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REVIEW ARTICLE

NEUROPATHY TARGET ESTERASE; ITS ROLE IN PHOSPHATIDYLCHOLINE REGULATION AND IMPLICATIONS FOR PATHO-PHYSIOLOGY

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ABSTRACT
Eukaryotic cells control the levels of their major membrane phospholipid, phosphatidylcholine (PC), by balancing synthesis with degradation via deacylation to glycerophosphocholine(GPC). Neuropathy target esterase (NTE) was originally identified as the target site for those organophosphates that caused a paralyzing delayed neuropathy with degeneration of long nerve axons. In adult animals NTE is present in the nervous system and a variety of non neural tissues. NTE mediated production of Glycerophosphocholine is also an abundant renal medullary organic osmolyte that protects renal medullary cells from the high interstitial concentrations of NaCl and urea to which they are normally exposed. This review article discusses the importance of NTE in metabolism and pathophysiology.

Key words: Phosphatidylcholine, Neuropathy, Glycerophosphocholine, osmotic, renal

INTRODUCTION:

Neuropathy target esterase enzyme was originally identified as a target of organophosphate compound that is involved in their induction of peripheral neuropathy in humans (1). It is an endoplasmic reticulum-associated protein, mostly exposed on the cytoplasmic face of the membranes (2). NTE is highly conserved in eukaryotes but has homologues in bacteria as well (3). The mechanism of organophosphate neurotoxicity is that certain organophosphates act as pseudosubstrates for serine hydrolases, and the covalent organophosphorylated intermediate formed by this reaction is hydrolyzed extremely slowly, there by inhibiting the enzyme. Loss of activity disrupts PC homeostasis, resulting in neuronal and glial death (4). The normal role of NTE in nerves is not yet completely understood.

NTE is also expressed outside of the nervous system in numerous organs, including the kidney, but its role in those tissues also is not yet fully characterized. GPC is one of the organic osmolytes normally accumulated by renal cells in response to high NaCl, both *in vivo* and in cell culture. Osmotic regulation of GPC by high NaCl has been studied in isolated renal inner medullary collecting duct cells, in cell cultures, and *in vivo (5).* It involves changes in both GPC synthesis and degradation. On the one hand, high NaCl inhibits the activity of GPC–choline phosphodiesterase, an enzyme that degrades GPC to choline and α-glycerophosphate; the slower degradation of GPC increases its concentration. On the other hand, high NaCl also raises phospholipase activity, enhances the rate of synthesis of GPC from PC in renal cells (6). This review article discusses the importance of NTE in metabolism and patho-physiological conditions.

Structural biology of NTE and role in the physiology: In the adult animals, NTE is primarily localized in the nervous system but is also found in a variety of non-neural tissues including intestine, placenta, and lymphocytes (1,7). Though NTE activity is not required for neurite initiation or elongation *per se*, it is essential for the optimal rate of neurite initiation (8). In rodents, constitutive deletion of the NTE gene is lethal during midgestation (9) and brain-specific deletion of NTE results in neurodegeneration (10), indicating that NTE is essential not only for embryonic development but also in adult neural function. It is also important for cell signaling and lipid trafficking.

The human NTE is a 1327 amino acid polypeptide that is anchored to the cytoplasmic face of endoplasmic reticulum (ER) by an amino terminal transmembrane segment (Tm) in both non-neural cells and neurons (10). NTE has two functional domains, an amino-terminal putative regulatory domain of about 700 residues and a carboxyl-terminal catalytic/esterase domain containing the active site serine residue (Ser966) (2). NTE is anchored in the ER via the Tm, while the regulatory- and

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catalytic domains relate with the cytoplasmic face of the ER (2).

NTE and osmotic regulation of GPC :

Levels of PC, the major membrane phospholipid of eukaryotic cells, are tightly regulated by coordination of its synthesis and degradation. In both the yeast and mammalian cells (11), PC synthesized by the CDP-choline pathway is deacylated by as yet unidentified phospholipases to form glycerophosphocholine. In principle, this deacylation at both sn-2 and sn-1 positions of PC could be mediated either by a single enzyme with phospholipase B activity or by sequential action of a phospholipase A_2 and a lysophospholipase. It has been reported that when mixed micelles of PC with detergent were incubated with the recombinant catalytic domain of NTE, fatty acid was liberated very slowly from the sn-2 position followed by fast deacylation of the resulting lysophospholipid (12). Several studies suggest that the transcription factor TonEBP stimulates NTE to produce GPC an osmoprotective molecule in the kidney. Accumulation of GPC within renal medullary cells helps protect them from the high interstitial concentrations of NaCl and urea to which they normally are exposed during operation of the renal concentrating mechanism. Its presence was discovered more than 50 years ago, and it was found to increase during antidiuresis in response to elevation of renal medullary interstitial NaCl and urea concentration (13). However, in the recent years the enzymes identified that regulate its abundance in response to changing levels of NaCl and urea. Knockdown of TonEBP/OREBP by a specific siRNA inhibits the high NaCl-induced increase of NTE mRNA, indicating that high NaCl elevates NTE mRNA through TONEBP/OREBP-mediated increase in its transcription, similar to other osmoprotective genes. Transcriptional activity of TonEBP/OREBP requires binding to a specific DNA element, ORE/TonE, whose consensus sequence is NGGAAAWDHMC(N). Between bp −900 and −1 in the 5'-flanking region of NTE, there are four DNA elements that fit this consensus. Thus high NaCl increases transcription of NTE, mediated by TonEBP/OREBP, and the resultant increase of NTE abundance and activity contributes to increased production and accumulation of GPC in mammalian renal cells in tissue culture and *in vivo* (14).

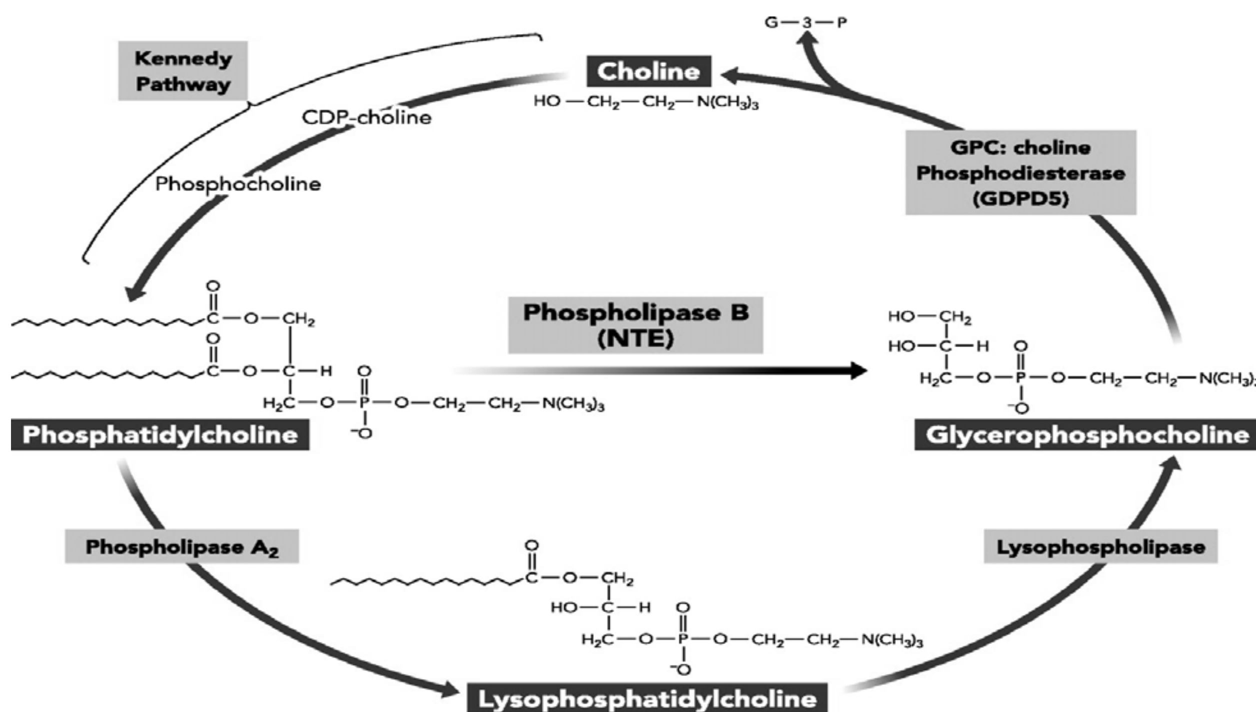

Figure 1: Courtesy: Gallazini and Burg, **Physiology. 2009; 24, 4,245-249**

Importance of GPC: Unlike phosphatidylcholine, GPC is soluble in water, and it crosses the blood brain barrier, and it requires lesser energy on the pathway to acetylcholine synthesis or the addition of specific brain fatty acids such as DHA. In humans, GPC taken by mouth is well absorbed and increases plasma levels of choline for up to nine and half hours (15). Research conducted with animals using radio labeled GPC suggests that GPC becomes incorporated into many other regulatory and structural molecules with various functions:

1) It offers a methyl group as a source for gene-level and other metabolic control.

2) GPC is a precursor to acetylcholine, which is used in the brain as a neurotransmitter and the rest of the body

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as a messenger/regulator (muscle contraction, organ functions, skin tone, blood vessel volume, platelet aggregation).

3) GPC can be incorporated into choline phospholipids such as phosphatidylcholine and sphingomyelin in every cell membrane and myelin sheath.

Phosphatidylcholine regulation and influence of NTE/ Phosphlipase A_2:

PC is the most abundant phospholipid of eukaryotic cells. PC homeostasis is regulated by a balance between the opposing actions of hydrolysis and synthesis (16). PC is mainly synthesized from the cytidine-5 -diphosphocholine (CDP)-choline pathway via a phosphorylcholine intermediate for condensation with diacylglycerol to produce PC (17-19). PC can be hydrolyzed by the phospholipase (s) A (PLA), PLB, and phospholipase D (PLD) (20). NTE is a specific PLB only hydrolyzing PC derived from the CDP choline pathway, whereas PC molecules formed through the methylation pathway are not degraded (21-22). Recent studies suggest that certain proteins and lipids may contribute during cellular perturbations like the imbalance in PC/PE homeostasis and play an important role in cellular responses (23).

The interplay between phospholipase (s) iPLA2β and iPLA2γ in the deacylation of neuronal mitochondrial phospholipids remains to be determined. NTE operates primarily at the cytoplasmic face of neuronal ER, but can also be detected in axons. Its substrate may be nascent PC delivered from the Golgi by Nir2 or another lipid-binding protein. Appropriately-presented PC may be the major determinant of NTE activity, but PKA-mediated inhibition may also coordinate its activity with the PC synthetic pathway. The phospholipase-B activity of NTE degrades PC to glycerophosphocholine plus two free fatty acids. By this activity, NTE reduces PC levels to facilitate the constitutive secretory pathway and so optimize export of materials from the neuronal soma. The association between NTE activity and PC synthesis operates not only in the neuronal soma, but also in the distal axon. Local PC homeostasis is probably essential for efficient vesicular transport within the axon, but details of the process are very limited. An intriguing observation relating to axonal PC homeostasis is that in genetic NTE-deficiency the centrally-projecting long spinal axon of dorsal root ganglion neurons undergoes distal degeneration while the peripherally-projecting axon of the same neurons appears to be spared. It is possible that this is because local iPLA2β activity compensates for NTE-deficiency. Experiments conducted in a yeast model suggest that there are three metabolic outputs of NTE activity that are relevant toward phospholipid homeostasis as follows: first, NTE reduces PC content, at least locally at the ER; second, along with *GDE1*-encoded glycerophosphocholine glycerophosphodiesterase, it controls PC resynthesis; and third it generates free fatty acids. For cells growing without inositol, the PC content of the ER membrane is very high, especially when choline is supplied at 37 °C. Phospholipid precursors are consumed, and PC becomes more abundant leading to increased lateral packing that could alter functions of some ER components. It is tempting to speculate that the interaction of Opi1p with Scs2p and the ER membrane is sensitive to this parameter. Increasing lateral packing attenuates the interaction of Opi1p with its anchors reducing phospholipid gene transcription. Contrarily, low lateral packing reinforces the interaction between Opi1p and ER membranes, promoting phospholipid gene transcription. NTE contributes toward phospholipid homeostasis by directly affecting membrane packing at the ER.

NTE-LysoPLA Activity Assays:

LysoPLAs are enzymes that hydrolyze lysolecithin to *sn*-glycero-3-phosphocholine. The assay procedure is a modification of a method for analysis of lysolecithin in human plasma and serum (24). LysoPLA activity is monitored continuously in an enzyme-coupled microplate assay. The reaction proceeds to form choline, hydrogen peroxide, and a colored derivative from the sequential action of three added enzymes (*sn*-glycero-3-phosphocholine phosphodiesterase, choline oxidase, and peroxidase) and two chromogenic agents. The enzyme assayed is either a LysoPLA or a phospholipase functioning as a LysoPLA (25).

NTE-LysoPLA activity is proportional to the paraoxon resistant and mipafox-sensitive increase in absorbance at 570 nm. More specifically, unless indicated otherwise, all reactants are added in 100 mM Tris buffer (pH 8.0) containing 1 mM calcium chloride and 0.01% Triton X-100. Reagent A contains 3-(*N*-ethyl-3-methylanilino)-2-hydroxysulfonate (3 mM), peroxidase (10 units/ml), *sn*-glycero-3-phosphocholine phosphodiesterase (0.0001 units/ml), and choline oxidase (10 units/ml). Reagent B contains 5 mM 4-aminoantipyrine. Reagents A (120 µl) and B (80 µl) are added to individual chambers containing Tris buffer as above (45 µl) in a 96-well polystyrene plate. Brain homogenate (15 µl) is added, followed by paraoxon in acetone (5 µl) and mipafox in 50 mM Tris–citrate (5 µl) to give 40 and 50 µM final concentrations, respectively. After a 20-min incubation, lysolecithin (250 µM final concentration) is introduced in Tris buffer (50 µl). The NTE-LysoPLA activity is measured by kinetic assay of absorbance at 570 nm for 10 min at 25°C by using a microplate reader (Molecular Devices). The activity is linear with regard to protein level and time.

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CONCLUSION:

The osmoregulatory role of the NTE is well defined with the GPC contributing as a major osmoprotective molecule during hyperosmotic stress in the kidney. Most of the work uptil now on NTE has focused on its catalytic function as an esterase and more recently as a phospholipase and lysophospholipase. In the future, the studies should expand the scope of work to encompass the cyclic nucleotide binding domains and their possible role in regulation of NTE or its interacting partners, which have yet to be defined. Future research work will also need to establish how mutated or chemically modified NTE leads to axonal degeneration and how these conditions are similar to that produced by conditional knockout of the gene in the central nervous system.

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