In mammalian brain, the inositol signal system serves as a major pathway linking serotonergic, muscarinic, adrenergic, histaminergic, and metabotropic receptors, and cholecystokinin, tachykinin, neurotensin, and platelet activating factor receptor systems (Agranoff and Fisher, 2001; Vadnal *et al*., 1997). Extracellular signaling occurs through a series of receptors and transducing proteins, such as GTP-binding protein (G_q), which activate membrane bound phospholipase C. Phospholipase C generates two second messengers- inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol(DAG)- in the cytosol, liberating calcium ions from the endoplasmic reticulum (ER) which then stimulate protein kinase C (Figure 1). Calcium ions activate a number of enzymes and receptors. IP_3 can be further phosphorylated by different inositol phosphate kinases to form highly phosphorylated forms such as IP_7 and IP_8, which exhibit profound biological activity. Recently, Snyder's group has shown (Saiardi *et al*., 2004) that a subset of proteins can be directly phosphorylated by IP_7 in an enzyme-free manner. These reactions reveal new avenues to control a bewildering array of biological functions especially in the brain (York and Hunter, 2004).

2. ENZYMATIC AND STRUCTURAL ASPECTS OF INOSITOL SYNTHASE

Eisenberg at NIH originally identified mammalian inositol synthase in crude rat tissue homogenates (Eisenberg, 1967). The biosynthesis of inositol in mammalian tissues takes place in two stages utilizing glucose 6-phosphate as the substrate. β-Glucose 6-phosphate is first isomerized to D-*myo*-inositol 3-monophosphate by inositol synthase (E.C. 5.5.1.4), an NAD^+ requiring enzyme (Eisenberg and Parthasarathy, 1987; Parthasarathy and Eisenberg, 1986; Mauck *et al*., 1980; Wong and Sherman, 1980). The second stage liberates free inositol from inositol 3-monophosphate in a hydrolytic reaction mediated by inositol monophosphatase 1 (IMPase 1), a Mg^{2+} requiring lithium- sensitive enzyme (Hallcher and Sherman, 1980; Naccarato *et al*., 1974). IMPase 1 differs from other nonspecific phosphatases such as alkaline and acid phosphatases in physical, structural and enzymatic characteristics and it cannot differentiate between the isomeric forms of inositol monophosphates (D-1 and D-3 monophosphates; Eisenberg and Parthasarathy, 1984). Basic enzyme characteristics, without the addition of any cations, were first studied in rat inositol synthase isolated from a variety of tissues (Maeda and Eisenberg, 1980; Eisenberg and Parthasarathy, 1987). Addition of any cations and addition of different cations

(final concentration 1 mM) stimulated synthase activity several fold. Both NH_4^+ and K^+ monovalent ions are stimulatory and divalent cations are inhibitory to inositol synthase activity (Eisenberg and Parthasarathy, 1987; Maeda and Eisenberg, 1980; Mauck *et al*., 1980). Lithium, a widely used mood stabilizer, sharply decreased the K_m of its activity. The chronic intake of lithium may have the ability to stimulate inositol synthase activity by decreasing its K_m thus facilitating the formation of inositol 3-phosphate from glucose 6-phosphate under conditions where there is a continued shortage of inositol, leading to inositol monophosphates accumulation. Inositol monophosphate accumulation during chronic lithium intake has been shown by Sherman *et al*. (1981a,b). The therapeutic nature of lithium may reside in its ability to decrease the K_m of inositol synthase activity, which supports the inositol depletion hypothesis proposed to explain the therapeutic effect of lithium. While some groups have recently challenged this hypothesis (Berry *et al*., 2004; Brambilla *et al*., 2004; Friedman *et al*., 2004;), recent observations by others in a number of cell types and isolated neurons (Harwood, 2005) support it. Inhibitors such as 2-deoxy-glucose 6-phosphate and glucitol 6-phosphate (Eisenberg and Parthasarathy, 1987; Maeda and Eisenberg, 1980) sharply decrease the activity of inositol synthase.

3. INTERMEDIATES OF INOSITOL SYNTHASE REACTION

The overall simplified reaction of inositol biosynthesis is: Glucose 6-phosphate → inositol +inorganic phosphate (Adhikari and Majumder, 1988; Eisenberg and Parthasarathy, 1987; Jin and Geiger, 2004; Jin *et al*., 2004; Parthasarathy and Eisenberg, 1986;). The first reaction converts glucose 6-phosphate to D-inositol 3-phosphate by synthase. Synthase action in the cyclization of glucose 6-phosphate is a rate limiting reaction (Hasegawa and Eisenberg, 1981). Biosynthetic formation of one molecule of free inositol is accompanied by the formation of one molecule of D-inositol 3-phosphate by inositol synthase (Eisenberg and Parthasarathy, 1987). Although NAD^+ is required, no net change in this coenzyme concentration is observed in the complete isomerization reaction leading from glucose 6-phosphate to inositol 3-phosphate (Parthasarathy and Eisenberg, 1986; Figure 2).

The mechanism of enzymatic isomerization of glucose 6-phosphate to D-inositol 3-monophosphate by synthase has been intensively studied for the past three decades (Eisenberg *et al*., 1964; Loewus *et al*., 1980; Parthasarathy and Eisenberg, 1986; Sherman *et al*., 1981a,b). Eisenberg and coworkers studied the formation of various intermediates in the reaction using electrophoretically homogeneous rat synthase (Eisenberg and Parthasarathy, 1987). The first intermediate they postulated to occur in the reaction was 5-ketoglucose 6-phosphate which was believed to be tightly bound to synthase leading to a head-to-tail intramolecular aldol condensation forming the second intermediate, *myo*-inosose-2 1-phosphate.

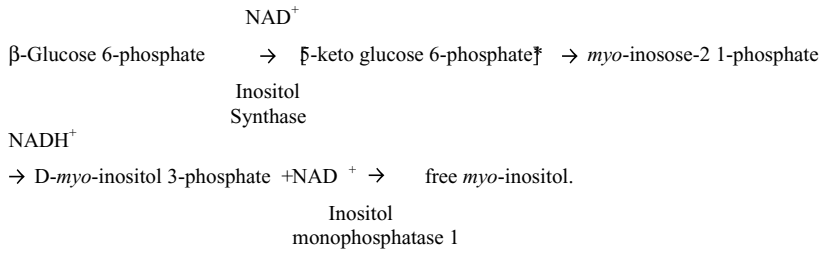

Figure 2. The pathway of biosynthesis of *myo*-inositol to free inositol by synthase reaction in mammalian tissues. * This is a postulated intermediate presumed to occur during the course of the reaction. Crystallographic studies with yeast synthase support the presence of this intermediate.

NADH generated from the oxidative step reduces the second intermediate to D-inositol 3-monophosphate. In the early work on the elucidation of the mechanism of synthase action it was noticed that the intermediates and coenzymes were tightly bound (this was prior to the advent of crystallographic studies and recombinant DNA analysis). Eisenberg and Parthasarathy (1987) disrupted the synthase reaction by adding large amounts of sodium borotritide (200-milliCurie NaB^3H_4/enzyme reaction), which enabled them to trap the intermediates in the form of reduced epimeric sugar alcohols representing the putative intermediates. The products thus obtained were a mixture of ^3H labeled *myo*-inositol and its 2-epimer, *scyllo*-inositol, which were derived from the unused substrate and a putative intermediate. The reduction of this intermediate (*myo*-inosose-2, 1-phosphate) by borotritide generated *myo*-inositol. The expected products from 5-keto glucose 6-phosphate conversion are D-[1,5-^{3}H]glucitol and its 5-epimer L-[1,5-^{3}H]iditol. In these experiments although abundant label was found in hydrogen-1 of glucitol (H1), no label was found in iditol and hydrogen-5 of glucitol (Figure 3). Therefore, it was concluded that 5-ketoglucose 6-phosphate had no finite existence and was very tightly bound to synthase and that cyclization to inosose-2, 1-phosphate occurs as quickly as its formation from glucose 6-phosphate. Experiments with purified rat synthase clearly demonstrated the formation of inosose-2, 1-phosphate that was sufficiently long-lived to be isolated and that the reduction by NADH must be the rate-limiting step, similar to the observations made using kinetic isotopic experiments. Synthase essentially closes the C-6 and C-1 bonds of glucose 6-phosphate, and with other isomerization reactions that lead from glucose 6-phosphate to the formation of fructose 6-phosphate and glucose 1-phosphate, accounts for almost all the branch points of glucose metabolism in nature. Besides the closure of the carbon-carbon bond, the transfer of hydrogen is a specific mechanism in the synthase reaction and has been corroborated by recent crystallographic and chemical studies (Stein and Geiger, 2000, 2002). The hydrogen shuttle

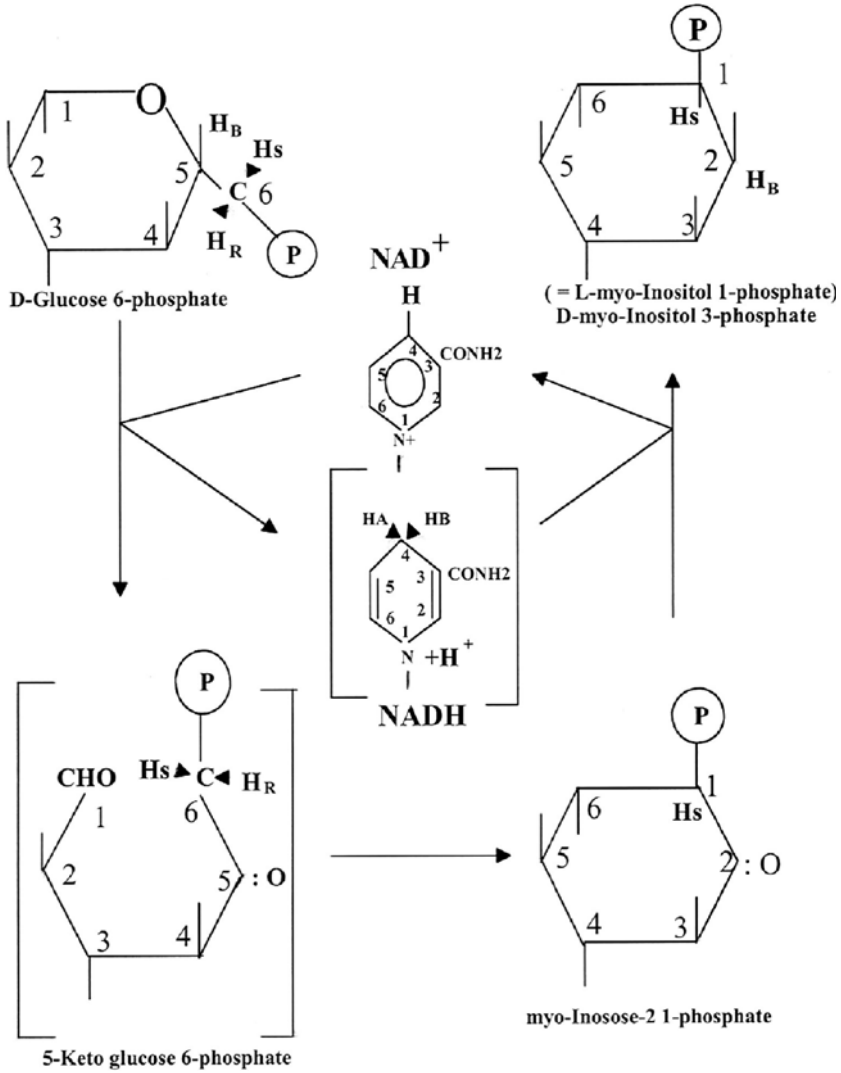

Figure 3. Isomerization of D-glucose 6-phosphate to D-*myo*-inositol 3-phosphate by inositol synthase. The hypothetical intermediate is within parenthesis.

between substrate and coenzyme leads to the oxidation at carbon-5 of glucose 6-phosphate by NAD^+ and reduction at carbon-2 of inosose-2 1-phosphate by NADH. Carbon-4 of reduced nicotinamide carries a pair of hydrogen atoms (H_A and H_B, see Figure 3), which can be stereoselectively, used for the synthase reaction. H_B of NADH is apparently transferred while H_A is not (Byun and Jenness, 1981). A similar stereo-selective approach occurs when C-6 and C-1 are linked

to form D-*myo*-inositol 3-phosphate. The paired hydrogen atoms at C-6 of 5-ketoglucose have hydrogen atoms Hs and HR. Hs is retained in the cyclization reaction while HR is lost to the solution after cyclization (Loewus *et al*., 1980). In 1995, Migaud and Frost synthesized inosose-2, 1-phosphate and observed that it not only acted as a substrate but was also a potent competitive inhibitor of inositol synthase. The crystal structures of *S. cerevisiae* (Stein and Geiger, 2000, 2002) and *M. tuberculosis* enzymes (Norman *et al*., 2002) have been studied at 2.0–2.5 Åresolutions. Both studies have unequivocally demonstrated the presence of bound NAD^+ at the active site.

4. STRUCTURAL ASPECTS OF INOSITOL SYNTHASE

Native mammalian inositol synthase is a trimer composed of 558 amino acids per subunit with a native molecular weight of 210,000 ± 2,000 as determined by chromatography, electrophoresis and ultra-centrifugal sedimentation equilibrium methods (Eisenberg and Parthasarathy, 1987; Maeda and Eisenberg, 1980; Mauck *et al*., 1980). The trimeric nature of mammalian inositol synthase is in sharp contrast to yeast and plant forms where it exists as a tetramer (Majumder *et al*., 1997 and 2003). The subunit molecular weight of inositol synthase is approximately 69,000 ± 600 (Eisenberg and Parthasarathy, 1987; Maeda and Eisenberg, 1980). Amino acid sequence analysis of rat testicular inositol synthase showed that the amino terminus was blocked precluding sequence analysis of the subunits after SDS-PAGE. Only cyanogen bromide (CNBr) was able to cleave the protein to generate two peptide fragments (EPAAEILVDSPDVIF and ESLRPRPSVYIPEFIAAN).

5. GENETIC REGULATION OF INOSITOL SYNTHASE

Early studies by Henry and co-workers (Carman and Henry, 1999; Culbertson *et al*., 1976; Chapter 6 in this volume) focused on the molecular aspects of the yeast inositol synthase gene (*INO1*), which is tightly regulated by inositol levels (Culbertson *et al*., 1976). When yeast is grown in media containing inositol, the *INO1* gene is completely repressed while its absence completely de-represses *INO1*. Expression of *INO1* is dependent on the upstream activation sequence, UAS_{INO}, which is the binding target for the heterodimer Ino2p/Ino4p (Culbertson *et al*., 1976; Klig and Henry, 1984; Hirsch and Henry, 1986). Binding is facilitated by limiting inositol levels, which derepresses *INO1* expression (Loewy and Henry, 1984) while repression is mediated by the transcriptional regulator Opi1p (Greenberg *et al*., 1982; Jiranek *et al*., 1998; Lopes and Henry, 1991). Widely used mood stabilizing agents such as lithium and valproate stimulate *INO1* gene expression, but valproate inhibits the synthesis of inositol 3-phosphate, thus

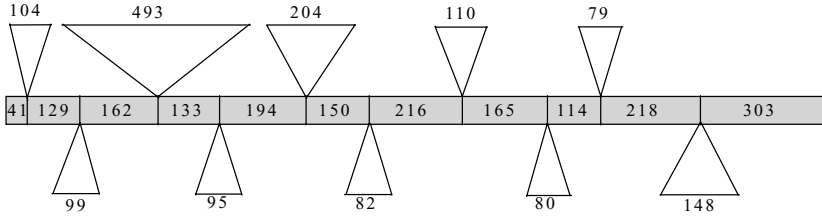

Figure 4. The gene structure of the human *ISYNA1*. The *ISYNA1* gene contains 11 exons (shaded) and 10 introns (triangles). The sizes of the exons (within boxes) and introns (above and below triangles) are indicated. The size of the first exon was determined after identification of the transcription start site (*tss*).

suggesting that inositol synthase may be a target for valproate action in yeast. Most of the information on human *ISYNA1* (the mammalian counterpart of *INO1*) is only available recently (AF220250 to AF220259; AF220530; AF314170; AF288525; AF251265; AH009098). The human gene spans 11 exons (Figure 4) and is located on chromosome 19p13.1; a related processed pseudogene is located on 4p15 (Figure 5). It is interesting that a recent genome-wide linkage analysis provides a strong support for an autism susceptible locus at 19p13.11 where *ISYNA1* resides (McCauley *et al*., 2005). mRNA structure and characterization indicate that it is ~1.8 kb long (Figure 6). Transcriptional start site (*tss)* analysis of *ISYNA1* mRNA from liver by an Inverse 5′ PCR protocol (Zeiner and Gehring, 1994) indicates that the *tss* is 20 bp upstream from the 3′ end of the non-coding, exon 1. However, a brain cDNA deposited in the NCBI database (AF220530) is longer at the 5′-end by an additional 21 bp. The differences between the two mRNAs with respect to their *tss* may be due to tissue-specific initiation sites, typical of many house-keeping genes possessing a G/C-rich promoter region. Indeed, the proximal 500 bp of the *ISYNA1* promoter, including exon 1, is extremely G/C rich (~70%). Majumder et al., (1997, 2003) have compared mRNA sequences from diverse species and have identified conserved amino acid motifs in inositol synthase. Given the importance of this gene in inositol signaling and that perturbances in cellular inositol homeostasis can lead to neurological symptoms, a detailed analysis of its promoter has been undertaken recently (discussed below; Seelan *et al*., 2004).

Functional expression of the human inositol synthase cDNA in yeast cells devoid of the *INO1* gene shows that it can complement inositol auxotrophy and excrete inositol (Ju *et al*., 2004). When grown in valproate (0.6 mM), these cells show a 35 and 25% decrease in synthase activity and inositol levels, respectively. However, valproate does not directly inhibit synthase activity at this concentration (0.6 mM) implying that this mood stabilizer works probably at the translational or transcriptional levels. Inositol synthase is present in a wide variety of organisms (protozoa, fungi, plants and mammals) and has been

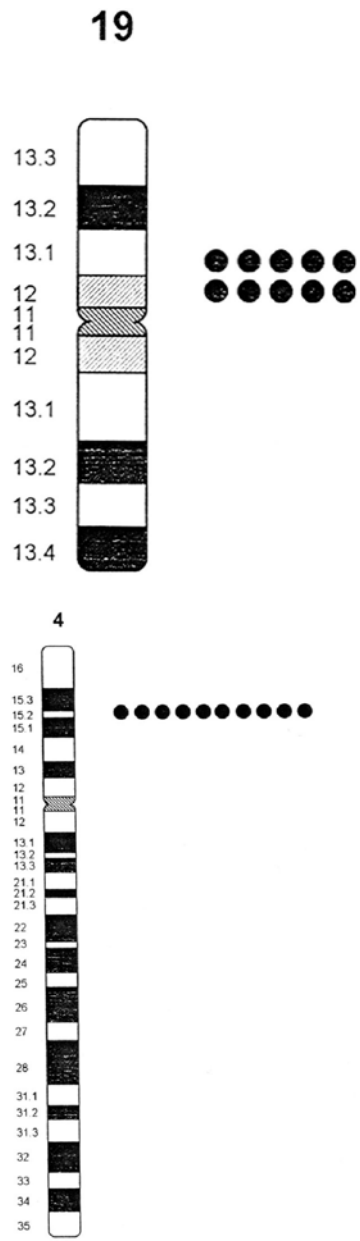

Figure 5. FISH mapping of *ISYNA1* gene. Each dot represents the double FISH signals detected on chromosome 19 and 4, after analysis of 100 metaphase chromosomal spreads. Chromosome 19p13.1 harbors the expressed gene and chromosome 4p15, the pseudogene.

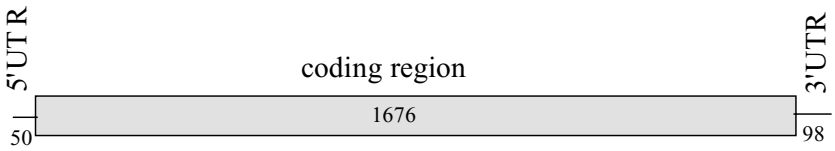

Figure 6. The cDNA structure of the human *ISYNA1* gene. The gene encodes a 1676 bp coding region with a 5′ UTR (untranslated region) of 50 bp and a 3′ UTR of 98 bp (GenBank Accession #AF220530).

purified from diverse sources (Majumder *et al*., 1997 and 2003). Inositol synthase is expressed in all tissues and has the highest expression in testis, followed by heart, pancreas, ovary and placenta; very low expression is observed in blood leukocytes, thymus, skeletal muscle, and colon (Guan *et al*., 2003). Paradoxically, it is only marginally expressed in the brain (Guan *et al*., 2003), an organ where inositol synthase is a target for the therapeutic intervention as a mood stabilizer (Agam *et al*., 2002). In contrast to *INO1* regulation in yeast, studies on *ISYNA1* show its regulation to be more complex. Rats administered therapeutic levels of lithium for 10 days show an increase in inositol synthase expression in the hippocampus, but not in the frontal cortex (Shamir *et al*., 2003). Inositol synthase activity in rat testes is significantly decreased in long-term diabetes when compared to controls (Whiting *et al*., 1979). Hasegawa and Eisenberg (1981) demonstrated decreased expression of inositol synthase activity in reproductive organs and liver of hypophysectomized male rats and in liver of thyroidectomized male rats, and these effects can be reversed by hormone treatments. It is also the target for estrogen regulation in the uterus of rats (Rivera-Gonzalez *et al*., 1998). A recent study (Guan *et al*. 2003) has shown that in HepG2 (liver-derived) cell lines, glucose and lovastain increase *ISYNA1* expression, the latter suggesting the involvement of a G-protein coupled signal transduction system. When HepG2 cells are cultured in the presence of lithium (10 mM), a 50% suppression in activity is observed. No effects were observed with inositol, estrogen, thyroid hormone or insulin. The lack of an inositol effect in HepG2 is surprising since the yeast gene is highly regulated by inositol levels; also, while lithium is inhibitory to the enzyme in HepG2 cells (Guan *et al*., 2003), it appears to stimulate expression in the rat hippocampus (Shamir *et al*., 2003). These contrasting effects may underscore some of the complexities associated with human inositol synthase regulation and may, in part, be attributed to tissue- or species-specific gene expression responses.

6. HUMAN *ISYNA1* PROMOTER ELEMENTS

Characterization of the core promoter of *ISYNA1* (Seelan *et al*., 2004) indicates that the promoter is up regulated by E2F1, a cell-cycle regulator. That E2F1 transactivates *ISYNA1* has been demonstrated by the following observations:

(i) the 5′-flanking region is stimulated by ectopic expression of E2F1; (ii) E2F1 induction is localized to the minimal promoter region between −261 and −34; sequences downstream of −34 do not significantly induce promoter activity; (iii) the promoter activity of the minimal promoter region shows a dose-dependent increase with exogenous expression of E2F1; (iv) the induction by E2F1 can be suppressed by the exogenous expression of Rb (retinoblastoma protein), a key negative modulator of E2F1 expression in cells (Dyson, 1998); and, (v) the presence of at least one functional E2F binding element at −117 (Seelan *et al*., 2004), as inferred by electrophoretic mobility shift assay (EMSA) and E2F1 antibody super-shift analysis.

Manual scan of the minimal promoter (−261 to –34) identified seven possible sequences with close homology to the consensus binding motifs for E2F1 (Tao *et al*., 1997). These are: TTCCGCC at −188, TGGCGG at −180, TCGGGC at −146, TTGGGCC at −117, TCCCGC at −90, TTCCCCG at −81, and TCGCGCG at −37 (Figure 7). Two of these sites at −81 and −90 were also identified with the program TFBind (Tsunoda and Takagi, 1999). EMSA and

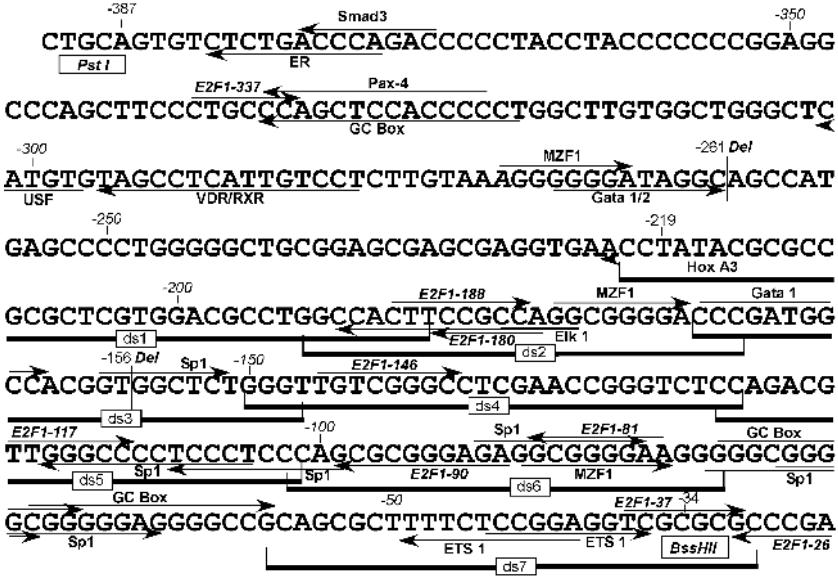

Figure 7. Features of the *ISYNA1* minimal promoter. The sequence of the minimal promoter between the *Pst* I (−387) and *BssH* II (−34) sites is shown. Potential transcription factor binding sites, obtained by various database searches are indicated by arrows and names. Putative E2F motifs are indicated with their nucleotide locations. −261Del and –156Del (vertical bars) indicate the site of truncation of the minimal promoter plasmid used for deletion analysis. ds1 – ds7 represent the oligos used for EMSA. ER, estrogen receptor; Elk 1, member of ETS oncogene family; ETS 1, E26 avian leukemia oncogene1; GATA 1, GATA binding protein 1; Hox A3, homeobox A3; MZF1, myeloid zinc finger 1; Pax-4, Paired box gene 4; Smad3, MAD homology 3; Sp1, transacting transcription factor 1; USF, upstream stimulating factor; VDR/RXR, Vitamin D receptor/Retinoid X receptor. A functional E2F element is located at –117 (Seelan *et al*., 2004; reprinted with permission from Elsevier).

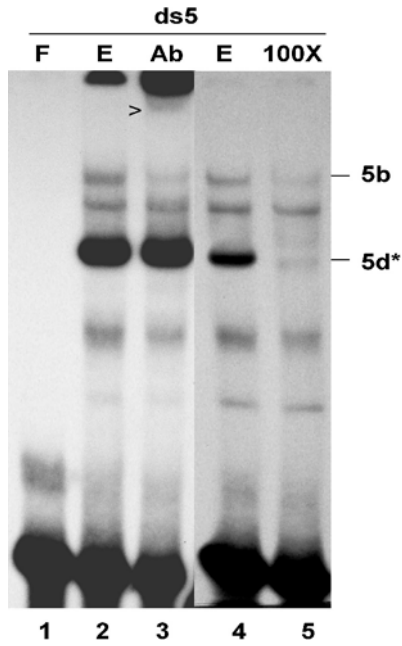

Figure 8. Analysis of E2F1 binding to ds5 of the *ISYNA1* minimal promoter by EMSA. ds5 binding was assessed with HeLa nuclear extracts alone (E), or preincubated with an antibody, KH129 (ActiveMotif, Carlsbad, CA), raised against E2F1 (Ab), or competed with a 100-fold molar excess of an unlabeled E2F oligo (100X), prior to the addition of labeled probe. F, free probe. The supershifted band by E2F specific antibody is denoted by an arrowhead. Complex 5b is the E2F1-specific complex while complex 5d* is a spurious band (Seelan *et al*., 2004; reprinted with permission from Elsevier).

antibody super-shift analysis, however, identified only one complex specific for E2F1 binding. This E2F1 mediated specific complex was competed partially by an excess of cold E2F oligo and was supershifted by an E2F1- specific antibody (Figure 8). Analysis of the oligo sequence responsible for the generation of this complex identified TTGGGCCC at −117 as a possible E2F binding sequence (Figure 7). This sequence has an 8/11 contiguous match with the context based E2F binding motif of Kel *et al*. (2001)–TTTSGGCSMDR. Interestingly, this E2F binding complex is also competed by an oligo containing the Sp1 motif, suggesting that Sp1 may also interact at this site (not shown). An analysis of the oligo sequence indicates that the E2F1 site overlaps an Sp1 element on the opposite strand (Figure 7). Thus the −117 element may be occupied by E2F1, Sp1, or by both simultaneously, and this may explain the partial competition observed using the E2F1 oligo in competition experiments.

Further detailed promoter analysis suggestes that E2F responsiveness is mediated by more than one E2F element in the minimal promoter. Because only one weak affinity E2F1 complex at −117 was identified experimentally, we

conclude that the transactivation of the *ISYNA1* promoter by E2F1 occurs through the cooperative interaction of several low-affinity binding sites present in the minimal promoter of *ISYNA*1 (Seelan *et al*., 2004). Some of these putative sites have been indicated in Figure 7. The significance of E2F regulation of *ISYNA1* promoter implies that this enzyme may have other important functions besides its known biosynthetic function. Alternatively, inositol biosynthesis may be intricately associated with several vital processes such as cell division, DNA synthesis or apoptosis. Interestingly, E2F1 plays a major role in the transcriptional regulation of E2F target genes in the adult testes (Wells *et al*., 2002), an organ where inositol synthase also has the highest activity (Guan *et al*., 2003).

We have also observed several other specific protein/DNA complexes binding to the minimal promoter of *ISYNA1*. Some of these complexes include Sp1. Sp1 recognizes the consensus sequence, GGGCGG (Kadonaga *et al*., 1986) and also loosely binds to GC-rich or GT-rich sequences (Suske,1999). EMSA analysis of the promoter revealed that several of the DNA/protein complexes are competed by the Sp1 oligo (Seelan *et al*., 2004). Both high affinity Sp1 binding (as inferred by the intense banding and ready competition) and weak Sp1 interactions were evident (Seelan *et al*., 2004).

7. PRECLINICAL AND CLINICAL STUDIES WITH INOSITOL

Allison and Stewart (1971) first demonstrated that lithium (acute model) depleted rat brain inositol by 30%. These studies along with others served as the basis for the "inositol depletion hypothesis" to explain lithium's mode of action in mood disorders (Berridge *et al*., 1989). However, chronic lithium studies with rats have shown conflicting results. Renshaw *et al*. (1986) observed an increase while Honchar *et al*., (1989) observed no change in inositol monophosphatase 1 levels in rat cerebral cortex. Whitworth and Kendall (1989) showed an increase in free inositol levels in rat cerebral cortex with no change in the striatum. Results from chronic lithium studies are difficult to assess because they had different experimental designs. In humans, inositol enters the blood stream and CSF quickly after oral intake (Groenen *et al*., 2003; Levine *et al*., 1993b, 1996;). Proton magnetic resonance studies also showed that brain inositol levels initially increased after oral inositol and subsequently returned to baseline levels. NMR techniques also show that brain inositol phosphates are increased after 7 and 14 days of lithium treatment (Yildiz *et al*., 2001). It should be noted that preliminary clinical studies on the beneficial effects of oral inositol in alleviating psychiatric symptoms have only been conducted with a limited number of patients and they have to be elaborated in a larger number of patients for the understanding of the full psychoactive effects of inositol. Some of the studies in various psychiatric conditions are summarized below:

7.1 Depression

Barkai *et al*. (1978) and Frey *et al*., (1998) have shown that depressed patients had significantly lower levels of CSF inositol, although others could not replicate this observation (Levine *et al*., 1995b). Placebo-controlled double-blind[2] study compared the effects of 12 gm/day of inositol versus glucose in a group of depressed patients, including both manic-depressive and major depressive disorders (Levine *et a*l., 1995b). All medications were stopped 3-7 days prior to the start of the study and patients received only inositol or the placebo, glucose. At 4 weeks, significant improvement was noted in the depressed group taking inositol versus placebo, demonstrating that inositol had a considerable anti-depressant effect. One patient experienced a hypomanic phase with placebo; no manic phases occurred with inositol. Laboratory analysis did not indicate any changes in liver, blood, or kidney functions. Based upon these studies and those of Chengappa *et al*. (2000) it can be considered that inositol can possibly be used as a psychotherapeutic agent in the treatment of depression. However, further studies with a larger number of patients are needed.

7.2 Obsessive-Compulsive Disorder (OCD)

Using 18 g/day of inositol, versus placebo, a double blind,[2] and crossover study was performed on 13 patients for six weeks. The Yale-Brown Obsessive Compulsive Scale[3] was used for analysis, and patients taking the inositol had significantly lower scores than those receiving placebo (Fux *et al*., 1996, 1999). Since inositol appears to be effective in depression, panic disorder and OCD, it has the clinical profile of a serotonin selective reuptake inhibitor (SSRI). Higher doses were used in this study compared to the depression and panic disorder studies previously described. Only a small number of patients were used.

7.3 Panic Disorder

Benjamin *et al*. (1995) conducted a double blind crossover study[2] with a group of 21 patients suffering from panic disorder. Four weeks of inositol

[2]Double blind cross: A clinical study in which neither the patients nor the investigating physician know the intervention to which the patients have been assigned. A double-blind design is considered to provide the most reliable data from a clinical trial. In a crossover study the subject is randomized into at least two treatment groups. After an appropriate wash out period each subject is reassigned to the alternate treatment group. A crossover study must have at least 2 treatment groups.

[3]Yale-Brown Obsessive Compulsive Scale is a reliable psychological measuring method to determine the severity of illness in patients suffering with obsessive-compulsive disorder with a range of severity and types of obsessive-compulsive symptoms.

(12 gm/day) or a proper placebo was administered. This study group contained patients with panic disorder with or without agoraphobia.[4] The results demonstrated that patients with panic disorder showed statistically lower frequency and severity of panic attacks and agoraphobia when treated with inositol. The number of panic attacks per week decreased from a baseline score of 9.7 ± 15 to 6.3 ± 9 after 4 weeks in the placebo group, compared to a significant reduction from 9.7 ± 15 to 3.7 ± 4 in the inositol treated group. Statistically significant differences were found in the number of panic attacks and phobia scores. Inositol was found to be an effective psychotherapeutic agent in the treatment of panic disorder in this initial study as well as in later studies (Palatnik *et al*., 2001). It should be noted that traditional treatments with SSRIs such as fluoxetine, tricyclic antidepressants (TCAs; *e.g*., imipramine), monoamine oxidase inhibitors (MAOIs; phenelzine) and triazolobenzodiazepines (*e.g*., alprazolam), exhibit significant side effects.

7.4 Schizophrenia

Levine *et al.* (1993a) conducted the first clinical study using inositol as a psychoactive agent in schozophrenia. Using 6 gms of inositol per day in a schizophrenic group, this double-blind add-on study found no significant therapeutic effects. These patients were maintained on their usual antipsychotic medication during the study period. Both the length of the study (days) and the dose of inositol (6 gm/day) were noted to be sub-optimal.

7.5 Post-Traumatic Stress Disorder (PTSD)

A double-blind crossover study was conducted on a group of patients suffering from post-traumatic stress disorder (Kaplan *et al*., 1996). Thirteen patients were studied, after excluding any history of drug abuse or organicity. Twelve grams of inositol per day or placebo (glucose) were used, after a washout period of two weeks, with patients remaining on study drugs for four weeks for each phase of the study. No other medications were allowed during this study. The outcome scales used were the Impact of Event Scale, SCL-90, HAM-D, and HAM-A, with no statistically significant effects noted on measures of intrusion or avoidance scales specific for PTSD. In general, inositol treatment did not demonstrate any therapeutic effect in the core symptoms of PTSD.

[4]Agoraphobia is a common phobic disorder. Most of the people suffering form this disorder show the fear of being alone in public places from which the person thinks escape would be difficult or help unavailable if he or she were incapacitated.

7.6 Attention Deficit Disorder (ADD)

Inositol has been tested in attention deficit disorder with hyperactivity (ADDH). Eleven children were given either inositol (200 mg/kg) or dextrose (placebo) in a crossover, double-blind study, over an eight-week period (Levine *et al*., 1995a). No other medications were allowed during the study. Of the eleven patients, eight had a history of responding to previous stimulant medications, one had been treatment-resistant, and two had not previously been treated. There was a trend towards worsening of the disorder with inositol versus placebo, and appeared to worsen core symptoms of this syndrome. Based upon these observations it was suggested that a low inositol diet might be of benefit in ADDH. The potential negative effects of dietary inositol in this condition require further cautious studies in children.

7.7 Autism

There has been an increasing interest in the use of serotonin reuptake inhibitors (SSRIs) in the treatment of autism (McDougle *et al*., 1996). Serotonin binds to several types of receptors including the 5-HT2, and 5-HT1c receptors, which are linked to inositol signaling. A double-blind crossover study was completed at a dose of 200 mg/kg per day, and the results demonstrated no significant improvement on inositol therapy (Levine *et al*., 1997).

7.8 Alzheimer's Disease

Pacheco and Jope (1996) described inositol system dysfunction in the brains of Alzheimers patients. A double-blind placebo controlled crossover study using a 6 gm/day dose of inositol (versus glucose) for 4 weeks demonstrated no significant changes in 11 Alzheimer's patients (Barak *et al*., 1996). The Cambridge Mental Disorders of the Elderly Examination (CAMCOG) overall scores demonstrated an improving trend with inositol, but results were not statistically significant. This study is encouraging, and needs to be repeated with larger patient numbers, higher doses, and for more extended times. A several month study would be desirable. It would be interesting to find out whether inositol as a natural brain sugar could delay the progression of Alzheimer's disease, or improve cognitive functions.

7.9 Other conditions

While Nemets *et al*. (2002) showed that 12 gm of oral inositol per day did not show any beneficial effects in premenstrual dysphoric disorder, 18 gms per day of inositol was therapeutic for patients suffering from bulimia nervosa and

binge eating (Gelber *et al*., 2001). In this study, the Global Clinical Impresion, the Visual Analogue Scale and binge eating criteria were followed for analysis. This finding is similar to the serotonin selective reuptake inhibitor used often with patients.

8. CONCLUSION

myo-inositol is a simple, naturally occurring sugar present in all mammalian cells and is composed of a cyclohexane ring with a hydroxyl group bound to each of the six carbons in a precise stereospecific orientation. It is the principal isomer of the brain and its presence in millimolar amounts contributes to osmoregulation. Although, it is not directly involved in the generation of energy, as is glucose, it is uniquely involved in brain signal transduction generating many inositol mono- and polyphosphates. The dietary route is one of the three pathways to maintain inositol homeostasis, the others being the receptor mediated salvage pathway involving IMPase 1 and a *de novo* pathway involving inositol synthase. In brain, inositol is a precursor for the formation of the inositol phospholipids, which on receptor stimulation produce two-second messengers, IP_3 and DAG. Inositol pathway has been implicated in the pathogenesis of bipolar disorder, with the mood stabilizers valproate and lithium targeting inositol synthase and IMPase 1, respectively. Recent observations of the inhibitory effect of valproate on inositol synthase suggest that this biosynthetic enzyme may be a potential therapeutic target for modulating brain inositol levels. Inositol synthase and its substrate/coenzyme (glucose 6-phosphate/NAD^+) are sufficient for the biosynthesis of inositol. Mammalian inositol synthase is a homotrimer and its isomerization of glucose 6-phosphate occurs through two intermediates as enzyme bound forms (5-ketoglucose 6-phosphate and inosose-2, 1-phosphate). The genomic structure, chromosomal localization, and characterization of the minimal promoter and identification of several transcription factor-binding elements in *ISYNA1* have been determined. The gene is located at 19p13.11, a candidate locus for autism spectrum disorder. Preclinical and selective clinical studies show that inositol can be used as a psychoactive agent and inositol synthase may be a promising target for designing novel anti-depressants thereby modulating brain inositol levels. These new mood modulators will potentially decrease the huge amounts of oral inositol taken to achieve therapeutic effects in neuropsychiatric disorders.

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