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COMMENTARY

DIETARY CHOLINE ALTERATION

IMPLICATIONS FOR γ-AMINOBUTYRIC ACID AND OTHER NEUROTRANSMITTER RECEPTORS

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Over a decade ago, it was first reported that acute choline administration alters acetylcholine synthesis in the brain [1, 2]. This finding led to hypotheses concerning treatment of neuropsychiatric disorders with exogenous choline, and to experimental studies addressing neurochemical effects of choline supplementation. A considerable literature has developed regarding the effects of choline administration on acetylcholine synthesis and degradation, although overall conclusions of these studies remain controversial. A more modest literature concerns the effects of modulation of dietary choline on brain function, and in particular neurotransmitter receptors. This commentary will address the effects of dietary choline on neurotransmitter receptors, primarily cholinergic and γ-aminobutyric acid-ergic (GABAergic) receptors.

Neurochemical effects of dietary choline modulation

Neurochemical effects of dietary choline modulation have been reported in detail. A brief summary will be provided here, with more detailed consideration in recent reviews [3, 4]. To review briefly the regulation of acetylcholine (ACh) synthesis in brain, a substantial literature indicates that the rate of ACh synthesis matches the rate of ACh release in neurons under physiological conditions [5]. ACh synthesis depends, in turn, on availability of choline either from exogenous sources or in some circumstances from endogenous sources such as the cellular membrane. A decrease in ACh synthesis due to an intervention leading to decreased availability of choline is likely to be reflected in decreased neuronal ACh content and thus decreased ACh release. Whether the converse occurs in the presence of choline supplementation remains uncertain. Since there is evidence that the choline transport mechanism is saturated at brain extracellular fluid choline concentrations under resting conditions [6], supplementation may not alter choline uptake and subsequent ACh synthesis and release. However, under conditions of increased neuronal activity, it appears likely that increased choline uptake can occur to provide for increases in ACh synthesis and release [7], and therefore supplemental choline may have an effect under these circumstances.

Substantial evidence indicates that supplemental choline is incorporated into ACh in the brain (e.g. Ref. 8). Evidence is conflicting that supplemental choline can enhance ACh synthesis; numerous studies can be cited indicating increases or no change in steady-state ACh concentrations after choline supplementation (see Refs. 3 and 4). Similarly conflicting evidence concerns effects of choline supplementation on ACh release or turnover [9, 10]. However, a number of studies indicate that choline supplementation can enhance ACh synthesis under conditions of increased demand, such as atropine administration [11, 12]. This may occur by increased uptake and direct use of choline, or alternatively through incorporation of supplemental choline into membrane phospholipids or some other compound. In one study, effects of choline pretreatment lasted longer than the increase in brain choline concentrations, suggesting that choline may be incorporated into another compound and later released for use in ACh synthesis [13]. However, some evidence also indicates that supplemental choline does not form a bound pool of choline which could be available for ACh synthesis [14].

In contrast, choline deficiency appears to have a direct effect on the ability of the brain to synthesize ACh, apparently due to decreased release of choline from bound stores [10]. Since membrane phospholipids appear to constitute a reservoir of free choline, choline deficiency may be associated with membrane depletion [15]. In summary, evidence from neurons at rest indicates that decreases in choline availability may directly reduce ACh synthesis and release, but it is unclear whether choline supplementation affects these processes. Under conditions of increased neuronal activity, choline supplementation appears to augment ACh synthesis and release.

With regard to receptor effects, these data indicate that dietary choline supplementation may act either directly by enhancing neurotransmitter synthesis, or indirectly perhaps by an effect on membrane phospholipids. Dietary choline deficiency may act both by acetylcholine synthesis and by altering cellular membranes. It should also be emphasized that

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effects may be different in conditions of increased neurotransmitter demand.

Cholinergic receptors

Initial reports of alterations in brain acetylcholine concentrations after choline administration indicated the possibility of *in vivo* neurotransmitter modulation. A reasonable corollary to these findings was investigation of choline effects on acetylcholine receptors. Initial studies by Morley *et al.* [16] used rats treated for 21 days with either choline-deficient or choline-supplemented diets. Nicotinic cholinergic receptors were assayed using α-bungarotoxin binding. Binding was increased significantly in cerebrum and forebrain (40–50%) in choline-supplemented compared to choline-deficient animals. Subsequently, the increase in binding was reported to be most pronounced in young (6 weeks) compared to older (6 months) rats [17]. Additional experiments indicated that little change in nicotinic receptors was observed in choline-deficient rats, but a substantial increase occurred in rats fed a supplemented diet [18]. Further, the increase in receptor binding occurred rapidly (24 hr) and resolved within several days after discontinuation of choline supplementation.

One report has also addressed the effects of choline supplementation on muscarinic cholinergic receptors [19]. In this study, mice received very long-term (18 months) supplementation of choline or phosphatidylcholine. No effects of choline supplementation were observed, but phosphatidylcholine-supplemented mice had a decrease in the number of receptors in cortex and hippocampus associated with an increase in phosphatidylcholine concentrations.

The mechanism for effects of dietary choline on cholinergic receptor density remains uncertain. Even if choline supplementation augmented ACh synthesis and release, in several systems chronic increases in agonist concentrations downregulate receptors in contrast to the increase in binding noted above [20, 21]. It could be speculated that alterations in ACh release may affect receptor expression leading to increased receptor density. Alternatively, choline supplementation might alter membrane phospholipids, in turn modulating receptor binding characteristics. However, alterations in the membrane environment might be expected to change binding characteristics, such as apparent affinity, but might not affect receptor density.

GABAergic receptors

Behavioral data indicate that chronic choline supplementation in rodents leads to alterations likely to involve the GABAergic system. Specifically, choline supplementation renders animals less susceptible to the sedative actions of pentobarbital [22], which appears to exert many of its effects at the $GABA_A$ receptor. In addition, choline supplementation attenuates the convulsant response to pentylenetetrazol, a $GABA_A$ receptor-associated chloride channel antagonist [23]. Finally, clinical studies indicate that choline supplementation decreases the duration of partial complex seizures [24], which in turn may be associated with alterations in GABAergic function.

On the basis of these findings, we evaluated the effects of dietary choline supplementation and deficiency on $GABA_A$ receptor binding and function in mice [25]. In these experiments, mice were fed diets containing 0% (deficient), 0.2% (basal) or 2.0% (supplemented) choline chloride for 28 days. Under these conditions, a significant (58%) increase was observed in plasma choline concentrations in mice fed supplemented diets, and a small, nonsignificant (20%) decrease in plasma choline was observed in mice fed choline-deficient diets. Behavioral studies using two paradigms sensitive to benzodiazepine effects, rotarod ataxia and open-field activity, indicated that mice fed choline-supplemented diets had a decreased response to a typical benzodiazepine, clonazepam, compared to mice fed a basal diet. Mice receiving choline-deficient diets had a slightly, but not significantly, greater response to clonazepam compared to basally-fed animals.

Benzodiazepine receptor binding was assessed *in vivo* and *in vitro* in these studies. In mice receiving choline supplementation, specific binding *in vivo* was increased in cortex and cerebellum by 20–25%. In mice fed a choline-deficient diet, binding was decreased by 20–60% in all brain regions evaluated. Results from *in vitro* binding studies in cortex indicated that the number of binding sites was increased in choline-supplemented mice, with no change in apparent affinity. Further, no change in GABA-stimulated binding was observed in either treatment group compared to mice receiving a basal diet. Binding at the putative chloride channel site labeled by [^{35}S]*t*-butylbicyclophosphorothionate ([^{35}S]TBPS) was also unchanged by choline-deficient or supplemented diets.

Function of the $GABA_A$ receptor can be evaluated by measuring uptake of chloride into membrane preparations stimulated by GABA or its analogs. Muscimol-stimulated chloride uptake into cortical synaptoneurosomes was similar in mice receiving basal and choline-deficient diets, but was increased significantly over a wide range of muscimol concentrations in mice fed a choline-supplemented diet. Overall, these results indicate that modulation of dietary choline can affect the $GABA_A$ receptor, and the benzodiazepine binding site and receptor function specifically. Chronic supplementation with choline, which elevates circulating choline levels, increases the density of benzodiazepine receptors in several brain regions and the function of the $GABA_A$ receptor in cortex. In contrast, although chronic deficiency of choline does not alter circulating choline levels, benzodiazepine binding was decreased in several brain regions.

Mechanism for choline effects

As noted above, it is possible that choline supplementation alters cholinergic binding through enhanced acetylcholine synthesis and release, which may, in turn, affect its post-synaptic receptor. It is unlikely that acetylcholine would affect the $GABA_A$ receptor, although it is possible that interactions between the two receptor systems exist. Another

possible mechanism for the effects of choline modulation is induced alterations in membrane phospholipids, with concomitant changes in receptor orientation or function.

Membrane lipids affect the primary interaction between neurotransmitter and receptor [26], as well as participating in modulation of signal transduction [27]. With regard to the $GABA_A$ receptor, several studies indicate that enhanced phospholipid metabolism through the treatment of membranes with phospholipase A_2 and C increases benzodiazepine binding [28–30]. In addition, phospholipase A_2 treatment decreases binding of the putative chloride channel ligand TBPS [31] and decreases barbiturate enhancement of benzodiazepine binding [30]. Although coupling of the GABA and benzodiazepine sites is not affected by phospholipase A_2 [30], exogenous phospholipase A_2 decreases GABA-stimulated chloride uptake in cortical synaptoneurosomes [32]. Phospholipase C incubation in protein-depleted membranes leads to an increase in GABA binding [33]. Finally, binding and function in solubilized $GABA_A$ receptors are optimized by incorporation of a natural lipid extract in addition to detergents [34]. These results indicate that *in vitro* alteration of the lipid environment can affect several binding sites and overall function of the $GABA_A$ receptor.

An additional modulatory role for membrane-derived phospholipids is indicated by experiments using exogenous phosphatidylserine. Hammond and Martin reported enhancement of benzodiazepine binding by exogenous phosphatidylserine (PS) [35], and similar results were reported both *in vitro* and using liposome administration *in vivo* by de Stein *et al.* [36]. In the latter study, *in vivo* PS administration appeared to be relatively specific for central benzodiazepine receptors; "peripheral-type" benzodiazepine receptors, α_1-adrenoreceptors, and muscarinic cholinergic receptors were unaffected. Thus, it is possible that membrane alterations which release PS or other phospholipids might modulate $GABA_A$ receptor and other neurotransmitter receptor characteristics.

The extent to which alterations in dietary choline affect membrane structure is uncertain, although several studies indicate that both membrane structure and phospholipid release may be affected. In a morphometric study, Bertoni-Freddari *et al.* [37] found that chronic choline supplementation in mice (10 months) augmented numerical and surface density of synapses, and decreased synaptic length compared to age-matched controls. Choline-deficient diets had no effect. Schmidt and Wecker [13] reported that choline supplementation may enhance the hydrolytic capacity of enzymes mediating phospholipid hydrolysis, whereas choline deficiency may have the opposite effect. Alterations in phospholipid metabolism might, in turn, alter membrane structure or phospholipid release, in either case affecting neurotransmitter receptors.

Future concerns

Modulation of neurotransmitter receptor function by dietary alterations offers a potentially novel approach to neuropsychiatric illness. Although initial enthusiasm concerning choline supplementation has not been supported clinically, additional information concerning effects of dietary choline modulation may suggest specific treatment approaches or illnesses to address. Studies in GABA receptors, for example, make it clear that changes in dietary choline can affect neurotransmitter receptors beyond the cholinergic system. In addition, this information may shed light on the role of membrane phospholipids in maintaining neurotransmitter receptor integrity and function. Techniques are available to address several important questions directly: studies in solubilized receptors may indicate the importance of choline alterations in the lipid environment, and studies in cultured neurons may provide controlled conditions in which to assess effects of choline on both phospholipid metabolism and neurotransmitter function. Similar approaches may be fruitful in the assessment of other dietary effects on neurotransmitter systems.

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