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Choline Administration Elevates Brain Phosphorylcholine Concentrations

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Abstract: The phosphorylcholine concentration of rat brain rises and falls in response to parallel changes in the concentration of circulating choline. A single oral dose of choline chloride (20 mmol/kg) elevated whole-brain concentrations of both choline and phosphorylcholine 5 h after administration; a greater proportion of exogenously administered choline was retained by the brain in its phosphorylated form than as the free amine. Striatal phosphorylcholine concentrations were elevated within 2 h of choline administration and continued to be significantly greater than control values for up to 34 h after treatment. The response of striatal choline levels to exogenous choline was of shorter duration than that of phosphorylcholine and was correlated with a significant increase in striatal acetylcholine concentrations. The consumption of a choline-free diet for 7 days lowered both serum choline and striatal phosphorylcholine concentrations, but had no effect on striatal choline or acetylcholine. These results suggest that choline kinase is unsaturated by its substrate *in vivo* and may thus serve to modulate the response of brain choline concentrations to alterations in the supply of circulating choline. **Key Words:** Choline—Phosphorylcholine—Acetylcholine—Precursors—Diet. **Millington W. R. and Wurtman R. J.** Choline administration elevates brain phosphorylcholine concentrations. *J. Neurochem.* **38**, 1748–1752 (1982).

Choline kinase (EC 2.7.1.32; ATP:choline phosphotransferase) catalyzes choline phosphorylation, the first step in the cytidine pathway through which choline is incorporated into phosphatidylcholine (Kennedy and Weiss, 1956). *In vitro* estimates of the kinetic constants of the enzyme indicate that it is unsaturated with its substrate; its K_m for choline (2.6 m*M*–Spanner and Ansell, 1979) is greatly in excess of the choline concentration of whole brain homogenates (35 μM) (Haubrich et al., 1975). Radiolabeled choline injected either intravenously (Jope and Jenden, 1979) or intracerebrally (Ansell and Spanner, 1968) is rapidly phosphorylated and accumulates in the brain as phosphorylcholine (PCh); however, it labels phosphatidylcholine and other phospholipid intermediates much more slowly. This observation suggests that PCh utilization by choline phosphate cytidylyltransferase is the rate-limiting step of the cytidine pathway, and not PCh synthesis by choline kinase. This hypothesis is further supported by *in vitro* estimates of the activities of the cytidine pathway enzymes. Choline phosphate cytidylyltransferase has the lowest *in vitro* activity and, therefore, is thought to be rate-limiting *in vivo* (Ansell and Spanner, 1977). Thus the rate of phosphorylation by choline kinase may be influenced by the availability of its precursor, choline, administration of which may result in the accumulation of its product, PCh.

Choline also serves as a precursor for acetylcholine (ACh) synthesis by central cholinergic neurons. Previous work from this (Cohen and Wurtman, 1975) and other laboratories (Haubrich et al., 1975; Racagni et al., 1975) has demonstrated

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Abbreviations used: ACh, Acetylcholine; PCh, Phosphorylcholine; TCA, Trichloroacetic acid.

that ACh synthesis depends, in part, on the availability of its precursor. The administration of choline to laboratory animals elevates plasma and brain choline and brain ACh concentrations (Cohen and Wurtman, 1975; Haubrich et al., 1975) and results in postsynaptic changes suggestive of enhanced transmitter release (Ulus and Wurtman, 1976; Haubrich and Pflueger, 1979). However, the quantitatively major fate of the choline entering the brain from the circulation is conversion to PCh, not to ACh (Jope and Jenden, 1979). The response of brain choline, and thus of ACh, to exogenous choline administration may as a result be influenced by the rate at which the choline is phosphorylated by choline kinase. If, indeed, the activity of choline phosphate cytidylyltransferase limits the rate of phosphatidylcholine synthesis, then it seems reasonable to assume that changes in the rate at which brain accumulates phosphorylcholine should cause parallel changes in the concentration and levels of PCh. We therefore initiated experiments to test whether PCh concentrations in rat brain are influenced by altering the supply of its precursor, choline.

MATERIALS AND METHODS

Male Sprague-Dawley rats (160–200 g; Charles River Breeding Laboratories, Wilmington, MA) used for these experiments were housed under a 12 + 12-h light-dark cycle (Vita-Lite, Duro-Test Corp., North Bergen, NJ). They consumed standard laboratory chow (Purina Rat Chow, St. Louis) except in experiments in which choline-free diets were provided. These were prepared in the M.I.T. diet facilities and were of the following composition (g/kg): casein, 90; dextrose, 103; dextrin, 103; sucrose, 84; Mazola corn oil, 70; agar, 17; vitamin mix (ICN Pharmaceuticals, Cleveland), 11; Rogers-Harper mineral mix, 11 (Rogers and Harper, 1975); water, 500. Some of the synthetic diets lacked choline; they were made by using a choline-free vitamin mixture. Others, used as controls in studies on the effects of choline deprivation, were made using vitamin mixtures that contained proportions of choline equal to those in the standard chow diet (0.183%). The animals were killed by decapitation for determination of serum choline concentrations; blood was collected from the cervical wound in chilled test tubes, and serum was separated by centrifugation at 5000 × *g* for 15 min. The rats were killed by head-focused microwave irradiation (Medical Engineering Consultants, Peabody, MA; 2.45 GHz, 2.4 kW, 3.0 s) for measurement of brain choline, acetylcholine, and phosphorylcholine.

Choline and ACh were analyzed by radioenzymatic assay (Goldberg and McCaman, 1973). Brain samples were homogenized in 10 volumes of 15% 1 *M* formic acid/acetone and then centrifuged at 5000 × *g* and 4°C for 15 min. A 250-μl aliquot of each supernatant fluid was lyophilized by vacuum centrifugation (Savant Instruments, Hicksville, NY), and the residue was reconstituted in 100 μl of 10 m*M* sodium phosphate buffer (pH 6.6). Choline and ACh were extracted into 300 μl of 3-heptanone containing sodium tetraphenylboron (5 mg/ml) (Aldrich Chemical Co., Cedar Knolls, NJ), and 200 μl of the organic phase was back-extracted into 300 μl of 0.4 *M* HCl. Duplicate 100-μl aliquots were then lyophilized for choline and ACh analyses. Choline was extracted from 20-μl serum samples without prior protein precipitation.

PCh was measured by a modification of the radioenzymatic choline/ACh assay. Brain samples were homogenized in 10 volumes of 10% trichloroacetic acid (TCA) and centrifuged for 20 min at 5000 × *g* and 4°C. A 250-μl aliquot of each supernatant was extracted three times with 500 μl of diethyl ether to remove the TCA, dried by vacuum centrifugation, and reconstituted in 50 μl of water. Choline and PCh were separated by thin-layer chromatography on cellulose plates (E. Merck Co., Darmstadt, F.R.G.) developed in butanol/water/ethanol/acetic acid (80:20:20:2, by vol). Choline and PCh standards were co-chromatographed on each plate and identified with Draggendorf's reagent to visualize choline and $FeCl_3$/sulfasalicylic acid (2:7) in 25% ethanol to visualize PCh. Plate recoveries were determined by internal standardization. Segments of the TLC plates containing PCh were scraped, eluted with 0.4 *M* HCl, and dried by vacuum centrifugation. PCh was hydrolyzed by incubating the samples at 37°C for 10 min with 4.0 units of alkaline phosphatase (Type VII, bovine calf intestine; Sigma Chemical Co.) dissolved in 30 μl of 25 m*M* Tris buffer (pH 8.0) containing 1.0 m*M* $MgCl_2$. Free choline was isolated from the incubation mixture by extraction with tetraphenylboron/3-heptanone and was assayed by radioenzymatic assay (Goldberg and McCaman, 1973).

RESULTS

Choline chloride administration to rats [20 mmol/kg (10 ml/kg), p.o.] elevated whole-brain concentrations of both choline and PCh 5 h after treatment (Fig. 1). The incremental increase of brain PCh (112.4 nmol/g) was greater than that of free choline (26.3 nmol/g), suggesting that a greater portion of exogenous choline was retained as phosphorylated choline than as the free amine.

We further characterized the relationship between choline availability and brain PCh concentration by determining the time course of the effects of choline administration on serum and striatal choline levels, and on striatal PCh and ACh (Fig. 2). We found that endogenous PCh concentrations were higher in rat striatum (697.8 ± 43.9 nmol/g) (±SEM) than in whole brain (228.2 ± 27.1 nmol/g), an observation consistent with a previous report (Jope and Jenden, 1979). Striatal PCh concentrations were significantly elevated 2 h after a single oral dose of choline chloride (20 nmol/kg) and remained elevated up to 34 h after treatment. Striatal choline concentrations were elevated 4 h after choline chloride administration, although the relative increase in striatal choline (from 31.3 ± 3.1 to 52.8 ± 5.9 nmol/g) was small compared with the 10–20-fold increase in circulating choline concentration present during this initial time period. At 8 h after choline administration, however, striatal choline levels had risen to 111.0 ± 8.3 nmol/g. This abrupt increase in free choline occurred while PCh

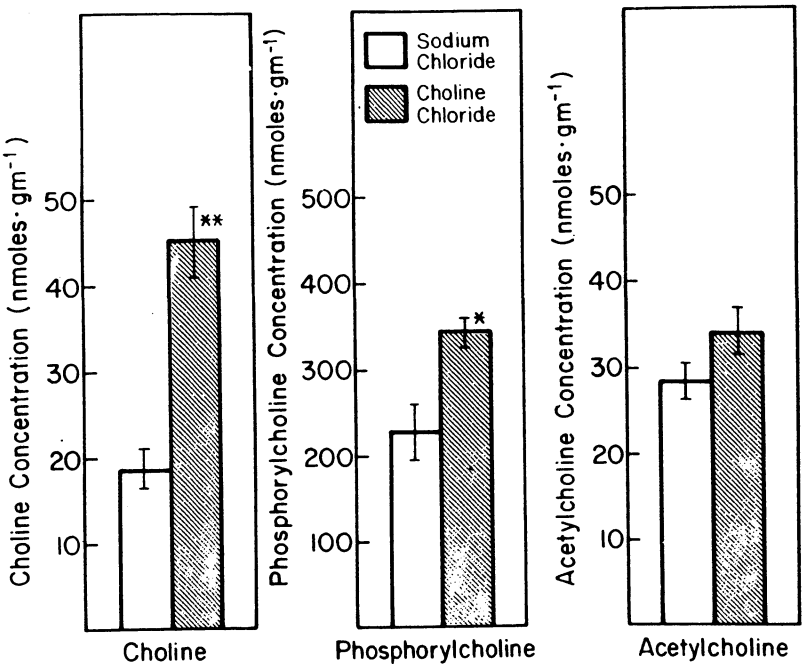

FIG. 1. The effect of oral choline administration on brain choline, phosphorylcholine, and acetylcholine concentrations. Groups of six rats received either choline chloride or sodium chloride (20 mmol/kg; 10 ml/kg) 5 h before death by microwave irradiation focused at the head. Brains were removed and divided by midline sagittal section. One half of each brain was analyzed for phosphorylcholine and the second half for both choline and acetylcholine by radioenzymatic assay. Data were analyzed by two-tailed *t*-test and are expressed as mean ± SEM. Double asterisk denotes $p < 0.01$, single asterisk $p < 0.05$—significantly different from control.

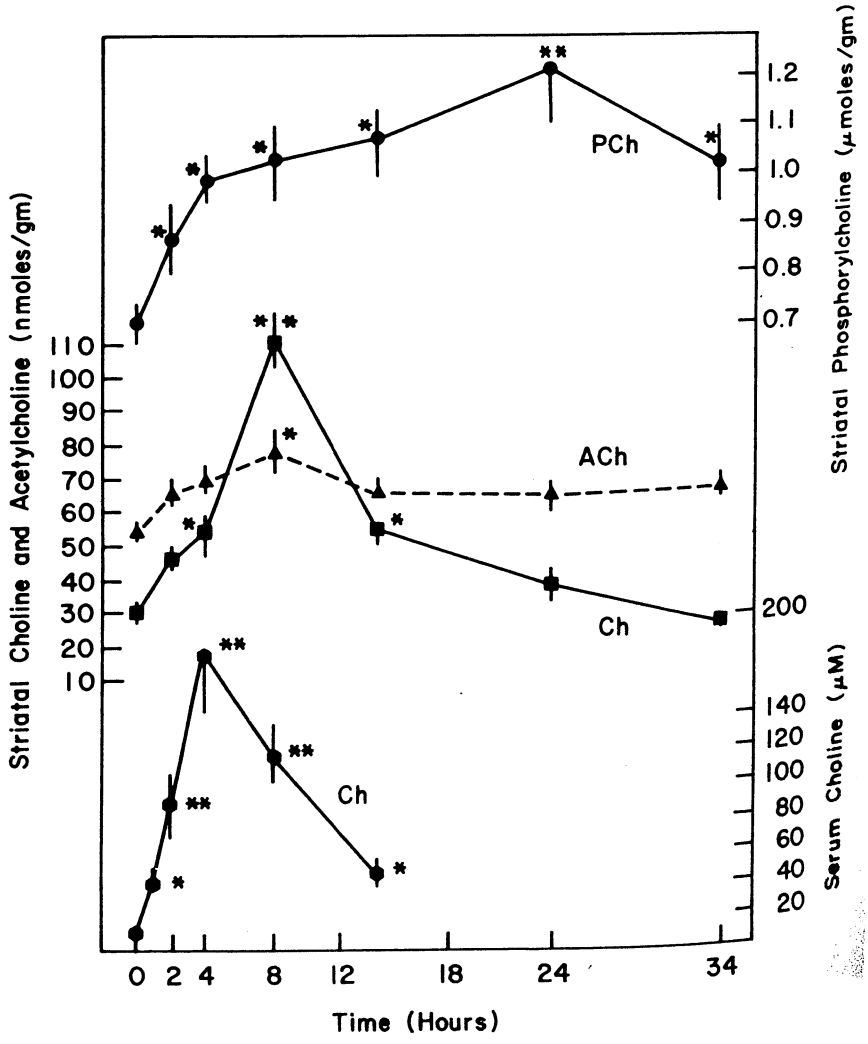

FIG. 2. Time course of the effect of oral choline chloride administration on serum choline and on striatal choline, phosphorylcholine, and acetylcholine concentrations. Groups of five to seven rats were given a single oral dose of either choline chloride or sodium chloride (20 mmol/kg; 10 ml/kg) and were killed at the indicated time intervals thereafter. For serum choline measurements, animals ($n = 5$) were killed by decapitation; the blood was collected in chilled test tubes, and sera separated by centrifugation. For striatal choline, phosphorylcholine (PCh) and acetylcholine (ACh) determinations, rats were killed by head-focused microwave irradiation; striata were removed and analyzed for either PCh or for choline and ACh. Values of choline, PCh, and ACh concentrations from control animals killed at 4, 14, and 24 h did not differ significantly from those killed at time zero. Data are expressed as mean ± SEM and were analyzed by analysis of variance followed by Neuman-Keul's a posteriori test. Double asterisk denotes $p < 0.01$, single asterisk $p < 0.05$—significantly different from control.

concentrations were maximally elevated. Striatal ACh levels were significantly elevated only at the 8-h time point.

Choline deprivation also affected brain PCh concentrations (Table 1). Striatal PCh concentrations among rats that consumed a choline-free diet for 7 days were significantly lower than those of rats that consumed an identical diet containing 0.183% choline chloride. Serum choline concentrations were also reduced among rats that consumed choline-free diets, but neither striatal choline nor ACh levels were significantly affected by dietary choline restriction.

DISCUSSION

The PCh concentration of rat brain rises and falls in response to parallel changes in the availability of circulating choline. The administration of a single oral dose of choline chloride produced a sustained elevation of striatal PCh, whereas a reduction of circulating choline concentrations as a consequence of dietary choline restriction lowered striatal PCh but not striatal choline or ACh concentrations. These studies were prompted by earlier reports that the PCh concentration of rat liver was elevated in rats killed 45 min after choline chloride injection (200 mg/kg, i.p.—Dawson, 1956) and reduced by the consumption of a choline-deficient diet for 2 days (Thompson et al., 1969). The results of the earlier studies taken together with data reported here suggest that choline kinase is unsaturated with its substrate *in vivo*, as predicted by *in vitro* estimates of its K_m (Spanner and Ansell, 1979).

Choline-induced elevations of brain PCh cannot readily be accounted for by assuming that PCh is transported intact from the circulation. In previous studies, we found that PCh is transported into brain by the blood-brain barrier choline transport carrier, but the affinity of the carrier for PCh (800 μM—Millington et al., 1978) is low relative to its affinity for choline (442 μM—Cornford et al., 1978). For significant amounts of circulating PCh to enter the brain, the plasma concentration of the compound must be sufficiently higher than that of choline to allow it to compete successfully for blood-brain barrier transport carrier sites; this does not appear to be the case. PCh does not circulate in rat serum in measurable concentrations (Dawson, 1955; unpublished data) and is rapidly hydrolyzed in blood (Riley, 1944). We found that serum PCh concentrations remained undetectable following a large oral dose of PCh, which raised serum choline concentrations 10-fold. (Our limit of detection would have been less than 1 nmol/ml.) Intravenously injected PCh (100 μmol/kg) was rapidly cleared from rat sera; its half-life was approximately 4 min. It is likely, therefore, that the incremental increase of brain PCh that follows choline administration results from PCh synthesis by the brain and not from PCh transport from the circulation.

The results of our study suggest that choline phosphorylation by brain choline kinase modulates the response of brain choline to pharmacologic treatment with choline chloride. This conclusion is supported by the observation that choline treatment elevates serum choline to a greater extent than striatal choline (Fig. 2), despite the fact that the blood-brain barrier choline transport carrier (K_t = 442 μM—Cornford et al., 1978) is not saturated by serum choline concentrations produced by choline administration. That striatal PCh accumulates under conditions of elevated circulating choline suggests that choline phosphorylation may attenuate the marked changes that would otherwise occur in the concentration of the free amine. This is further supported by the finding that PCh levels appear to reach a maximum 8 h after oral choline chloride administration, possibly as a result of end-product inhibition of choline kinase (Sung and Johnston, 1967). At that time, the rate of PCh accumulation had diminished, while choline accumulation increased to an extent sufficient to elevate striatal ACh levels significantly. The unsaturation of choline kinase may, thus, modulate the pharmacologic response of brain ACh concentrations to exogenous choline administration.

Our finding that serum, but not brain, choline is reduced by the consumption of a choline-free diet is congruent with previous reports (Cohen and Wurt-

TABLE 1. *The effect of dietary choline deprivation on serum choline and on striatal choline, phosphorylcholine, and acetylcholine concentrations*

Treatment	Serum choline (μM)	Striatal choline (nmol/g)	Striatal PCh (nmol/g)	Striatal ACh (nmol/g)
Control diet	13.0 ± 0.3	24.4 ± 2.8	689.0 ± 67.5	53.0 ± 2.5
Choline-free diet	8.4 ± 1.5[a]	21.0 ± 3.1	527.6 ± 39.2[a]	52.9 ± 2.9

Groups of 12 rats consumed diets containing either 0.183% or no choline for 7 days. Six animals from each group were decapitated for collection of sera. The remaining animals were killed by microwave irradiation focused at the head; striata were removed and analyzed by radioenzymatic assay either for phosphorylcholine (PCh) or for choline and acetylcholine (ACh). Data are expressed as mean ± SEM.

[a] $p < 0.05$—differs from control.

man, 1976; Haubrich et al., 1976; Wecker and Schmidt, 1979). We have further demonstrated that brain PCh levels were reduced in parallel with the fall in circulating choline, suggesting—as proposed by Haubrich et al. (1976)—that brain choline concentrations may be maintained within narrow limits at the expense of larger pools of PCh and perhaps other phospholipid precursors.

It remains to be established whether changes in brain PCh have any effect on phospholipid synthesis. The phosphatidylcholine content of neonatal rabbit lung (Kotas and Wiles, 1977) and of isolated rat hepatocytes (Sundler and Akesson, 1975) can be increased by enhanced availability of choline, but there is little basis for extrapolating these results to brain, because phospholipids of lung and liver are used for specialized purposes. The cytidine pathway may, nonetheless, be an obligatory fate for PCh. PCh is a poor substrate for the low-affinity choline transport carrier (Diamond and Kennedy, 1969); therefore, choline phosphorylation may serve to retain choline intracellularly. Alkaline phosphatase hydrolyzes brain PCh (Strickland et al., 1956), but not at physiologic pH, and, moreover, is thought to be an ectoenzyme; i.e., a membrane-bound enzyme on the outer surface of the plasma membrane (Spanner and Ansell, 1979). A large pool of PCh, which expands and contracts parallel to changes in the supply of circulating choline, may thus render phosphatidylcholine synthesis relatively insensitive to, rather than susceptible to, changes in precursor supply consequent to fluctuations in its dietary consumption.

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