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REVIEW

Inositol and higher inositol phosphates in neural tissues: homeostasis, metabolism and functional significance

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Abstract

Inositol phospholipids and inositol phosphates mediate well-established functions in signal transduction and in Ca^{2+} homeostasis in the CNS and non-neural tissues. More recently, there has been renewed interest in other roles that both *myo*-inositol and its highly phosphorylated forms may play in neural function. We review evidence that *myo*-inositol serves as a clinically relevant osmolyte in the CNS, and that its hexakisphosphate and pyrophosphorylated derivatives may play roles in such diverse cellular functions as DNA repair, nuclear RNA export and synaptic membrane trafficking.

Keywords: affective disorder and treatment, diphosphoinositol polyphosphates, inositol hexakisphosphate, lithium, Na^+/myo-inositol transporter, phytate.

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The prominent role of inositol phospholipids in signal transduction events within the CNS is now well established (for reviews, see Fisher and Agranoff 1987; Fisher *et al*. 1992). In addition, roles for phosphoinositides as facilitators of a diverse array of cellular events, such as membrane trafficking, maintenance of the actin cytoskeleton, regulation of cell death and survival, and anchors for plasma membrane proteins, have been documented (for reviews, see Toker and Cantley 1997; Low 2000; Vanhaesebroeck *et al*. 2001). In contrast, with the notable exception of inositol 1,4,5-trisphosphate and its pivotal role in the mobilization of intracellular calcium, less emphasis has been placed on the functional significance of the water-soluble components of the phosphoinositide pathway, i.e. *myo*-inositol and its phosphorylated derivatives. To date, seven of the 63 possible inositol phosphate esters have been assigned putative physiological roles (Irvine and Schell 2001), and many others have been detected in a variety of organisms. In addition, at least four pyrophosphorylated inositol phosphates and enzymes that synthesize (Huang *et al*. 1998; Saiardi *et al*. 2001b) and degrade them (Safrany *et al*. 1998; Caffrey *et al*. 2000) in brain have been identified. Given their structural diversity, it appears likely that cellular functions of other inositol phosphates remain to be discovered.

In this review, we focus on the role that *myo*-inositol itself and some of its highly phosphorylated derivatives (namely, inositol hexakisphosphate and the diphosphoinositol polyphosphates) may play in neural function. The CNS is an atypical tissue in that it possesses relatively high concentrations of *myo*-inositol as well as the means to synthesize it. *myo*-Inositol serves not only as a precursor molecule for inositol lipid synthesis, but also as a physiologically important osmolyte. Alterations in brain and CSF inositol concentrations have been reported in a number of pathological conditions. In addition, oral administration of

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Abbreviations used: DIPP, diphosphoinositol polyphosphate phosphatase; HMIT, H^+/*myo*-inositol symporter; IMPase, inositol monophosphatase; IP_6, inositol-1,2,3,4,5,6-hexakisphosphate; mIPMK, mammalian inositol polyphosphate multikinase; MW, molecular weight; PI, phosphatidylinositol; PI4P, phosphatidylinositol-4-phosphate; $PI(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate; $[PP]_2$-IP_3, 5,6-bisdiphosphoinositol-(1,3,4)-trisphosphate; PP-IP_4, 5-diphosphoinositol-(1,3,4,6)-tetrakisphosphate; PP-IP_5, 5-diphosphoinositol-(1,2,3,4,6)-pentakisphosphate; $[PP]_2$-IP_4, 5,6-bisdiphosphoinositol-(1,2,3,4)-tetrakisphosphate; SGLT, Na^+/glucose cotransporter; SMIT, Na^+/*myo*-inositol transporter; VSOAC, volume-sensitive organic osmolyte anion channel.

inositol has been claimed to be of therapeutic benefit in the treatment of several neuropsychiatric disorders. Inositol hexakisphosphate (IP_6; phytate) is the most abundant of the inositol phosphates in both neural and non-neural cells. In common with *myo*-inositol, IP_6 is particularly prevalent in the CNS. IP_6 binding sites have been identified (Sasakawa *et al*. 1995), and a number of functions highly relevant to the CNS have been proposed. IP_6 was held to be the most highly phosphorylated inositol-containing molecule until the discovery of pyrophosphate-containing derivatives, which has added yet another level of complexity to our understanding of both the metabolism and the function of inositol phosphates. The diphosphoinositol polyphosphates are readily synthesized by enzymes derived from the CNS and exhibit a rapid turnover, and are thus likely to play significant roles in neural function.

Inositol

Historical overview

A crystalline product derived from muscle extracts and having the empirical formula of a carbohydrate was discovered some 150 years ago (Scherer 1850) and was termed 'inosit', from *inos* the Greek root for muscle, and which was translated into English as 'inositol'. It was later predicted to be one of nine possible stereoisomers of hexahydroxycyclohexane (Bouveault 1894), of which seven were correctly predicted to be optically inactive and of the remaining two to be an enantiomeric pair (Fig. 1). The inositol isomer described by Scherer was found to be naturally abundant, and was eventually identified as its hexakisphosphate ester, phytic acid, a common component of many foods, especially grains. To distinguish the name of the prevalent isomer in

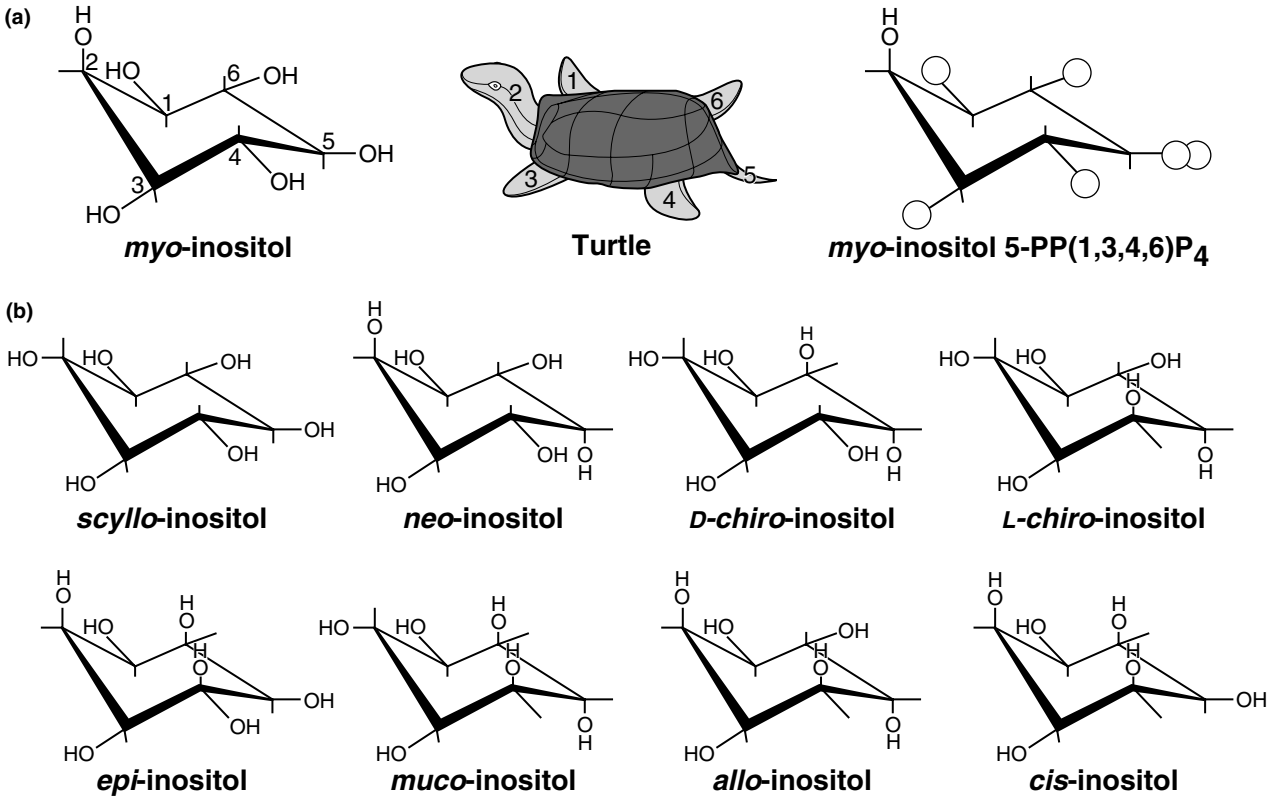

Fig. 1 Structures and nomenclature of the inositols. Of the nine possible isomers of hexahydroxycylcohexane, *myo*-inositol is by far the most prevalent in nature. The term 'cyclitol' can be used to refer to the nine inositols collectively. When the term inositol is used without a prefix, it is inferred to be the *myo* isomer. Viewed in its favored chair configuration (a), *myo*-inositol has one axial and five equatorial hydroxyl groups. A convenient mnemonic device for visualizing the molecule three dimensionally is to consider it as a turtle (IUPAC-IUB Commission on Biochemical Nomenclature (CBN) 1992) in which the D1 position is the right front limb and the head (axial) is position D2. Looking down from above and continuing counter-clockwise, the left front limb becomes D3, etc. A pyrophosphoryl inositol polyphosphate is shown to illustrate the usefulness of the 'turtle' in visualizing this molecule. It should be cautioned that inositol phosphates, like inositol, are not rigid molecules, and actual conformations are expected to reflect the regional ionic environment within the cellular milieu. (b) Names and structures of the other eight cyclitols. About a tenth of brain cyclitol is *scyllo*-inositol, and *neo*-inositol has been detected in trace amounts in brain.

nature from the eight other isomeric inositols, it was renamed *myo*-inositol. When one uses the term inositol without a prefix, it is generally presumed that one is referring to the *myo* isomer, whereas the term 'inositols' refers to all nine. Inositol was one of a number of 'bios' growth factors established to be required by some microorganisms (Eastcott 1928). A nutritional deficiency in laboratory animals could not be easily demonstrated, presumably because of the ubiquitous presence of inositol in the diet, and the ability of some tissues to convert D-glucose-6-phosphate to D-inositol-3-phosphate, which could then be dephosphorylated to inositol. In addition, dietary phytate can in some instances give rise to free inositol, either by the action of ingested dietary phytases or from endogenous phytase activity in the intestinal mucosa. Cultured human cell lines that are dependent on inositol have been identified, some of which can synthesize inositol, but presumably not in sufficient quantities to support maximal growth (Eagle *et al*. 1960).

Although the human diet typically provides 1 g/day of inositol (Holub 1986), approximately 4 g/day could be synthesized by the kidneys (Clements and Diethelm 1979; see Fig. 2). Brain and testis also synthesize inositol (Hauser and Finelli 1963), but the kidney is by far the major organ involved in its catabolism and excretion. Inositol degradation occurs via a specific oxygenase that cleaves the polyol ring with the resulting production of D-glucuronic acid (Charalampous 1958; Arner *et al*. 2001). The latter is then metabolized in the liver to D-xylulose 5-phosphate and enters the pentose phosphate pathway (see Fig. 2).

A special function for inositol in the nervous system was first suggested by the discovery of a relatively high inositol content in the brains of many species (Young 1934; Long 1961). Folch found that cephalin contained lipid-bound inositol (Folch and Woolley 1942), which was eventually shown to be one of three phosphoinositides: phosphatidylinositol (PI), phosphatidylinositol-4-phosphate (PI4P) and phosphatidylinositol-4,5-bisphosphate [PI(4,5)P_2]. Evidence that the inositol lipids are metabolically highly active was first noted in manometric studies (Sloane-Stanley 1953), and later by means of ^{32}P labeling (Eichberg and Dawson 1965). Eventually, the high rate of turnover could be explained by evidence that they were components of the phosphoinositide-mediated signal transduction system (Berridge 1987).

Most of the other eight isomers of *myo*-inositol, largely the result of chemical or microbial action on *myo*-inositol hexaphosphate produced by plant grains, have been isolated as phosphate esters from soil. A number of derivatized cyclitols, mainly methyl ethers, are also present in plants (Posternak 1965). *Scyllo*-inositol is present in human brain in quantities estimated to be from 5 to 12% that of *myo*-inositol (Seaquist and Gruetter 1998). Rat and rabbit brains contain an epimerase that converts *myo*- to *scyllo*-inositol via a *myo-scyllo*-inosose intermediate (Sherman *et al*. 1968a,b),

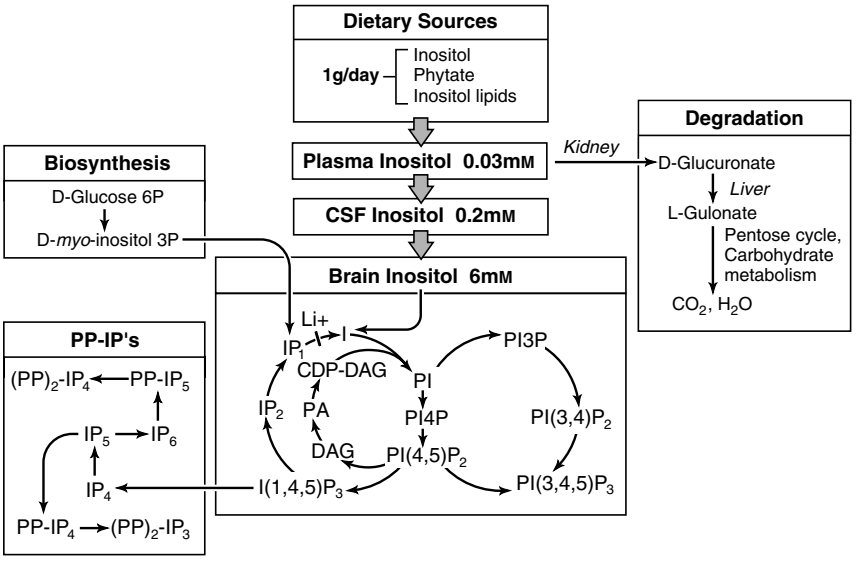

Fig. 2 Sources and metabolic fates of inositol in humans. The inositol intake in the human diet is estimated to be about 1 g per day, arising from free inositol, or derived by hydrolysis of ingested phytate or to a lesser extent, of inositol lipids. Some tissues, including brain endothelium, have the ability to form inositol from glucose 6-P. CSF is enriched seven-fold in inositol relative to blood (plasma), whereas the brain inositol concentration is some 200-fold higher. Inositol levels in neuronal and glial cells derived from various species can be between three and four times that of whole brain. Inositol degradation is exclusively via conversion to glucuronic acid in the kidney, followed by further degradation in the liver. Free inositol is required for synthesis of phosphatidylinositol (PI). Its formation from higher inositol phosphates is blocked by Li^+ (see text). The formation of IP_5-IP_8 is depicted in Fig. 3.

but it is not known whether brain *scyllo*-inositol is synthesized *in situ* or how much of it is transported into the brain via the blood (Spector 1978). Levels of both *myo* and *scyllo* isomers can be measured non-invasively in the human brain by means of ^{1}H-spectral shift NMR. *Neo*-inositol has been reported to be present in mammalian brain, but at much lower concentrations (Sherman *et al*. 1971). Small quantities of labeled *chiro*-inositol are reported in the rat brain following intraperitoneal injection of ^{3}H-labeled *myo*-inositol (Pak *et al*. 1992). At present, phosphates of inositols other than *myo*-inositol have not been reported in brain or other animal tissues. However, it has been recently learned that *Entamoeba histolytica* contains *neo*-inositol phosphates (Martin *et al*. 2000).

Distribution of inositol in CNS and other neural tissues

Inositol in tissues and other biological samples can be isolated from other polyols by TLC and, following derivatization, separated by GLC, and then identified by retention times and quantified by means of a variety of detectors (cited in Kouzuma *et al*. 2001). It can also be selectively measured in a mixture by means of specific bacterial dehydrogenases (Kouzuma *et al*. 2001). With the exceptions of the testis, kidney and lens of the eye, the mammalian CNS contains significantly higher concentrations of inositol than non-neural tissues. As is the case in non-neural cells (Sigal *et al*. 1993), it is likely that inositol in neural tissues is not in a single compartment (Wolfson *et al*. 2000). If the assumptions are made, for purposes of comparison, that each wet weight gram or mL of brain contains 100 mg of protein, and that its dry weight comprises 20% of wet wight, then intracellular concentrations of inositol present in whole brain, gross anatomical regions or peripheral nerves can be calculated to range from 2 to 15 mM. These concentrations of inositol exceed those present in the CSF and plasma by 25–50- and 50–100-fold, respectively (see Table 1). Within the CNS, relatively similar concentrations of inositol are reported for distinct regions of either human (Shimon *et al*. 1998) or rat brain (Patishi *et al*. 1996). However, at least in human brain, the absolute values may not remain constant throughout life because concentrations of inositol in temporal cortex decline by > 50% between the ages of 20 and 90 (Stokes *et al*. 1983). Inositol is also readily lost from brain tissue when cell integrity is compromised. Thus when slices are prepared from cerebral cortex, hippocampus or cerebellum, there is a 50–60% loss of inositol (Table 1).

It remains uncertain to what extent inositol is preferentially concentrated within neuronal or glial cells. It has been suggested that inositol is primarily (Glanville *et al*. 1989), or even exclusively (Brand *et al*. 1993), localized to the glia. However, inositol concentrations in some neuronal populations may equal or exceed those observed for glia. Concentrations of inositol approaching 10–20 mM have been reported for the Purkinje cell layer of the cat cerebellar flocculus (Godfrey *et al*. 1982), and for the giant neurons of Deiter's nucleus in the rabbit (Sherman *et al*. 1977). These values are similar to those obtained for cultured human NT2-N neurons or SH-SY5Y neuroblastoma cells (Novak *et al*. 1999). In other neuronal types, inositol concentrations are distinctly lower. For example, the concentration of inositol in cultured rat cerebellar granule cells is < 25% of that in NT2-N neurons (Novak *et al*. 1999), and HCN-2 human neurons are reported to be less than 1 mM in inositol (Koch *et al*. 1999).

Molecular mechanisms for maintenance of inositol concentrations

In addition to the recycling of inositol phosphate second messengers, cells maintain their intracellular concentration of inositol via three distinct mechanisms: transport of inositol across the plasma membrane via specific carrier molecules, *de novo* synthesis of D-inositol 3-phosphate from D-glucose 6-phosphate, and the efflux of inositol, which is mediated via a volume-sensitive organic osmolyte channel.

Na^+/myo-inositol transporter

Concentrations of inositol in neural cells typically exceed those in plasma by a wide range, from 2- to 500-fold (Sigal *et al*. 1993). This concentration of the polyol is accomplished primarily by a saturable Na^+-dependent uptake mechanism that exhibits a high affinity for inositol and is mediated by a specific sodium *myo*-inositol transporter (SMIT) molecule, originally cloned from canine MDCK cells (Kwon *et al*. 1992). From its cDNA, a protein of 718 amino acids with 12 membrane-spanning domains is predicted (molecular weight; MW = 79.5 kDa). Both the amino and carboxyl termini of SMIT are cytoplasmic, and several potential phosphorylation sites for either protein kinase A or protein kinase C are found in the cytoplasmic domains. Canine SMIT exhibits 40–50% homology with the Na^+/D-glucose cotransporters of rabbit intestinal mucosa and kidney proximal tubules (Hediger *et al*. 1987; Coady *et al*. 1990). Human SMIT is highly homologous to the canine transporter (Berry *et al*. 1995). The gene encoding human SMIT (SLC5A3) appears to be a complex transcriptional unit that consists of at least five exons subject to alternate splicing (Porcellati *et al*. 1998), and is overexpressed in Down's syndrome (see below).

The transport characteristics of cloned renal SMIT were examined in detail following expression in *Xenopus* oocytes (Hager *et al*. 1995). Uptake is pH-dependent and requires two Na^+ ions for each molecule of inositol transported. Half-maximal uptake occurs at 50 μM, and phlorizin is a potent competitive inhibitor. *Myo*- and *scyllo*-inositols are the preferred substrates for transport, with the rank order efficacy for transport for a number of carbohydrates being *myo*-inositol = *scyllo*-inositol > L-fucose > L-xylose > L-glucose, D-glucose, 3-*O*-methyl-D-glucose, 2-deoxy-

Table 1 Concentrations of *myo*-inositol in neural tissues

Cell/tissue	Inositol concentrations		Reference
	Reported	Calculated (mM)[a]	
Human brain			
frontal cortex	5.8 mmol/kg wet wt	5.8	Shimon *et al.* (1998)
occipital cortex	5.8 mmol/kg wet wt	5.8	Shimon *et al.* (1998)
cerebellum	5.1 mmol/kg wet wt	5.1	Shimon *et al.* (1998)
temporal cortex (age 20)	60 μmol/g protein	6.0	Stokes *et al.* (1983)
temporal cortex (age 90)	27 μmol/g protein	2.7	Stokes *et al.* (1983)
temporal cortex	4.4 μmol/g wet wt	4.4	Stokes and Hawthorne (1987)
Human			
CSF	22 ng/μL	0.12	Shetty *et al.* (1995a)
plasma	2.8 ng/μL	0.02	Shetty *et al.* (1995b)
sural nerve	26 000 pmol/mg protein	2.6	Sundkvist *et al.* (2000)
NT2-N neurons	195 nmol/mg protein	17.4[c]	Novak *et al.* (1999)
HCN-2 neurons	< 1 mM	< 1	Koch *et al.* (1999)
SK-N-SH neuroblastoma	60 nmol/mg protein	6.0	Stubbs and Agranoff (1993)
SH-SY5Y neuroblastoma	112 nmol/mg protein	11.2	Novak *et al.* (1999)
1321 N1 astrocytoma	80 nmol/mg protein	8.0	Novak *et al.* (1999)
Rat brain			
whole brain	11.5 mmol/kg wet wt	11.5	Lohr *et al.* (1988)
whole brain	6.6 μmol/g wet wt	6.6	Palmano *et al.* (1977)
whole brain	5.8 mmol/kg wet wt	5.8	MacGregor and Matschinsky (1984)
cerebral cortex	2.1 mmol/kg wet wt	2.1	Patishi *et al.* (1996)
cerebral cortex	21 mmol/kg dry wt	4.2	Sherman *et al.* (1986)
cerebral cortex	2.3 mmol/kg wet wt	2.3	Belmaker *et al.* (1998)
cerebral cortex (gray)	61 nmol/mg protein	6.1	Novak *et al.* (1999)
cerebral cortex (white)	80 nmol/mg protein	8.0	Novak *et al.* (1999)
cerebral cortex (slices)	21 nmol/mg protein	2.1	Stubbs and Agranoff (1993)
cerebral cortex (slices, washed)	9 nmol/mg protein	0.9	Stubbs and Agranoff (1993)
hypothalamus	3.7 mmol/kg wet wt	3.7	Patishi *et al.* (1996)
hypothalamus	33 mmol/kg dry wt	6.6	Sherman *et al.* (1986)
hypothalamus	4.4 mmol/kg wet wt	4.4	Belmaker *et al.* (1998)
cerebellum	3.4 mmol/kg wet wt	3.4	Patishi *et al.* (1996)
cerebellum	31 mmol/kg dry wt	6.2	Sherman *et al.* (1986)
cerebellum	3.1 mmol/kg wet wt	3.1	Belmaker *et al.* (1998)
cerebellum (slices)	38.3 nmol/mg protein	3.8	Heacock *et al.* (1993)
cerebellum (slices, washed)	17.0 nmol/mg protein	1.7	Heacock *et al.* (1993)
cerebellum: molecular layer	29 mmol/kg dry wt	5.8	MacGregor and Matschinsky (1984)
cerebellum: granular layer	23 mmol/kg dry wt	4.6	MacGregor and Matschinsky (1984)
cerebellum: granular layer	22 mmol/kg dry wt	4.4	Sherman *et al.* (1977)
cerebellum: medullary layer	27 mmol/kg dry wt	5.4	MacGregor and Matschinsky (1984)
hippocampus	3.6 mmol/kg wet wt	3.6	Patishi *et al.* (1996)
hippocampus	34 mmol/kg dry wt	6.8	Sherman *et al.* (1986)
hippocampus	3.1 mmol/kg wet wt	3.1	Belmaker *et al.* (1998)
hippocampus (slices)	30.3 nmol/mg protein	3.0	Heacock *et al.* (1993)
hippocampus (slices, washed)	11.5 nmol/mg protein	1.15	Heacock *et al.* (1993)
caudate	2.8 mmol/kg wet wt	2.8	Patishi *et al.* (1996)
caudate	19 mmol/kg dry wt	3.8	Sherman *et al.* (1986)
caudate	2.7 mmol/kg wet wt	2.7	Belmaker *et al.* (1998)
Rat			
serum	0.1 μmol/mL	0.1	Palmano *et al.* (1977)
serum	63.7 μM	0.06	MacGregor and Matschinsky (1984)
sciatic nerve	3.1 μmol/g wet wt	3.1	Palmano *et al.* (1977)

Table 1 (Continued)

Cell/tissue	Inositol concentrations		Reference
	Reported	Calculated (mM)[a]	
sciatic nerve	2.7 mmol/kg wet wt	2.7	MacGregor and Matschinsky (1984)
cerebellar granule cells	41 nmol/mg protein	4.1	Novak *et al.* (1999)
astroctyes: cerebral cortex	120 nmol/mg protein	12.0	Strange *et al.* (1994)
astrocytes: cerebral cortex	25.6 μg/mg protein	14.2	Isaacks *et al.* (1994)
astrocytes: cerebral cortex	26.6 μg/mg protein	14.9	Isaacks *et al.* (1999a)
C_6 glioma	28 nmol/mg protein	31.0[c]	Strange *et al.* (1991)
Rabbit			
giant neurons of Deiter's nucleus	44 mmol/kg dry wt	8.8	Sherman *et al.* (1977)
dorsal root ganglion	21 mmol/kg dry wt	4.2	Sherman *et al.* (1977)
spinal cord, anterior horn	56 mmol/kg dry wt	11.2	Sherman *et al.* (1977)
Cat			
molecular layer of cerebellar flocculus[b]	17.4 mmol/L	17.4	Godfrey *et al.* (1982)
auditory nerve	10 mmol/L	10.0	Godfrey *et al.* (1982)
facial nerve	14.5 mmol/L	14.5	Godfrey *et al.* (1982)
vestibular nerve	12 mmol/L	12.5	Godfrey *et al.* (1982)
Mouse			
whole brain	4.8 mmol/kg wet wt	4.8	Thurston *et al.* (1989)
Guinea pig			
cerebral cortex	48 mmol/kg dry wt	9.6	Sherman *et al.* (1986)

[a]Inositol intracellular concentrations are calculated on the basis that each g wet wt of tissue contains 100 mg of protein. Dry weight is assumed to comprise 20% of wet weight (Long 1961). [b]See reference for additional brain regions. [c]Calculated on the basis of measured intracellular volumes.

D-glucose > D-xylose. In contrast, the specificity for transport by the Na^+/glucose cotransporter (SGLT1) is D-glucose, α-methyl-D-glucopyranoside, D-galactose, D-fucose, 3-*O*-methyl-D-glucose > D-xylose, L-xylose, 2-deoxy-D-glucose > *myo*-inositol, L-glucose, L-fucose. Uridine is transported by an Na^+/nucleoside transporter, which exhibits considerable homology to both SGLT1 and SMIT (61% and 47%, respectively), although neither SMIT nor SGLT1 is able to transport uridine.

SMIT, as determined by expression of its mRNA, is widely distributed throughout the CNS and is found in both neural and non-neural cells. There are striking regional differences in the expression of SMIT mRNA, with highest levels observed in the choroid plexus, pineal, hippocampus, locus cœruleus and Purkinje cells. Although the distribution of mRNA for SMIT is distinctly uneven, all brain regions possess the transcript for the transporter (Inoue *et al*. 1996). In contrast, only relatively small differences in V_{max} (0.2–0.5 nmol/mg protein/min) have been reported for SMIT measured in astrocyte preparations derived from major brain regions, including cerebral cortex, hippocampus, cerebellum, diencephalon and tegmentum (Isaacks *et al*. 1997, 1999c; Lubrich *et al*. 2000). K_m values for the high affinity transport of inositol in these distinct regions are also relatively similar (13–50 μM; see Table 2). In terms of development, expression of both the mRNA and SMIT protein is highest in fetal brain and declines postnatally (Guo *et al*. 1997). This pattern of expression may be linked to the high concentration of inositol in fetal CSF (Battaglia *et al*. 1961). As observed for the adult brain, mRNA for SMIT is diffusely distributed throughout the fetal brain and spinal cord with positive signals observed for both neural and non-neural cells. The developmental pattern for SMIT in kidney is completely opposite of that observed for brain: renal SMIT mRNA expression is low during embryogenesis and increases significantly after birth (Guo *et al*. 1997).

Both the expression and activity of SMIT can be regulated by a number of factors, the best documented of which is osmotic stress. This has been demonstrated both *in vivo* (Ibsen and Strange 1996) and *in vitro* for cultured glia (Strange *et al*. 1991, 1994; Isaacks *et al*. 1997), neuroblastoma (Wiese *et al*. 1996) and human NT2-N neurons (Novak *et al*. 1999). In response to hyperosmolar stress (> 330 mOsm), the expression of SMIT mRNA increases several-fold, as does the activity of the transporter. *SMIT* gene expression can be regulated by five tonicity-responsive enhancers scattered throughout the 5′-flanking region of the gene (Rim *et al*. 1998). The increase in rat SMIT mRNA occurs only after several hours and precedes increased activity of the transporter (Ibsen and Strange 1996). The latter, which is associated with an increase in the V_{max} of SMIT without a change in K_m, is blocked in the presence of

Table 2 Kinetic characteristics of Na^+/*myo*-inositol transporter in neural tissues

Cell/tissue	K_m (μM)	V_{max} (nmol/mg protein/min)	Reference
Human			
NT2-N neurons	36	0.06	Novak *et al.* (1999)
1321 N1 astrocytoma	40	0.18	Batty *et al.* (1993)
Rat			
Astrocytes			
Cerebral cortex	13–18	0.16	Isaacks *et al.* (1997)
	26	0.19	Isaacks *et al.* (1999c)
	50	0.47	Lubrich *et al.* (2000)
Cerebellum	27	0.20	Lubrich *et al.* (2000)
Hippocampus	50	0.30	Lubrich *et al.* (2000)
Diencephalon	27	0.19	Lubrich *et al.* (2000)
Tegmentum	48	0.21	Lubrich *et al.* (2000)
C6 glioma	15	0.12	Paredes *et al.* (1992)
B50 neuroblastoma	14	0.02	Reboulleau (1990)
Fetal brain cells	35	0.01	Fruen and Lester (1991)
Rabbit			
Peripheral nerve	83	0.01	Greene and Lattimer (1982)
Mouse			
Astrocytes (cerebral cortex)	25	0.06	Wiesinger (1991)
NB41A3 neuroblastoma	46	0.15	Wiese *et al.* (1996)
Neuroblastoma	12	0.01	Yorek *et al.* (1986)

cycloheximide, thereby indicating a requirement for *de novo* protein synthesis. Conversely, when cells are returned to iso-osmotic conditions, SMIT mRNA levels decrease rapidly and inositol is slowly lost by passive efflux. Whereas SMIT transcripts are broadly distributed throughout the brain, there are marked regional differences in the magnitude of increased expression of SMIT mRNA in response to hypertonicity (Ibsen and Strange 1996; Bitoun and Tappaz 2000). Following increases in plasma osmolarity, significant increases in SMIT mRNA levels were observed for the rat cerebral cortex, habenula and nucleus accumbens, but little increase occurred in the caudate-putamen. Similarly, plasma hypertonicity increases SMIT mRNA in the inferior, but not in the superior, colliculi (Ibsen and Strange 1996). Increases in mRNA for SMIT were also observed following the intraperitoneal administration of kainic acid, a treatment that results in an intensive neuronal discharge and a concomitant increase in the concentration of intracellular electrolytes. The increases in SMIT mRNA were most pronounced in the frontal and parietal cortex (Nonaka *et al*. 1999).

SMIT expression is also regulated by inositol. Thus, when cultured astrocytes obtained from the cerebellum, cerebral cortex, hippocampus or tegmentum were pre-treated with 400 μM inositol for 1–15 days there was a time-dependent reduction of SMIT activity, which reached its maximum (approximately 30%) at day 8 (Lubrich *et al*. 2000). In contrast, SMIT activity in astrocytes obtained from the diencephalon is not down-regulated by inositol. The latter observation, along with the differential regulation of SMIT activity by corticosterone and hyperosmolarity (Lubrich *et al*. 2000), may suggest the presence of distinct SMIT isoforms in different brain regions. SMIT may also be regulated by anti-bipolar therapeutic drugs such as Li^+, valproate or carbamazepine, each of which is reported to reduce the activity of the transporter (Lubrich *et al*. 2000). The activity of SMIT may also be regulated by protein kinase C, the activation of which results in an inhibition of the transporter in human astrocytoma cells (Batty *et al*. 1993) and human NT2-N neurons (Novak *et al*. 2000). In contrast, activation of protein kinase A does not alter the activity of SMIT in NT2-N neurons (Novak *et al*. 2000).

H^+/myo-inositol symporter

A novel mammalian inositol transporter has recently been identified from the screening of databases for sequences related to SGLT. However, this molecule does not transport glucose or other hexoses, but is an H^+-*myo*-inositol symporter (HMIT; Uldry *et al*. 2001). HMIT cDNA encodes a 618 amino acid polypeptide with 12 predicted transmembrane domains (MW approximately 75–90 kDa, depending upon the extent of glycosylation). HMIT is expressed almost exclusively within the CNS, primarily within astrocytes, but it is also present in some neuronal populations. Of interest are the pH-dependencies of the two transporter types. Thus whereas SMIT is progressively inhibited by a reduction in pH (Matskevitch *et al*. 1998), HMIT is maximally active at

low pH. Although the physiological significance of HMIT has yet to be established, this transporter might mediate an additional regulatory mechanism of inositol homeostasis.

Inositol synthesis

Inositol is synthesized *de novo* from glucose in a number of organs, including brain, testis, kidney and liver (Hauser and Finelli 1963). The trimeric enzyme D-inositol-3-phosphate synthase catalyzes the irreversible cyclic aldol condensation of glucose-6-phosphate to yield D-inositol-3-phosphate (L-inositol-1-phosphate; Eisenberg 1967; Culbertson *et al*. 1976; Maeda and Eisenberg 1980). The reaction requires nicotinamide adenine dinucleotide (oxidized) (NAD), is stimulated by NH_4^+ and K^+, and is inhibited by Li^+. The D-inositol-3-phosphate produced is then hydrolyzed by the Mg^{2+}-dependent Li^+-inhibitable enzyme inositol monophosphatase (IMPase). No synthase activity could be detected in human NT2-N neurons (Novak *et al*. 1999). This result is consistent with previous immunocytochemical data localizing the brain enzyme primarily to the capillary endothelium (Wong *et al*. 1987).

Inositol efflux

In response to hypotonic stress, inositol efflux occurs from both neurons and glia via a facilitated diffusion mechanism that involves a non-specific Cl^- channel. Based upon electrophysiological studies, the latter has been designated as the volume-sensitive organic osmolyte anion channel (VSOAC) and mediates the rapid efflux of triethylamines and amino acids, in addition to inositol (Jackson and Strange 1993; Goldstein and Davis 1994; Ruhfus and Kinne 1996; Jackson and Madsen 1997). VSOAC-mediated inositol efflux is dependent upon the non-hydrolytic binding of ATP and can be regulated by $G_{\alpha i}$, protein kinases A and C, tyrosine kinases and lipoxygenases (Strange *et al*. 1993; Ruhfus *et al*. 1996; Karihaloo *et al*. 1997; Song *et al*. 1998; Novak *et al*. 2000). VSOAC, the molecular characteristics of which have yet to be identified, is characterized on the basis of its inhibition by Cl^- channel blockers such as niflumic acid and 4,4′-diisothiocyanatostilbene-2,2′,disulfonic acid, as well as a number of *cis*-unsaturated fatty acids (Strange *et al*. 1993; González *et al*. 1995; Ruhfus and Kinne 1996; Isaacks *et al*. 1999a).

Putative roles for inositol in neuropathological conditions

A number of conditions have been identified in which alteration in inositol disposition may play a role, for example either as a physiologically important osmolyte or as a precursor molecule for phosphoinositide synthesis.

Disorders of volume regulation in the nervous system

In functioning neural and non-neural cells, electrolytes are transported across the plasma membrane, and the composition of osmotically active particles inside the cell is in a constant state of flux. Unless the osmolyte concentration is tightly regulated, bulk flow of water across the plasma membrane may result in damaging changes in cell volume. To maintain cell volume, acute changes in tonicity are regulated by changes in the transport of Na^+, K^+, H^+ or Cl^- across the plasma membrane, whereas chronic changes in tonicity are offset by the transport of 'compatible' or 'non-perturbing' organic osmolytes, primarily inositol (Strange 1992; Lang *et al*. 1998).

At least part of the pathogenesis of Down's syndrome may be related to the function of inositol as an osmolyte in the nervous system. Band q22 of human chromosome 21, which contains the gene encoding SMIT, is triplicated (Berry *et al*. 1995), and the inositol concentrations in the brain and CSF are 30–50% higher than in controls (Shetty *et al*. 1995b; Shonk and Ross 1995; Berry *et al*. 1999; Huang *et al*. 1999). Fetal CSF, which has a 10-fold higher concentration of inositol than that of the adult mammal (Battaglia *et al*. 1961), is even further enriched in Down's syndrome, and the excess of the polyol has been linked to osmolyte and electrolyte imbalances in the developing CNS. Inositol concentrations are also elevated in Ts65Dn mice, which possess three copies of mouse chromosome 16 – homologous to human chromosome 21 (Shetty *et al*. 2000). The triplication of SMIT results in the predicted 50% increase in inositol uptake in the trisomic fetal cortical neurons (Acevedo *et al*. 1997).

Under conditions of extended hypernatremia, the brain accumulates inositol to offset water loss (Lohr *et al*. 1988; Lien *et al*. 1990; Lee *et al*. 1994). If the hypernatremia is corrected too rapidly, the sudden osmotic gradient produces cerebral edema, which may cause seizures or even death as the swelling brain is driven against the unyielding skull (Gullans and Verbalis 1993; Jackson and Madsen 1997). Accordingly, protracted hyponatremia results in a depletion of brain inositol reserves, and the administration of isotonic fluids can produce cerebral dehydration, shrinkage and myelinolysis (Thurston *et al*. 1989; Videen *et al*. 1995).

Inositol transport is also altered in stroke, a condition in which neurons around a focal ischemic zone become hypoxic, with a concomitant loss of ATP. The latter results in a rise in intracellular electrolyte concentrations and in an increase in intracellular osmolyte loads caused by the accumulation of catabolites (Venkatachalam and Buja 1994). Both of these events contribute to cytotoxic edema, which would ordinarily be countered by inositol efflux (Nonaka *et al*. 1998). However, because VSOAC activity requires ATP, efflux is diminished, thereby exacerbating neuronal swelling (Jackson *et al*. 1994). Physical trauma to the brain can compromise the blood–brain barrier, with a concomitant leakage of ions and macromolecules into the cerebral extracellular space, which in turn results in vasogenic edema and the triggering of an up-regulation of SMIT mRNA and cellular inositol uptake (Yamashita *et al*. 1997).

Alterations in inositol transport are also implicated in hepatic encephalopathy. This condition is associated with hyperammonemia, which results in astrocyte swelling and a loss of cerebral inositol (Haussinger 1994). In cultured astrocytes incubated with NH_4^+, there is a marked reduction in inositol uptake, which reflects both a reduction in the number of transporter molecules and their affinity for inositol. Ammonia also increases the fast efflux of inositol from these astrocytes (Isaacks *et al*. 1999b). Both the reduction in inositol influx and the accelerated efflux of inositol may represent adaptive changes to counteract the ammonia-induced swelling of astrocytes.

Bipolar disorder: Li^+ and the inositol depletion hypothesis

The discovery that lithium selectively blocks the action of IMPase (Hallcher and Sherman 1980) was successfully exploited by Berridge and colleagues in concert with the use of [^{3}H]inositol to demonstrate ligand-stimulated activation of the phosphoinositide-mediated second messenger system in intact cells (Berridge *et al*. 1982). They further hypothesized that the well-known psychotherapeutic action of Li^+ in mania could be attributed to its block of IMPase (Berridge *et al*. 1989), with a concomitant reduction in the synthesis of phosphoinositides. Li^+ administered *in vivo* leads to an accumulation of inositol monophosphate (Allison *et al*. 1976). When brain slices are incubated in the presence of Li^+, receptor activation results in a substantial increase in the formation of inositol mono- and bisphosphates, whereas conversely, the production of inositol tris- and tetrakisphosphates is reduced (Batty and Nahorski 1985; Kennedy *et al*. 1989, 1990; but see also Lee *et al*. 1992). The depletion hypothesis proposes that Li^+ also decreases free inositol that is required as cosubstrate with cytidine diphosphodiacylglycerol (CDP-DAG, see Fig. 2) for PI formation via PI synthase. Depletion of the inositol in the presence of Li^+ could then slow the metabolic sequence; whereby PIP_2, the precursor of the messenger molecules I(1,4,5)P_3 and DAG, is regenerated. In keeping with this possibility, a reduction in PIP_2 supply has been observed in a non-neural cell line (CHO) transfected with the cDNA for the M_1 muscarinic receptor (CHO-M_1) following chronic receptor activation in the presence of Li^+ (Jenkinson *et al.* 1994). An appealing aspect of the inositol depletion hypothesis is the uncompetitive nature of the Li^+ block of IMPase: the inhibition is enhanced by its substrate, inositol monophosphate. Thus, in 'hyperactive' cells, with rapid phosphoinositide recycling and a concomitantly larger production of inositol monophosphates [I(1)P, I(3)P and I(4)P], greater inhibition by Li^+ is proposed to occur, with a lesser block in 'normal'′ cells. However, in experimental animals administered Li^+ at doses equivalent to human therapeutic levels, there is only a small decrease in brain inositol concentration (Allison and Stewart 1971), which remains above the established K_m for PI synthase (Benjamins and Agranoff 1969; Ghalayini and Eichberg 1985; Imai and Gershengorn 1987). In contrast, the Li^+ block leads to a 20-fold increase in total brain inositol phosphates, and it has been proposed that it is this increase that mediates the therapeutic action of Li^+, rather than inositol depletion (Agranoff and Fisher 2001). Some neuronal populations may contain as much as 10–20 mM inositol, a finding that would seem to further lower the probability that inositol levels in such neurons can be lowered sufficiently in the presence of Li^+ to slow the phosphoinositide cycle. This objection would be overcome if the inositol recycled in the phosphoinositide cycle were in an intracellular pool that excluded the bulk of brain inositol. Alternatively, Li^+ may target populations of neurons in which inositol concentrations are constitutively low. Experimental demonstration of Li^+-mediated accumulation of CDP-DAG, cosubstrate with inositol in the PI synthase reaction, would indicate depletion of the inositol substrate. However, for CDP-DAG to accumulate experimentally, prior depletion of cellular inositol is required, as occurs in the preparation of brain slices (Godfrey 1989; Heacock *et al*. 1993). In the case of cultured cells, the ability of Li^+ to augment receptor-mediated increases in CDP-DAG accumulation is cell-specific. Thus, whereas Li^+ strongly potentiates CDP-DAG accumulation in both cerebellar granule cells and CHO-M_3 cells following carbachol addition, no such increase is observed for SH-SY5Y cells – a result attributed to the high concentration of inositol in this neuroblastoma cell line (Gray *et al*. 1994). A number of studies have reported a reversal of a biological action of Li^+ by inositol. For example, inositol is reported to reverse teratogenic actions of Li^+ in developing *Xenopus* embryos (Busa and Gimlich 1989). In this case, further investigations revealed the inhibition of glycogen synthase kinase by Li^+, and evidence was presented to indicate that this metabolic site, rather than IMPase, accounts for the deleterious effect that Li^+ has on development (Klein and Melton 1996).

Involvement of inositol in other neurological disorders

A role for inositol, either direct or indirect, has been proposed for a number of other disorders of the CNS. For example, intraperitoneal administration of inositol to *curly tail* mice, a genetic model of folate-unrelated neural tube defects, significantly reduced the incidence of spina bifida in fetuses (Greene and Copp 1997). The beneficial effects of inositol were mimicked by the administration of activators of protein kinase C and could be prevented by Li^+. It is suggested that inositol administration results in a stimulation of phosphoinositide turnover, which in turn up-regulates the β-retinoic acid receptor, a gene that is expressed at reduced levels and linked to neural tube defects. Administration of inositol can also ameliorate the symptoms associated with experimental diabetic peripheral neuropathy. The human condition is associated with decreased Na^+-K^+-ATPase activity and slowing of nerve conduction velocity, both of

which are reversed in fucose-fed rats upon dietary supplementation with inositol (Yorek *et al*. 1993). The inhibition of inositol uptake in peripheral nerves has been implicated in the etiology of the disease. The proposed pathway involves hyperglycemia-induced activation of aldose reductase, leading to sorbitol accumulation and subsequent inhibition of inositol uptake, which results in a lowered cellular inositol concentration (Gillon and Hawthorne 1983). Inositol uptake is also reduced in fibroblast cell lines obtained from patients with ataxia telangiectasia, an autosomal recessive disorder characterized, among other symptoms, by a progressive degeneration of the cerebellum (Yorek *et al*. 1999). Finally, although inositol concentrations are unaltered in Alzheimer's disease (Stokes and Hawthorne 1987), it has recently been shown that *myo*-inositol can form a stable complex with Aβ42, a soluble form of amyloid present in plaques. *Epi*- and *scyllo*-inositols (but not D-*chiro*-inositol) were also capable of forming complexes. The interaction of inositol with Aβ42 results in a complex that is non-toxic to both PC-12 cells and primary human neurons (McLaurin *et al*. 2000).

Use of inositol in the treatment of human neuropsychiatric disorders

Barkai *et al*. (1978) reported that significantly lower concentrations of inositol were present in the CSF of both unipolar and bipolar depressed patients than were in healthy control subjects. Some 15 years later, it was demonstrated that oral administration of high doses of inositol (6 g/day) could increase the concentration of inositol in the CSF by as much as 70% (Levine *et al*. 1993). This observation prompted investigations into the use of inositol in treatment of depression. Oral administration of 12 g/day of inositol reportedly resulted in a significant improvement in depression, as ranked by the Hamilton Depression Rating Scale, whereas the discontinuation of treatment with inositol led to a rapid relapse in a significant number of patients (Levine *et al*. 1995; Levine 1997). Inositol administration is also claimed to be effective in the treatment of both panic disorder (Benjamin *et al*. 1995) and obsessive compulsive disorder (Fux *et al*. 1996). The reported beneficial effects of inositol became evident only after 4–6 weeks of treatment, a time frame similar to that required for most therapeutic agents, including Li^+. Although there is clear NMR evidence that oral administration of inositol is reported to result in a 20% transient increase in inositol concentrations within the brain (Moore *et al*. 1999), the mechanism underlying a possible therapeutic improvement remains obscure. An unexplored possibility is that the increased production of glucuronic acid that accompanies a large intake of inositol increases the excretion of P 450-hydroxylated xenotoxic or autotoxic metabolites as their glucuronides.

Inositol administration is reported to be either ineffective or even contraindicated in conditions such as attention deficit hyperactivity disorder (Levine 1997), schizophrenia (Levine *et al*. 1993), Alzheimer's disease (Barak *et al*. 1996) and autism (Levine *et al*. 1997).

Inositol hexakisphosphate

Inositol hexakisphosphate (IP_6 or 'phytic acid') was originally identified some 80 years ago as the principal phosphate storage molecule in plants (1–3% by weight of whole grains and cereals). Subsequently, it has been determined that IP_6 is ubiquitously distributed in animal cells, and is one of the most abundant of the inositol phosphates. Separation and quantitation of highly phosphorylated inositol phosphates from biological samples were initially problematical because high salt concentrations are required for elution from ion-exchange HPLC columns, which complicates the detection and analysis. Many of these problems can be circumvented by the use of the metal dye detection method (Mayr 1990). High voltage electrophoresis separation at low pH (Seiffert and Agranoff 1965) separates IP_6 and IP_5 isomers from one another in a few minutes and has proven useful in biological and soil samples (Cosgrove 1980). ^{31}P NMR analysis has been employed in the identification of higher inositol phosphates (Scholz *et al*. 1990), and $^{31}P/^{1}H$ two-dimensional NMR has also been applied successfully to the analysis of phytate hydrolysates (Johnson and Barrientos 1995). Concentrations of IP_6 in distinct brain regions range from 10 to 15 μM (Yang *et al*. 2001). Intracellular concentrations of IP_6 are estimated to be in the range of 10–100 μM for animal cells, whereas in the slime mold *Dictyostelium*, it can be 10 times higher (Shears 2001). Intracellular availability of phytate is limited by the insolubility of its Ca^{2+}/Mg^{2+} salts and its avid electrostatic binding to protein. Apart from some indications that the concentrations of IP_6 can change during the cell cycle, there are few documented examples of situations in which the concentration of IP_6 changes significantly following cell stimulation. This had led to the suggestion that the metabolic turnover of the IP_6 is sluggish – certainly quite distinct from that observed for other inositol phosphates, such as $I(1,4,5)P_3$. However, this impression may be misleading as more than one metabolic pool of IP_6 may be present in cells. Moreover, as discussed below, there is strong evidence for the rapid phosphorylation of IP_6 into the diphosphoinositol polyphosphates 'IP_7' and 'IP_8', and these products in turn can be dephosphorylated back to IP_6 (see Fig. 3). Thus a portion of the intracellular IP_6 may represent an intermediate, rather than an end point, in the metabolism of inositol phosphates.

The way in which IP_6 is synthesized in mammalian cells is not yet established with certainty. Unlike the situation that exists in plants and *Dictyostelium*, in which IP_6 can be synthesized from inositol (via D-inositol 3-phosphate) following a series of positionally selective, sequential kinase reactions (see Irvine and Schell 2001), there is no evidence for the existence of this pathway in mammalian cells. In the latter, it appears more likely that the synthesis of IP_6 proceeds

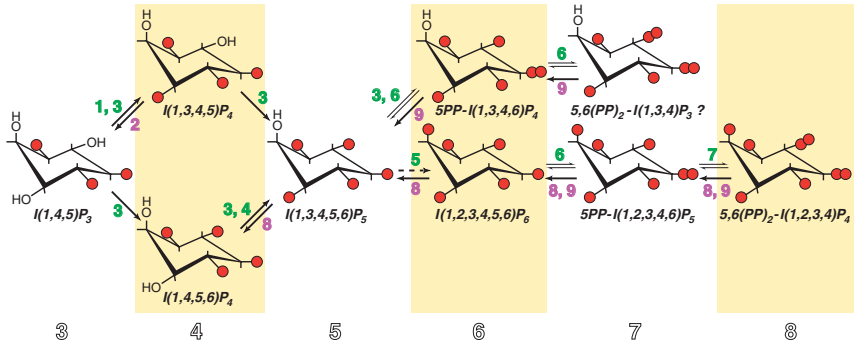

Fig. 3 Biosynthesis and degradation of higher inositol phosphates. Large numerals below vertical bars correspond to the number of phosphate molecules in each inositol phosphate depicted. Small colored numerals refer to enzymes as follows. Phosphotransfereases (kinases and synthases) are in green and phosphatases are in mauve. 1, inositol-(1,4,5)P_3-3-kinase; 2, inositol-(1,3,4,5)P_4-3-phosphatase [The I(1,3,4,5)P_4-3′-phosphatase activity in brain (Höer *et al.* 1990) is secondary to 5′-phosphatase action, yielding I(1,3,4)P_3, which can re-enter the synthetic pathway following conversion via kinases (not shown) to IP_5. Loss of the 3-phosphate activity is also catalyzed by PTEN (Maehama and Dixon 1998).]; 3, inositol polyphosphate multikinase; 4, inositol-(1,4,5,6)P_4-3-kinase; 5, inositol-(1,3,4,5,6)-2-kinase (the presence of this enzyme in brain has not yet been confirmed); 6, diphosphoinositol polyphosphate synthase ('IP_6 kinase'); 7, bisdiphosphoinositol polyphosphate synthase ('IP_7 kinase'); 8, multiple inositol polyphosphate phosphatase.

via the action of a newly discovered inositol polyphosphate multikinase (mIPMK; Saiardi *et al.* 2001a), which can sequentially phosphorylate I(1,4,5)P_3 in both the 3′ and 6′ positions to yield I(1,3,4,5)P_4 and then to yield I(1,3,4,5,6)P_5 (see Fig. 3). Alternatively, I(1,3,4,5)P_4 can be synthesized via the action of an I(1,4,5)P_3 3-kinase, a highly specific enzyme that is enriched in neural tissues and is likely to have evolved from mIPMK (Irvine and Schell 2001). Direct evidence for the presence of an IP_5 2-kinase (the enzyme that would complete the synthesis of IP_6) in mammalian cells is currently lacking, although such an enzyme has been documented in yeast (Ives *et al.* 2000).

A diverse array of putative physiological functions has been assigned to IP_6. For example, in non-neural tissues, IP_6 has been suggested to be anti-neoplastic (Jariwalla 1999), to prevent kidney stone formation (Grases *et al.* 1998) and to act as a cellular antioxidant (Graf *et al.* 1987). In plants, IP_6 was found to regulate stomatal pore closing (Lemtiri-Chlieh *et al.* 2000). Interest in the role that IP_6 may play in the CNS stems from the fact that a large number of high-affinity IP_6 binding sites can be detected in membrane preparations from distinct brain regions (Sasakawa *et al.* 1995). Autoradiographic studies have indicated that the distribution of these IP_6 binding sites is consistent with their localization to neuronal cell bodies. IP_6 has also been shown to bind with even higher affinity to a number of purified proteins, several of which are closely associated with membrane trafficking events. These include the vesicle adaptor proteins AP-2 (Voglmaier *et al.* 1992) and AP-180 (Ye *et al.* 1995), arrestins (Gaidarov *et al.* 1999) and synaptotagmin (Fukuda *et al.* 1994). As a consequence, roles for IP_6 in receptor regulation (Sasakawa *et al.* 1995), vesicle trafficking (Gaidarov *et al.* 1996) and neurotransmitter release (Llinas *et al.* 1994; Ohara-Imaizumi *et al.* 1997) have been proposed. In addition, IP_6 has been shown to bind with high affinity to myelin proteolipid protein and L-type Ca^{2+} channels. It has also been proposed to regulate PKC and protein phosphatases (see Shears 2001 and references therein). However, because IP_6 is so highly negatively charged, it has a propensity to bind non-specifically, and thus the assignment of physiological functions to IP_6 must be made with caution. In this regard, Shears (2001) has proposed a series of criteria that would distinguish IP_6-specific from IP_6-non-specific actions, including the ability of IP_6 to elicit the response in the presence of physiologically relevant concentrations of Ca^{2+} and/or Mg^{2+}, the inability of Ca^{2+} chelators to mimic the effect of IP_6, demonstration that other inositol phosphates, especially the diphosphoinositol polyphosphates, are either inactive or exhibit a lower potency and a lesser ability of inositol hexakissulfate or other conformers of IP_6 (e.g. *neo-* and *scyllo*-inositol hexaphosphates) to mimic the action of IP_6. When these criteria are rigorously applied, the purported ability of IP_6 to regulate a number of physiological events becomes less certain. For example, several of the assays in which high-affinity IP_6 binding to proteins has been demonstrated, e.g. to myelin proteolipid protein (Yamaguchi *et al.* 1996), were conducted in Ca^{2+}-free media. Furthermore, in several instances, the diphosphoinositol polyphosphates appeared to bind more avidly than IP_6 to a proposed target protein, e.g. AP-180 (Ye *et al.* 1995). Calcium chelators have also, on occasion, been found to mimic the effect of IP_6, e.g. as in a proposed extracellular neuromodulatory role for IP_6 (Vallejo *et al.* 1987; Sun *et al.* 1992). Nonetheless, an IP_6-dependent protein kinase that phosphorylates pacsin/syndapin I, a protein involved in synaptic vesicle recycling, has recently

been identified that on the whole satisfies most of the stated criteria (Hilton *et al*. 2001).

Two putative roles of IP_6 linked to nuclear function have emerged recently. IP_6 is reported to activate a repair mechanism for radiation- and chemically induced double-stranded breaks in DNA via activation of a DNA-dependent protein kinase (Hanakahi *et al*. 2000). The latter, which is part of a multimeric DNA end-joining complex, possesses a putative binding domain for IP_6. Other inositol phosphates, as well as inositol hexakissulfate, are less effective, and the effect of IP_6 can be observed at physiological Mg^{2+} concentrations (Hanakahi *et al*. 2000). A second proposed nuclear role for IP_6, based upon mutation studies in yeast, is the regulation of nuclear mRNA transport. York *et al*. (1999) have identified four yeast genes involved in mRNA export, three of which encode for enzymes involved, either proximally or distally, in IP_6 synthesis. Deletion of IP_6 kinase (see below) does not impair mRNA export, thereby eliminating the involvement of the diphosphoinositol polyphosphates (Saiardi *et al*. 2000). Additional support (albeit correlative) for a link between IP_6 availability and mRNA export comes from studies with mammalian cells in which transfection with the SopB protein (a *Salmonella* virulence protein, which can act as an inositol phosphatase) results in a reduction of both parameters (Feng *et al*. 2001), although results obtained with SopB need to be interpreted with caution because of the multiple effects of SopB on cellular processes (see Shears 2001). These results, taken collectively, nonetheless raise the intriguing prospect that IP_6 plays a key role in nuclear function.

Diphosphoinositol polyphosphates

Much of our present knowledge regarding the metabolism of these inositol polyphosphates is derived from studies with ^{3}H- or ^{32}P-labeled substrates and cloned recombinant enzymes. Although there existed previous evidence from anion-exchange chromatography for the presence of inositol-containing compounds that were more polar than IP_6 (Europe-Finner *et al*. 1991), the first clear-cut identification of inositol phosphates containing energy-rich pyrophosphoryl residues was obtained in 1993 from two independent laboratories. Investigations with cell-free lysates obtained from *Dictyostelium* or AR4–2J pancreatoma cells that had been incubated with radiolabeled IP_6 (Menniti *et al*. 1993; Stephens *et al*. 1993) or, alternatively, intact hepatocytes incubated with [^{3}H]inositol (Glennon and Shears 1993), led to the identification of three diphosphoinositol polyphosphates: diphosphoinositol tetrakisphosphate (PP-IP_4), diphosphoinositol pentakisphosphate (PP-IP_5, also known as 'IP_7') and bisdiphosphoinositol tetrakisphosphate ([PP]$_2$-IP_4, also known as 'IP_8'; see Fig. 3). The concentrations of the diphosphoinositol polyphosphates are relatively low in mammalian cells (1 μM), but are up to 300 times higher in *Dictyostelium* (Stephens *et al*. 1993). Structural analyses utilizing metal-dye-detection HPLC procedures reveal that in mammalian cell lines the major isomers are likely to be 5-PP-IP_4, 5-PP-IP_5 and 5,6-(PP)$_2$-IP_4 (Albert *et al*. 1997).

The precursor for PP-IP_4 synthesis is IP(1,3,4,5,6)P_5. The latter can then be phosphorylated to PP-IP_4 by the action of mIPMK (Saiardi *et al*. 2001a). Alternatively, any one of the three identified IP_6-kinase isoforms derived from distinct genes and cloned from mammals (Voglmaier *et al*. 1996; Saiardi *et al*. 2000, 2001a) can also phosphorylate IP_5 to PP-IP_4 (and PP-IP_4 to (PP2)-IP_3 – see Fig. 3). However, IP_6 is the preferred substrate for the enzyme. The three IP_6 kinases (K_1, K_2 and K_3; MW = 49, 49 and 46 kDa, respectively) exhibit 45–65% similarities in amino acid sequences, and all are highly enriched in brain (Saiardi *et al*. 2001a). The K_m for IP_6 is approximately 1 μM, whereas the enzyme exhibits a much lower affinity for its cosubstrate, ATP (1–2 mM). When transiently transfected in a non-neural cell, human embryonic kidney cell 293 (HEK293), the K_2 and K_3 isoforms are preferentially localized to the nuclei and cytoplasm, respectively, whereas the K_1 isoform is found at both subcellular locations. A selective kinase that preferentially phosphorylates PP-IP_5 to (PP)$_2$-IP_4 ('IP_7' kinase) has also been purified from brain (Huang *et al*. 1998), but has not yet been cloned. This enzyme is enriched in the cytoplasm, and has a MW of 56 kDa and exhibits a 10-fold higher affinity for PP-IP_5 than for IP_6 (1 vs. 10 μM). Both IP_6 and PP-IP_5 kinases can act reversibly under physiological conditions and thereby act as ATP synthases. These observations have prompted the suggestion that diphosphoinositol polyphosphates may act as primary phosphate donors to acceptors other than ADP, such as proteins (Voglmaier *et al*. 1996; Huang *et al*. 1998), but none have as yet been identified.

Diphosphoinositol polyphosphates are very active metabolically. This becomes evident when their breakdown is prevented by the inclusion of fluoride to inhibit phosphatase action (Menniti *et al*. 1993). By use of this F^- 'metabolic trap' technique, it has been calculated that 20–50% of the cellular IP_6 reservoir cycles through the diphosphoinositol polyphosphates per hour. A similar flux occurs between IP_5 and PP-IP_4 (Menniti *et al*. 1993). The observation that diphosphoinositol polyphosphates turn over rapidly has stimulated interest in their potential physiological roles. Most emphasis to date has been placed on the possibility that membrane trafficking events are regulated by these pyrophosphorylated inositol phosphates. In yeast, deletion of the IP_6 kinase results in a disruption of vacuole biogenesis (Saiardi *et al*. 2000). In mammals, PP-IP_5 binds tightly (K_d = 20 nM) to AP-3 (a clathrin adaptor protein) and is a potent inhibitor of AP-3-dependent assembly of clathrin cages (Ye *et al*. 1995). Clathrin is a major protein in brain (~1% of total), and its assembly is a key event in endocytosis. PP-IP_5 also binds with high affinity to AP-2, another adaptor protein that promotes the assembly of clathrin cages (Shears *et al*. 1995). Because dephosphorylation of

PP-IP_5 to IP_6 should result in a less potent inhibition of clathrin assembly, this metabolic step could conceivably result in the activation of endocytosis (Safrany *et al*. 1999). Of possible relevance is the recent observation that PP-IP_5 (like IP_6) can stimulate a protein kinase that selectively phosphorylates pacsin/syndapin I (Hilton *et al*. 2001), a protein involved in synaptic vesicle recycling. In addition, PP-IP_5 binds with high affinity to coatomer, a cytosolic multimeric complex that also controls membrane trafficking events between the Golgi complex and endoplasmic reticulum (Fleischer *et al*. 1994; Ali *et al*. 1995).

The turnover of diphosphoinositol polyphosphates may also be regulated, at least in some instances, by agonist occupancy of cell surface receptors. For example, in a smooth muscle cell line, either the activation of β-adrenergic receptors, or the addition of a cell-permeant analog of cAMP, results in a 70% reduction of $(PP)_2$-IP_4 concentration (but not that of either PP-IP_4 or PP-IP_5; see Safrany and Shears 1998). Atypically, this is not mediated via protein kinase A activation. A sustained depletion of intracellular Ca^{2+} in hepatocytes following the addition of thapsigargin has also been shown to reduce the steady state concentrations of diphosphoinositol polyphosphates by 50%. However, it seems unlikely that receptor activation of phospholipase C regulates the turnover of the diphosphoinositol polyphosphates, as the addition of vasopressin to hepatocytes does not alter the steady state concentrations of either PP-IP_5 or $(PP)_2$-IP_4 (Glennon and Shears 1993).

There are three potential enzymatic routes for the dephosphorylation of the diphosphoinositol polyphosphates (Fig. 3). The first is the reversal of the IP_6- and PP-IP_5-kinase reactions, as outlined above. Secondly, the multiple inositol polyphosphate phosphatase can carry out the dephosphorylation (Craxton *et al*. 1997). This enzyme, first cloned from liver, is also enriched in brain. More recently, a group of enzymes known as the diphosphoinositol polyphosphate phosphatases (DIPP) have been identified. One of these is reported to be regulated by Li^+ (Hua *et al*. 2001). The first member of this family to be isolated was an 18-kDa, Mg^{2+}-dependent enzyme that exhibited high affinity for both PP-IP_5 and $(PP)_2$-IP_4, and was present in brain along with other tissues (Safrany *et al*. 1998). It was subsequently demonstrated that PP-IP_4 is also a substrate for DIPP (Caffrey *et al*. 2000). Two additional isoforms of DIPP (MW = 21 kDa) have been identified, DIPP2α and DIPP2β, the latter being distinguishable by the presence of an additional glutamine residue. This change results in an enzyme that is 65–80% less active against PP-IP_5, PP-IP_4 or $(PP)_2$-IP_4 than is the α-isoform (Caffrey *et al*. 2000). The purification and molecular cloning of DIPPs has revealed them to contain a Nudix (*nu*cleoside *di*phosphate linked to some other moiety, *X*) motif. Nudix, also known as a MutT motif, is a 23 amino acid residue domain found in enzymes that protect cell integrity against oxygen radicals. The DIPPs are unusual in that they are the first example of a MutT-type enzyme that can readily metabolize a class of substrates other than nucleoside diphosphates. However, the diphosphoinositol polyphosphates do not appear to be general substrates for other MutT-type enzymes (see Safrany *et al*. 1999).

Conclusions

Although the first indication of the importance of inositol-containing phospholipids in cell signaling events was obtained nearly 50 years ago (Hokin and Hokin 1953, 1955), discovery of new roles for both the phosphoinositides and the water-soluble inositol phosphates continue unabated. In this review, we have focused on the importance of *myo*-inositol, the basic building block, and its water-soluble phosphates, in CNS function. Not only does inositol serve as precursor for the synthesis of inositol phospholipids, but it also plays an essential role in the regulation of cell volume within the CNS. The versatility of function of inositol and its phosphates is further illustrated by the case of IP_6 and disphosphoinositol polyphosphates, highly phosphorylated forms of inositol that are readily synthesized in the brain and are now linked to both the regulation of nuclear function and to endo/exocytosis.

Inositol plays an essential role in eukaryotes, and the presence of inositol-containing phospholipids has been documented in some eubacteria and Archebacteria. Thus, it is likely that inositol was one of the earliest organic molecules to evolve, and that during the course of evolution it has been adapted to serve in many functions. Given the variety of possible inositol phosphates, it is likely that additional roles for inositol and its phosphates in the nervous system are yet to be uncovered.

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