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Choline : The Essential Nutrient for Brain Development†**Jan Krzysztof BLUSZTAJN Ph. D.**

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Introduction

All cells use choline as the precursor of particular membrane phospholipids, including phosphatidylcholine (PC), choline plasmalogens and sphingomyelin. Cholinergic neurons utilize choline for an additional purpose—synthesis of their neurotransmitter, acetylcholine (ACh). Diet supplies most of the choline used by brain cells for both of those functions. The relationships between dietary choline and brain function have been the subject of many investigations^(1,2), and over the last fifteen years a large amount of data obtained by workers in various laboratories has shown that choline supplementation increases ACh levels and release and modulates postsynaptic events in a manner consistent with enhanced cholinergic transmission⁽³⁾ [for a review]. In addition since cholinergic neurotransmission is important in the physiology of memory, choline has been used as a possible memory improvement drug⁽⁴⁾. In this minireview, I. shall describe some of the recent findings indicating that, during the perinatal period, the brain is especially sensitive to the supply of choline and that numerous physiological mechanisms exist to insure adequate provision of choline early in life.

Choline supply during perinatal period.

During early development of mammals, changes in the availability of choline to tissues occur because of variations in dietary intake of

choline and because of changes in its metabolism. In the fetus and the neonate, large amounts of choline are needed for the formation of membrane phospholipids in rapidly dividing and growing cells and there are physiological mechanisms which ensure that these demands are met. The fetus is supplied with choline by the placenta which contains a specific transporter⁽⁵⁾ and synthesizes choline *de novo*⁽⁶⁾. Concentrations of circulating choline are 6–7-fold higher in the fetus and neonate than they are in the adult^(7,8). Consistent with high plasma choline levels, brain choline concentrations are more than two times higher in the neonate than in the adult^(7,8), due also to the greater transport of the amine from plasma to the central nervous system⁽⁹⁾. Choline consumption by the neonate is high because milk is a rich source for choline^(10–13). Interestingly, choline levels in milk are highest at the beginning of lactation, reaching, in humans, 700 μM (i.e. 100 fold higher than the levels found in maternal plasma) and establishing a plateau of 200 μM by the tenth day⁽¹¹⁾. While adult rat liver oxidizes choline via the action of choline dehydrogenase, the liver of neonates contains little of this enzyme, whose expression is turned on between postnatal days (P) 20 and P 40. This causes a prolonged half-life of choline in the neonate⁽⁸⁾. The rate of *de novo* synthesis of choline within liver by phosphatidylethanolamine N-methyltransferase is also relatively slow in the neonate, and turns on during the first week of life⁽¹⁴⁾. In contrast, brain contains a highly active form of this enzyme which is present only during the first 5 days of postnatal life⁽¹⁵⁾. For this reason the *de novo* brain biosynthesis of choline is

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highest in the baby.

Choline supplementation during perinatal period enhances visuospatial memory.

The high demand for choline in the developing brain and the physiological mechanisms which have evolved to supply the growing brain with this nutrient suggest that manipulations in the availability of choline during the perinatal period ought to result in changes in brain development. This hypothesis has been tested in a series of studies by Meck and Williams who found that there are two sensitive periods in development during which treatments with modest amounts of supplemental choline (increasing daily intake by 60~250%) result in long-lasting facilitation of visuospatial memory. In order to supplement growing fetuses with choline, these authors treated pregnant rats with this nutrient provided in the drinking water. Newborn rats were also treated with daily injections of choline. The first sensitive period occurs during embryonic days (E) 12~E 17, and another during P 16~P 30⁽¹⁶⁻¹⁸⁾. Choline supplementation during these periods elicits an improvement in choice performance in a 12-arm radial maze (a visuospatial task) at all stages of training. These effects of perinatal choline treatment on memory appear to be permanent, as both working and reference memory performance continue to show facilitation relative to controls even in aged animals at 26 months of age⁽¹⁹⁾.

Mechanism of action of choline.

The two sensitive periods correlate with the birth and differentiation of cholinergic neurons (prenatal) and with synaptogenesis (postnatal). The neurogenesis of cholinergic cells of the basal forebrain occurs between E 12 and E 17 in the rat, following a caudal to rostral gradient such that the peak of neurogenesis of the nucleus basalis occurs on E 13, of the vertical limb of the diagonal band on day E 15, and of the septum on E 15~16^(20,21) (and references therein). These groups of cells are believed to be involved in processes of memory⁽²²⁻²⁴⁾,

and they are especially vulnerable in Alzheimer's disease⁽²⁵⁻²⁷⁾. Thus, supplemental choline might enhance ACh synthesis in the newly born cholinergic neurons, or it might provide a precursor for PC in these cells leading, perhaps, to increased cell size or cell number. It is worth noting that in cultured cells PC content depends on extracellular choline concentrations, and PC content of such cells affects their viability and growth⁽²⁸⁾. Although the prenatal brain has only a minute percentage of the choline acetyltransferase and high-affinity-choline-uptake activities present in the adult⁽²⁹⁾, its ACh concentrations are 15~30%⁽²⁹⁾ of those found in the adult. It is possible that this ACh acts locally as a trophic factor. Indeed, ACh has been shown to have neurotrophic effects, inhibiting neurite outgrowth in retinal ganglion cells^(30,31) and dendritic growth in hippocampal neurons⁽³²⁾, influencing cortical cytoarchitecture during development⁽³³⁾, and stimulating mitosis *in vitro* of cells derived from E 16~P 7 brain but not in cells derived from the cerebrum at other ages⁽³⁴⁾. Increased ACh levels due to prenatal supplementation with choline may enhance neurotrophic responses. Since immature cholinergic neurons may not express high-affinity choline uptake⁽²⁹⁾, their ACh synthesis may be more sensitive to elevations of extracellular choline levels than that of the adult cells in which the high-affinity choline uptake system's affinity for choline may be the rate-limiting factor in determining ACh synthesis⁽³⁾. In addition to ACh exerting trophic actions by acting on muscarinic receptors, another metabolite of choline, sphingosylphosphorylcholine (lysosphingomyelin), has recently been described as a potent mitogen^(35,36). It is possible that choline supplementation might cause elevated levels of sphingosylphosphorylcholine in the brain and thus affect neurogenesis and/or myelination.

The postnatal period of development of the brain cholinergic system in the rat is characterized by the establishment of projections and synapses. These processes occur at the fastest rate approxi-

mately during the second and third weeks after birth and are largely complete by P 30^(29,37-39). The expression of cholinergic muscarinic receptors occurs during the same period; however the coupling of phosphoinositide turnover to these receptors is enhanced during the first week of life relative to the adult⁽⁴⁰⁻⁴³⁾. At this age the brain grows rapidly⁽⁴⁴⁾ and PC is needed for membrane synthesis. Lipid-synthesizing activity during the second week of life in the rat brain is by far the fastest; specifically, choline incorporation into PC by the CDP-choline pathway⁽⁴⁵⁾, cholesterol synthesis⁽⁴⁶⁾ and sulfate incorporation into sulfatides⁽⁴⁷⁾, and elongation of fatty acids⁽⁴⁸⁾ are all highest during that period, as is myelin synthesis⁽⁴⁹⁾. Thus the second choline-sensitive period is somewhat delayed relative to the fastest period of cholinergic development and of lipid synthesis postnatally.

Though it is clear that a variety of extracellular signaling molecules that bind to plasma membrane receptors evoke a change in phospholipid turnover [in particular that of phosphatidylinositides⁽⁵⁰⁾, other phospholipids, including PC and sphingomyelin, are also affected (see refs. 36,51 for reviews)]. In cholinergic cells the effects of neuronal stimulation on phospholipid metabolism are not only important to the functions of their membranes but also, through the link between phospholipids and ACh via choline, to the regulation of the synthesis of their neurotransmitter⁽⁵²⁾. Receptor-evoked PC breakdown leads to formation of several classes of compounds which may act as intracellular or extracellular signals⁽⁵¹⁾. These include arachidonic acid, a precursor of prostaglandins and other eicosanoids; phosphatidic acid, which has been implicated as a possible second messenger molecule⁽⁵³⁾, and diacylglycerol, an activator of a regulatory enzyme, protein kinase C (PKC). Activation of PKC occurs also without diacylglycerol in the presence of unsaturated fatty acids⁽⁵⁴⁾. Breakdown of PC to unsaturated fatty acids catalyzed by phospholipase A₂ also produces

lysoPC. Recent data indicate that lysoPC may act synergistically with diacylglycerol and calcium to activate PKC⁽⁵⁵⁾ and that different PKC isozymes are differentially modulated by this lipid⁽⁵⁶⁾. PKC may be a component of biochemical events involved in generation of long term potentiation (LTP) in hippocampus⁽⁵⁷⁾, a process which has been postulated to underlie memory formation. In rats, iontophoretically applied unsaturated fatty acids enhance LTP^(58,59) and diets containing these fatty acids cause an apparent activation of PKC concomitant with spatial memory enhancement⁽⁶⁰⁾ [and references therein]. It is possible that fatty acid released from phospholipids are the endogenous mediators of PKC activation in LTP. Another choline-containing phospholipid, platelet-activating factor, has been postulated to act as the retrograde messenger of LTP⁽⁶¹⁾. Choline administration might cause increases in brain PC levels. This PC may serve as a storage pool of fatty acids or may act as the precursor of platelet-activating factor, both of which are released during LTP.

Conclusions.

From the discussion above it is apparent that metabolites of choline subserve many diverse functions in the brain. Administration of supplemental choline may thus affect not only the cholinergic function but also other properties of cells within the central nervous system.

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