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Galactose-1-phosphate is a regulator of inositol monophosphatase: a fact or a fiction?

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Summary Classic galactosemia is due to the deficiency of galactose-1-phosphate uridyl transferase and is transmitted as an autosomal recessive disorder. Patients suffering from classic galactosemia display acute symptoms such as poor growth, feeding difficulties, jaundice, hepatomegaly etc., which disappear when the individual is on galactose free diet. However, these patients continue to suffer from defects such as neurological disturbances and ovarian dysfunction, due to the accumulation of galactose-1-phosphate, which is a normal intermediate of galactose metabolism. The biochemical mechanism of galactose-1-phosphate mediated toxicity is still an enigma. Recent experiments strongly suggest that galactose-1-phosphate is also a substrate for inositol monophosphatase (IMPase). Phosphatidylinositol bisphosphate {PI(P)2} dependent signaling serves as a second messenger for several neurotransmitters in the brain. Therefore, the brain is critically dependent on IMPase for the supply of free inositol in order to sustain {PI(P)2} signaling. Circumstantial evidence strongly supports the possibility that being a substrate, galactose-1-phosphate could modulate IMPase function in vivo. The implication of this idea is discussed in relation to classic galactosemia as well as bipolar disorder, which has been thought to be due to the hyper-activation of {PI(P)2} mediated second messenger pathways(s).

INTRODUCTION

Galactose metabolic pathway, commonly referred to as the Leloir pathway, is evolutionarily conserved from bacteria to humans. It involves the sequential activities of (Fig. 1) galactokinase (GALK), galactose-1-phosphate uridyl transferase (GALT), and UDP-galactose 4-epimerase (GALE). Studies on galactose metabolism in *Escherichia coli* and *Saccharomyces cerevisiae* have mainly focused on the regulation of gene expression while in humans the emphasis has been on understanding the inborn errors of galactose metabolism. Deficiency of GALT causes toxicity in many organisms, due to the accumulation of galactose-1-phosphate, which is a normal intermediate of galactose metabolism (1–4). Increasing experimental evidence mainly from studies conducted in yeast suggests that galactose-1-phosphate is a substrate for IMPase as well as UDP-glucose pyrophosphorylase (5,6). In view of these results, I hypothesize that galactose-1-phosphate, an otherwise normal intermediate of galactose metabolism is a regulator of IMPase, and discuss its implications on inositol mediated signaling mechanism in brain and its possible relevance.

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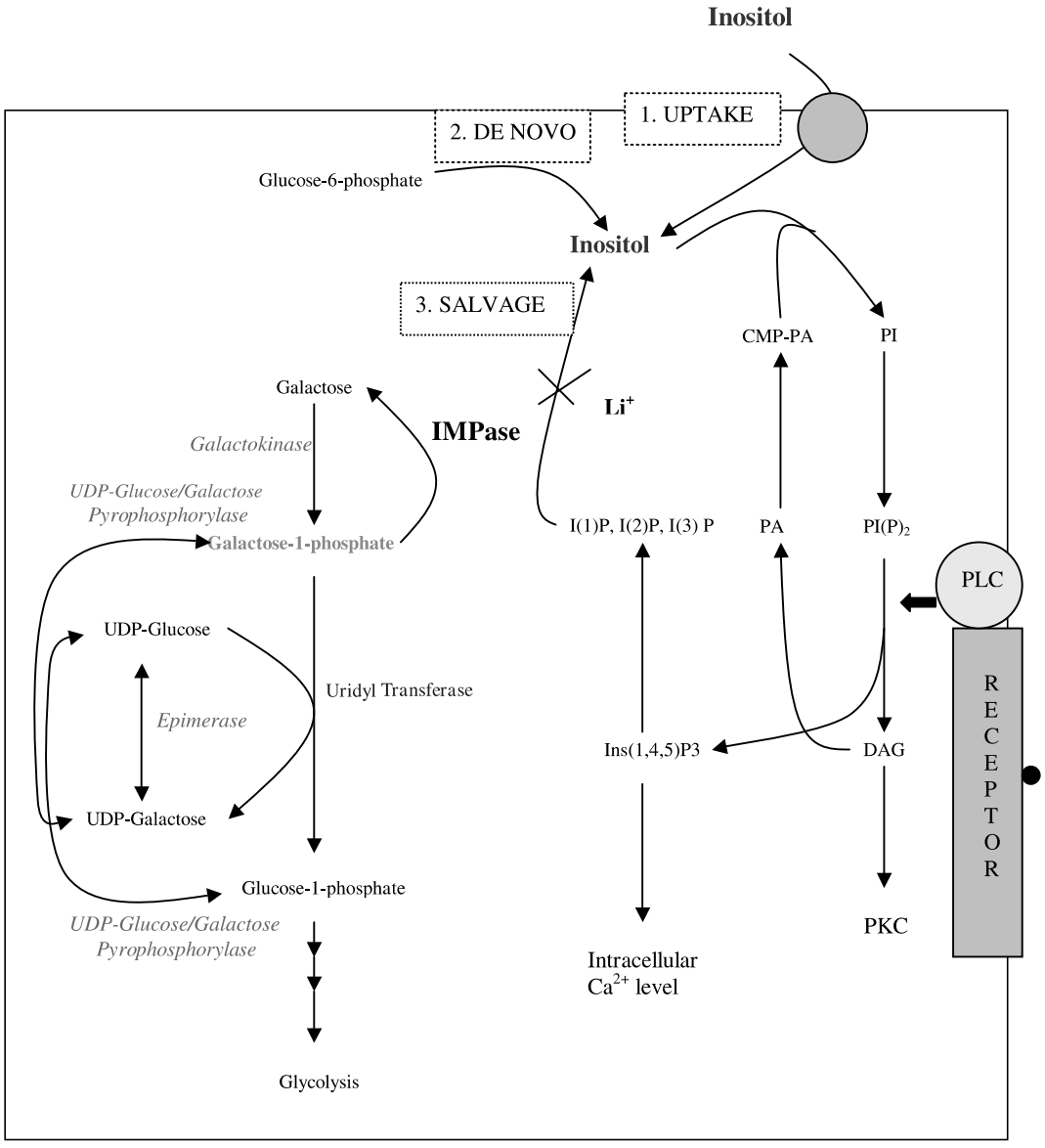

Fig. 1 Schematic representation of the cross talk between galactose metabolism and phosphotidylinositolbisphosphate {PI(P)2} signaling pathway. The second messanger diacylglycerol (DAG) and inositol 1,4,5 triphosphate {Ins(1,4,5)P3} are formed by the hydrolysis of phosphotidylinositol 4,5 bisphosphate {PI(P)2} in response to agonist stimulated activation of phospholipase C. DAG and Ins(1,4,5)P3 activate protein kinase C and mobilize intracellular Ca^{2+}. The recycling of DAG to phosphotidylinositol proceeds via phosphatidic acid and cytidinemonophosphorylphosphatidate (CMP-PA). Ins (1,4,5)P3 is also recycled back to free inositol by sequential dephosphorylation to form Ins (1)P, Ins(3)P, and Ins(4)P which are then hydrolysed to free Inositol by IMPase PI synthase catalyzes the synthesis of PtdInsP from free Inositol and CMP-PA. IMPase can also hydrolyse galactose-1-phosphate formed during the normal metabolism of galactose. The de novo synthesis of galactose-1-phosphate occurs through the reaction catalyzed by UDP-glucose pyrophosphorylase, epimerase followed by UDP-glucose pyrophosphorylase as shown.

CLASSIC GALACTOSEMIA: AN UNSOLVED PROBLEM

Galactosemia, an inborn error of galactose metabolism is caused by the lack of any one of the enzymes of the Leloir pathway. It is transmitted as an autosomal recessive disorder and occurs at a frequency of 1:30,000 to 1:60,000. It has been categorized into three types: galactokinase deficiency galactosemia, epimerase deficiency galactosemia, and classic galactosemia due to the deficiency of galactose-1-phosphate uridyl transferase. Classic galactosemia is the most common, well studied and yet not well understood in terms of the biochemical basis of the diverse phenotypes (1–4).

Classic galactosemic infants on unrestricted galactose ingestion, suffer from acute symptoms such as poor

growth, feeding difficulties, jaundice, hepatomegaly, cerebral edema, sepsis due to *E. coli* infection, cataract, and renal tubular dysfunction. The above symptoms disappear when the individual is on galactose restricted diet immediately after birth. However, these individuals continue to suffer from defects from cognitive functioning, speech and language deficits, learning disability, progressive disease with ataxia and dystonia, ovarian dysfunction, and devastating neurological sequale (1–4). Persistence of long-term defects even after withdrawal of galactose from the diet is thought to be due to the endogenous production of galactose (7). It is also suggested that the toxicity initiated in utero could impair the proper development of brain and other organ systems making it difficult to reverse these phenotypes by simply withdrawing galactose form the diet (8,9). Based on a wealth of information and the fact that the patients with galactokinase deficiency galactosemia (in this case galactose and galactitol accumulate) do not develop the dietary independent complications as observed in classic galactosemia, it is believed that the primary cause for the long-term defect in classic galactosemia, is due to the block in the further metabolism of galactose-1-phosphate (10,11). The long-term clinical findings which primarily emanate from this metabolic block have been difficult to delineate at the biochemical level due to the lack of appropriate experimental models.

Based on a large body of experimental results, following broad possibilities have been considered (1–4,12,13): (a) the accumulation of galactose-1-phosphate could act as a energy sink, and/or could inhibit the enzymes involved in the carbohydrate metabolism resulting in the diverse phenotype; (b) a defect in the biosynthesis of glycoproteins and/or glycolipids due to an impairment in the synthesis of UDP-galactose, which is an important sugar donor for the assembly of complex polysaccharides, glycoproteins, and glycolipids; (c) a defect in the inositol mediated second messenger pathway resulting in the cellular dysfunction. While the first two possibilities have attracted the attention of many investigators, the third possibility is controversial (13) due to conflicting results. However, recent experiments indicate that the disturbance in inositol metabolism could be a likely possibility than originally thought, in the development of long-term syndrome observed in classic galactosemic patients.

INOSITOL METABOLISM IN CLASSIC GALACTOSEMIA

It was observed that brain autopsy samples of two new borns affected by classic galactosemia showed a reduction of up to 80% free inositol (Ins) and phosphotidylinositol (PtdIns) as compared to a normal individual (14,15). A reduction of upto 30% of free Ins and PtdIns was observed in galactose intoxicated rats (16). However, a significant reduction in free inositol could not be observed under similar experimental conditions (17–19). Based on these conflicting reports, it was suggested that only a marked increase in blood and tissue galactose (that occurs only in untreated galactosemic patients) would be likely to result in a physiologically relevant decrease in inositol concentrations. Therefore, it was suggested that it is unlikely that the long-term defects observed in galactosemic patients on galactose restrictive diet are due to a defect in inositol dependent signaling mechanism (13). Contrary to this, it was suggested that the diminished turnover of phosphotidylinositol in a critical fraction of phosphatidylinositol pool that causes cellular dysfunction rather than an absolute decrease in free inositol (20,21). Warfield and Segal (18) suggested that although the concentration of inositol and phosphotidylinositol appear to be essentially same in galactose fed animals as compared to the controls, it is possible that a metabolite of galactose affects the membrane bound or soluble phosphohydrolase without affecting the de novo synthesis or the other pathways of PtdIns metabolism.

Recent studies on IMPase support the view of Warfield and Segal (18). Over-expression of human IMPase alleviates galactose toxicity in a galactose-1-phosphate uridyl transferase deficient yeast (5). This result was interpreted in that the accumulation of galactose-1-phosphate in a transferase deficient yeast, could act as a substrate for IMPase, thereby interfering with its normal function of hydrolysis of inositol monophosphate (see below for details). Accordingly, over-expression of human IMPase overcomes this defect, implicating that IMPase is a potential target for galactose-1-phosphate mediated toxicity. The above view is strengthened by the observation that galactose-1-phosphate is as good a substrate as inositol monophosphate for IMPase obtained from human and rat brain (22). Based on this, the authors suggested that the functional concept of IMPase must be expanded to include a possible role in vivo in the regulation of brain galactose metabolism.

GALACTOSE-1-PHOSPHATE IS A COMMON INTERMEDIATE

Recent experimental evidence suggests that galactose-1-phosphate is a substrate for human as well as yeast UDP-glucose pyrophosphorylase (5,6). Pulse feeding of galactose in yeast followed by determination of metabolite concentrations indicated that the levels of galactose-1-phosphate oscillate and the authors suggest that this could be the result of the activity of UDP-glucose pyrophosphorylase (23). Over-expression or disruption

of this enzyme is lethal to yeast (24), probably because of a disturbance in the galactose metabolic network (24). In humans, UDP-glucose pyrophosphorylase is suggested to be involved in the de novo synthesis [Fig. 1] of galactose from glucose-1-phosphate (7,25). Galactose-1-phosphate toxicity observed in classic galactosemic patients who are on galactose restricted diet has been attributed to the activity of UDP-glucose pyrophosphorylase. The fact that humans have retained the ability to synthesize galactose-1-phosphate despite its toxicity in individual lacking galactose-1-phosphate uridyl transferase, probably points out a strong evolutionary selection to maintain this metabolic pathway.

It is clear from the foregoing that at least three enzymes (IMPase, galactose-1-phosphate uridyl transferase, and UDP-glucose pyrophosphorylase) in addition to other phosphatases (26) could play a critical role in maintaining the concentration of galactose-1-phosphate with in narrow limits. Surprisingly mice disrupted for galactose-1-phosphate uridyl transferase also accumulate galactose-1-phosphate and yet do not show any symptoms of toxicity as seen in galactosemic patients (27). It is conceivable that the 'knock out' mice might have excess IMPase or UDP-glucose pyrophosphorylase to overcome the galactose-1-phosphate toxicity as demonstrated in yeast (5,6). It is also possible, that unlike humans, that galactose-1-phosphate may not have any additional regulatory function in mice and accumulation of this may not lead to toxicity. Alternatively, a 'two hit' model has been proposed to explain the above paradox (3), which states that both galactitol and galactose-1-phosphate should accumulate to cause toxicity in classic galactosemic patients.

GALACTOSE-1-PHOSPHATE IS A REGULATOR OF INOSITOL MONOPHOSPHATASE

Brain is critically dependent on the receptor mediated phosphotidylinositol (1,4)P2 dependent signaling, since it serves a second messenger system for several neurotransmitters (28–31). Phosphotidylinositol (1,4)P2 is hydrolysed to two second messangers viz., diacylglycerol and Ins (1,4,5)P3 by phospholipase C, in response to cell stimulation. These second messengers exert their affects by activating protein kinase C and mobilizing intracellular Ca^{2+}, respectively. The generation of these two second messengers in response to cell stimulation depends on the continuous regeneration of the precursor phosphotidylinositol (1,4)P2, which in turn is dependent on the availability of free inositol. This is mainly achieved by recycling inositol monophosphates by IMPases, since brain has limited capacity for de novo synthesis and it cannot take up inositol from circulation due to blood–brain barrier (29,32). Thus, regulation of IMPase is critical for maintaining a controlled supply of inositol depending upon the physiological need and any variation in this process could result in abnormal brain function.

Hyperactivation of phosphatidylinositol signaling has been implicated in Bipolar disorder, a recurrent long-term mood disorder characterized by the presence of both depressive and manic phase (30,32). The 'inositol depletion hypothesis' proposed by Berridge et al. (31) states that, Li^{+} a well characterized uncompetitive inhibitor of IMPase used as a drug for the treatment of bipolar disorder reduces the free inositol available for the regeneration of PIP2, thereby attenuating the signal transduction. While the inositol depletion hypothesis has been widely accepted, it has not been proven beyond doubt that Li^{+} acts only through IMPase.

Since IMPase is the key determinant in providing free inositol and the cells have to quickly adapt to changing intra- and extra-cellular melieu in response to signals, IMPase is likely to be regulated at the biochemical level. Following lines of circumstantial evidence support the hypothesis that galactose-1-phosphate is a regulator of IMPase: (a) galactose-1-phosphate is also synthesized by the activity of UDP-glucose pyrophosphorylase (Fig. 1), even in a cell lacking galactose-1-phosphate uridyl transferase, suggesting that galactose-1-phosphate could have has additional role(s), than being only an intermediate of galactose metabolism (7); (b) over-expression of IMPase can suppress galactose toxicity in yeast (5); (c) galactose-1-phosphate is as good a substrate for IMPase as inositol monophosphate under in vitro conditions (22). Two distinct genetic loci for IMPase have been identified in humans, suggesting that its function may also be regulated at the genetic level (33–35).

HYPOTHESIS

Inositol monophosphatase is the rate limiting enzyme in the recycling of inositol in the InsPtd signaling pathway (30) and hence its activity can be regulated at the biochemical level. I hypothesize that, galactose-1-phosphate is a physiological regulator of IMPase and accordingly the concentration of galactose-1-phosphate is maintained with in narrow limits by the activities of various enzymes. Any physiological condition that disturbs this state can in principle bring about cellular dysfunction by altering the inositol monophosphate hydrolyzing activity of IMPase. According to this view, the long-term neurological symptoms observed in classic galactosemia could be the result of increased galactose-1-phosphate which down regulates IMPase by competitively inhibiting its normal function of hydrolyzing inositol monophosphate, thereby impairing the PtdIns signaling system. Recent studies have shown that

Li^+ causes toxicity to yeast only when it is using galactose as the sole carbon source, at a concentration generally used for therapeutic purpose (*36*). The toxicity has been attributed to the accumulation of galactose- and/or glucose-1-phosphate as a consequence of inhibition of phosphoglucomutase by Li^+. Therefore, it is conceivable that the therapeutic action of Li^+ could be an indirect affect of galactose-1-phosphate accumulation than a direct affect on IMPase. Alternatively, the affect of Li^+ could be a cumulative one. As a corollary to the above hypothesis, a decrease in galactose-1-phosphate, below the normal levels, would result in increased free inositol due to the 'up regulation' of IMPase activity. Such a change, in principle could contribute significantly towards the development of bipolar disorder. The above idea is supported by a recent observation that a mutation in yeast that caused up regulation of UDP-glucose pyrophosphorylase, reduced galactose-1-phosphate concentration below than observed in a yeast deficient in galactose-1-phosphate uridyl transferase (*6*). The above observations clearly point out that the maintenance of the concentration of galactose-1-phosphate through different metabolic pathways could play a critical role in the normal physiology of a cell. This view is consistent with the observation that bipolar disorder is multi-genic (*37*). The hypothesis that galactose-1-phosphate is a physiological regulator predicts that exogenous administration of galactose (administration of galactose has been shown to cause an accumulation of galactose-1-phosphate) should have the same affect as Li^+ in the treatment of bipolar disorder, since galactose-1-phosphate would 'down regulate' IMPase function.

CONCLUSIONS

A major challenge in modern biology is to establish genotype–phenotype relationship. Despite significant efforts, the biochemical basis of the pathophysiology of classic galactosemia and bipolar disorder has remained an enigma. Understanding the consequences of genetic disorders involving metabolic intermediates appear to be far more complex than initially imagined, since the intermediates can 'criss cross' between metabolic pathways as has been suggested. Secondly, the concentration of the metabolites keep changing depending upon the physiological status, the cell type and developmental state making it difficult to establish a cause and effect relationship. A full understanding of these complex disorders can only come from studying these processes from a molecular to systems level. As illustrated in this paper, comparative biochemistry can provide us valuable insights into the metabolic network. Modern techniques which provide us unprecedented power to delineate complex biological processes coupled with experimental systems that are amenable to genetic analysis have to be employed to get an in-depth understanding. The idea that galactose and inositol metabolic pathways functionally interact may provide us clues to develop novel experimental strategies to understand the biochemical basis of both galactosemia and bipolar disorder. Probably, the long-term defects observed in classic galactosemic patients is the price humans have to pay for recruiting galactose-1-phosphate as a regulator of IMPase.

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