Free and bound choline blood levels after phosphatidylcholine

In six normal subjects we investigated the effects of oral phosphatidylcholine (lecithin) on the concentrations of plasma choline, erythrocyte choline, and choline-containing lipids. Plasma choline levels rose 1 hr after treatment and remained elevated for 8 hr, with peaks at 3 and 4 hr after phosphatidylcholine. Erythrocyte choline levels also rose, although the rise was slightly delayed relative to plasma choline. There was no change in the plasma choline-containing lipid concentration. These results demonstrate that, in normal subjects, oral phosphatidylcholine induces prolonged rises in plasma and erythrocyte choline concentrations and is therefore useful when such effects are desired.

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Clinical and laboratory research in the field of central nervous system cholinergic pharmacology has been seriously hampered by the lack of a long-lasting cholinomimetic agent that is active in the brain. Based on the contention that increased levels of choline will increase the concentration of choline in the brain since the choline transport system across the blood brain barrier (K_T approximately 440 μM) is not saturated by the normal plasma choline concentration of 10 to 20 μM,[6] Haubrich et al.[10] and Wurtman et al.[5, 20] suggested the use of large doses of choline to promote acetylcholine (ACh) synthesis. Systemic administration of choline may lead to a rise in choline levels in the brain,[5, 10] but a resultant elevation in levels and utilization of ACh is controversial (for review, see Jenden[14]).

Recently choline has been partially superseded by the use of phosphatidylcholine* (PCh), which does not give rise to the strong "fishy" odor obtained after choline treatment.[4, 11] PCh has been reported to be beneficial in patients with tardive dyskinesia[8, 22] and is undergoing clinical evaluation in a number of other diseases.[2–4, 7, 18, 19] Since the clinical use of the

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*Although the term lecithin has recently been used in reports of investigations of phosphatidylcholine therapy, lecithin has long been used to indicate an impure mixture of phosphatides. We therefore use phosphatidyl to more accurately identify the compound under investigation.

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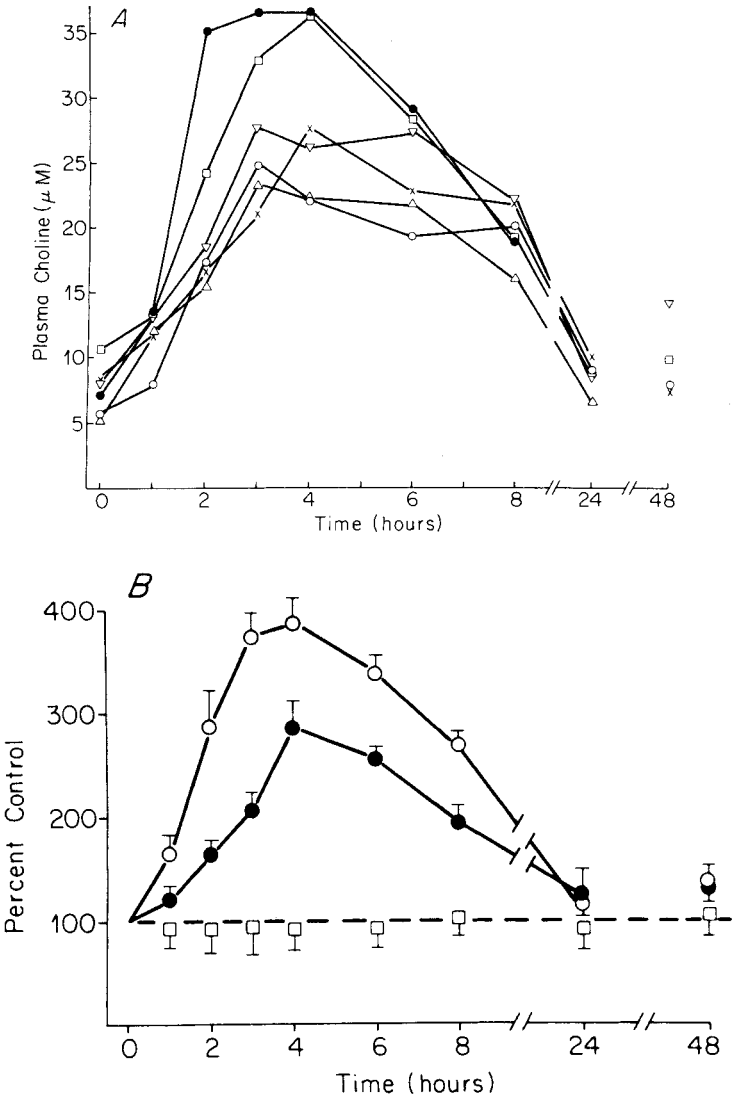

Fig. 1. *A*, Plasma choline concentration. *B*, Average percent changes in plasma choline (○), plasma lipid bound choline (□), and erythrocyte choline (●) obtained from six normal subjects after oral PCh (15 gm/70 kg body weight). The initial plasma choline, erythrocyte choline, and plasma lipid bound choline concentrations were 7.4 ± 2.0, 15.3 ± 4.9, and 996 ± 234 ($\bar{x}$ ± SD).

drug is expanding rapidly, we studied the PCh kinetics in six normal subjects. Wurtman et al.[21] and Hirsch et al.[12] reported that human consumption of a meal supplemented with 100 gm of impure PCh granules (equivalent to 2.3 gm choline) increased serum choline concentration. This effect was reported to last for at least 12 hr, at which time serum choline was at its highest level.[12, 21] More recently Zeisel et al.[23] reported that 25 gm of 80% pure PCh quadrupled plasma choline concentration with a peak level at approximately 5 hr after treatment. We report here the effects of PCh consumption by normal subjects on plasma choline, erythrocyte choline, and choline-containing lipids in the plasma. Since this pool may also be a precursor of brain choline and ACh,[1, 15] choline-containing lipids were measured to determine whether consumption of a large amount of PCh altered the release of lipid bound choline into the blood. Eryth-

rocyte choline was measured as an index of changes of the choline concentration in an intracellular pool, and also because it has been shown to rise after lithium[16] and in some patients with affective disorders.[9, 16]

Materials and methods

Six subjects were studied. Each took 15 gm/70 kg body weight (total dose = 11.1 to 16.7 gm PCh) Phospholipon-100 (approximately 94% PCh; American Lecithin) at 8:00 to 9:00 A.M. after an overnight fast. The PCh was suspended in 0.45% NaCl and consumption was followed by a small amount of milk. After the 2-hr blood sample the subjects ate a breakfast of orange juice, coffee or tea, and two slices of toast with jam and a pat of butter. After the 4-hr blood sample was drawn the subjects ate a lunch with low PCh content.

At the indicated times blood was drawn into a heparinized syringe and centrifuged at 4° at 3000 *g* for 5 min. Choline in the plasma and erythrocytes was extracted in 15% 1N formic acid in acetone (v/v) that contained precisely known quantities of [$^2H_9$]-choline as internal standard. Choline-containing lipids in the plasma were extracted in 20 vol $CHCl_3$:MeOH (2:1) containing [$^2H_9$]-PCh as internal standard. [$^2H_9$]-PCh was labeled in the choline moiety by predeuteration of the methyl groups. Free choline was hydrolyzed from the lipids by incubation in 1N KOH for 1 hr at 95°. Choline was isolated by ion-pair extraction with dipicrylamine into dichloromethane and measured by gas chromatography–mass spectrometry as previously described.[14] Choline was also measured by gas chromatography using a nitrogen detector with homocholine (3-hydroxypropyltrimethylammonium iodide) as the internal standard after extraction as described by Kosh et al.[17] The results of the two assays were almost identical with correlation coefficients of 0.98 for choline in both plasma and erythrocytes.

Results

Oral doses of 15 gm PCh/70 kg body weight increased plasma choline concentrations to levels three to four times those before treatment (Fig. 1, *A*). Increased concentrations were apparent 1 hr after treatment in all subjects.

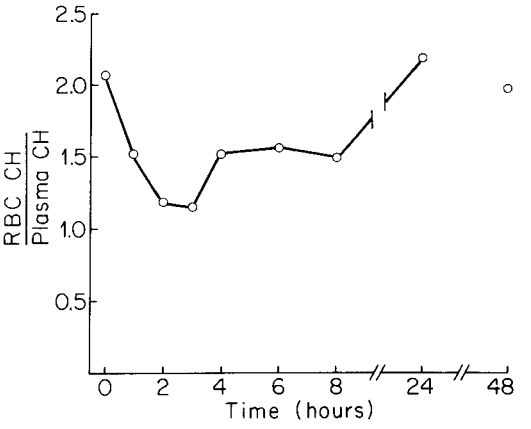

Fig. 2. Average ratios of erythrocyte choline to plasma choline in six subjects after oral administration of PCh (15 gm/70 kg body weight).

Plasma choline levels peaked between 3 and 4 hr after PCh. The duration of the peak plasma choline level varied among the subjects, e.g., in two subjects there was a well-defined peak while in two others there was a plateau between 3 and 8 hr after treatment. Plasma choline concentrations were near normal, although still slightly elevated, 24 hr after treatment.

Erythrocyte choline concentrations rose in approximate parallel to the rise in plasma choline (Fig. 1, *B*), but the increase of choline in erythrocytes was delayed relative to the plasma choline and reached maximum concentrations between 4 and 6 hr after PCh. The results from one subject were omitted from the calculations because his initial erythrocyte choline concentration was 170 μM, approximately 10 times that of the other subjects. The high erythrocyte choline level was confirmed in a repeat determination 1 mo later. The cause of this abnormally high level is under investigation.

The ratio of the choline concentration in erythrocytes to that in plasma is shown in Fig. 2. This indicates that, although the intracellular erythrocyte choline concentration rose, it did not do so to the same extent as plasma choline.

Lipid bound choline in the plasma did not change after PCh (Fig. 1, *B*).

Discussion

These results demonstrate that, in man, PCh raises plasma choline concentration for a longer

period than an equimolar dose of choline. The only side effect noted was mild nausea in two subjects approximately 6 hr after treatment. The extended effect of, and minimal side effects attributed to, PCh suggest that it is clinically preferable to choline as a means of elevating plasma choline levels.

The only cell easily accessible for measuring intracellular choline concentration in man is the erythrocyte. PCh induced prolonged elevation of erythrocyte choline concentration to much the same extent as plasma choline. This is presumably a direct result of the increased plasma choline concentration since the erythrocyte has a transport system with a relatively low affinity for choline (40 μM) that is not saturated by the normal plasma choline concentration of approximately 10 μM. These results in erythrocytes indicate that at least one intracellular pool of choline increases after PCh. As shown in Fig. 2, however, the intracellular erythrocyte choline concentration did not rise to the same degree as plasma choline. The ratio of erythrocyte choline to the plasma choline level may be a useful index for monitoring intracellular choline changes after PCh or choline. The blood brain barrier also has a low affinity for choline (K_T = 440 μM) so that choline transport from the plasma into the brain should also be increased by PCh treatment.[6] Whether or not increased brain choline will alter the activity of cholinergic neurons remains to be determined. Although this hypothesis remains unresolved, choline loading may have a greater effect in pathologic states where there is evidence of cholinergic hypofunction. The beneficial effects of PCh therapy reported[4] and the prolonged increase in choline levels, with minimal side effects, following PCh suggest that it is a drug of choice for promoting cholinergic function by precursor loading.

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