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SHORT COMMUNICATION

Effects of oral choline administration on serum and CSF choline levels in patients with Huntington's Disease[1]

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THE ADMINISTRATION of choline (Ch) by injection (COHEN & WURTMAN, 1975) or orally (COHEN & WURTMAN, 1976) increases blood Ch, brain Ch, and brain acetylcholine (ACh) levels. The extent to which Ch administration can affect Ch or ACh levels in human brain has not been tested; moreover, to our knowledge, only one report describes Ch levels in human body fluids after Ch administration (AQUILONIUS & ECKERNÄS, 1975). Such data have increased in importance now that Ch administration has been proposed as a treatment for human brain diseases thought to be associated with inadequate central cholinergic tone (DAVIS *et al.*, 1975*a*,*b*; GROWDON *et al.*, 1976). We now describe changes in blood and cerebrospinal fluid (CSF) Ch levels that occurred in human subjects during a period of oral Ch administration. All of these subjects had Huntington's Disease (HD) and were receiving the Ch as part of a larger clinical study designed to examine the utility of Ch in the therapy of HD. (This disorder may involve a deficiency in central cholinergic function (MCGEER *et al.*, 1973; BIRD & IVERSEN, 1974; STAHL & SWANSON, 1974).

MATERIALS AND METHODS

Nine subjects with established HD participated in the study. The diagnosis of HD was based on an autosomal dominant pattern of inheritance in the family plus characteristic neurologic signs, including personality change, mental deterioration, slurred speech, involuntary muscular contractions (chorea), and unsteady gait. All patients gave informed consent according to the provisions of a protocol on HD approved by the Massachusetts Institute of Technology Subcommittee on the Use of Humans as Experimental Subjects.

Patients hospitalized at the MIT Clinical Research Center received a diet that provided 2200–2500 kcal/day, 85–95 g protein, 240–260 g carbohydrate, and 100–120 g fat. Neither the quantity of food consumed nor the content of free or bound (i.e. lecithin) Ch was measured. Blood obtained by routine venepuncture was allowed to clot and then centrifuged; sera were frozen within 30–60 min of collection. Cerebrospinal fluid was obtained from the lumbar subarachnoid space by conventional lumbar puncture methods and immediately frozen.

After pretreatment blood and CSF samples were obtained, the patients took Ch either as the chloride or bitartrate salt mixed in 30 ml of water sweetened with fruit juice, sugar, and honey. Initially, they drank 1–2 g of Ch (calculated as the base) four times a day; the daily dosage was increased every 2 days. The maximum dose achieved varied, but ranged from 2–5 g, four times a day (8–20 g/day total dose). After each dose increment, serum Ch levels were measured at 9 a.m. (1 h after a Ch dose) for comparison with previous values. One day during the treatment period, four patients received only the 8 a.m. dose: blood samples were taken at 10 a.m., 12 noon, 2 p.m., and 4 p.m. on that day and assayed for Ch. A repeat lumbar puncture was performed when each patient reached his maximum dose. In all cases, CSF was collected 1 h after Ch ingestion, and at the same time of day as the pretreatment sample. Samples of CSF were also obtained from five subjects without known brain disease who were undergoing myelography to evaluate symptoms suggestive of herniated lumbar disc disease. In each instance, the myelogram was normal, and the excess fluid from routine CSF laboratory determinations was frozen for Ch analysis. Blood and CSF Ch levels were measured by a modification (COHEN & WURTMAN, 1975) of the technique of SHEA & APRISON (1973).

RESULTS

Serum Ch levels increased in all patients during the period of Ch administration. Pretreatment serum Ch levels obtained at 9 a.m. on the day after admission ranged between 10.7 and 15.2 nmol/ml, with a mean of 13.6 $\pm$ 1.7 nmol/ml (Table 1). On the second day of treatment, serum Ch levels in the same patients (measured at 9 a.m., 1 h after 2 g of Ch were administered) ranged from 22.9 to 27.4 nmol/ml, with a mean of 25.8 $\pm$ 1.7 nmol/ml.

The increase in serum Ch levels was directly proportional to the amount of Ch ingested. Five patients took Ch doses of 2, 4, and 5 g four times a day on separate days, and serum Ch was determined each day 1 h after one of the Ch doses. We found a dose-related linear increase in serum Ch levels ($r = 0.90$).

During the course of therapy, four patients drank a single dose of Ch (3–5 g) at 8 a.m. and received no more Ch the rest of the day; four blood samples were taken at subsequent 2-h intervals, the last at 4 p.m. Blood Ch levels were found to increase markedly and remain high, so that even at 8 h after the Ch dose, they were twice as high as pretreatment levels. This prolonged elevation was independent of the amount of Ch ingested, as the decay curves for serum Ch concentrations were similar whether subjects took a 3-, 4-, or 5-g dose.

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Abbreviations used: ACh, acetylcholine; Ch, choline; CSF, cerebrospinal fluid; HD, Huntington's Disease.

TABLE 1. EFFECT OF ORAL Ch ADMINISTRATION ON BLOOD Ch LEVELS IN PATIENTS WITH HUNTINGTON'S DISEASE

Patient	Serum Ch (nmol/ml) Before treatment	During treatment with 2 g Ch 4×/day*
1	10.8	27.4
2	15.0	27.4
3	13.8	25.0
4	14.0	27.1
5	14.8	23.7
6	10.7	25.0
7	14.1	22.9
8	13.8	27.4
9	15.2	26.1
Mean ± S.E.M.	13.6 ± 1.7	25.8 ± 1.7†

* Blood samples were collected 1 h after a Ch dose on the second day of treatment.
† $P < 0.001$, Student's t-test.

Choline levels were measured in the CSF of eight of the nine patients with HD (Table 2); patient No. 1 did not have a lumbar puncture. The mean Ch level before treatment was 1.8 ± 0.1 nmol/ml. During treatment, at a time when each patient was taking his maximum dose of Ch, the mean Ch level rose 74% to 3.1 ± 0.3 nmol/ml. No significant correlation was noted between the Ch levels in a particular subject's serum and CSF, either before or during treatment. Highest CSF Ch levels tended to be observed in samples from subjects receiving the highest Ch dose.

The mean CSF Ch level measured before Ch treatment in patients with HD was 1.8 ± 0.1 nmol/ml; in control subjects, the mean CSF Ch level was 2.2 ± 0.3 nmol/ml. This difference was not statistically significant.

DISCUSSION

These data indicate that Ch ingestion by humans produces dose-related increases in serum Ch levels that persist for at least 8 h. This increase is similar in magnitude to that reported by AQUILONIUS & ECKERNÄS (1975) in two patients with HD, even though they employed slightly different dosage schedules. Such prolonged elevations in serum Ch were unexpected, since plasma Ch in rats had been shown to turn over with a half-life of less than 1 min (FREEMAN *et al.*, 1975). The prolonged elevation in human serum Ch concentration may reflect slow Ch absorption, species differences in Ch metabolism, differences in the fates of Ch administered orally and intravenously, or, possibly, a rapid flux of Ch from tissues into blood. Preliminary studies in rats indicate that blood Ch levels also remain markedly elevated for 8 h after the Ch base is administered by stomach tube (HIRSCH *et al.*, 1976).

Circulating Ch is a major source of brain Ch, and brain Ch levels bear a linear relationship to the amount of Ch infused intravenously into rats (FREEMAN *et al.*, 1975). Part of the Ch taken up into the brain is utilized for ACh synthesis. Since the enzyme that catalyzes this reaction (choline acetyltransferase) normally is not saturated with its substrate, Ch administration can accelerate brain ACh synthesis and increase ACh levels in rats (COHEN & WURTMAN, 1975, 1976). Although we were unable to measure Ch and ACh levels in the brains of our human subjects who received Ch, our observations do show that such treatment can elevate Ch levels in the blood that goes to the brain. These data, therefore, provide the biochemical basis for administering pharmacologic doses of Ch to patients with brain diseases that may be associated with deficient central cholinergic tone.

Oral Ch administration also significantly elevates CSF Ch levels. Neither the sources of CSF Ch nor the relationship of CSF Ch to the Ch pools available for brain ACh synthesis is fully understood. Cerebrospinal fluid Ch could derive from at least three sources: (1) directly from the blood (GARDINER & DOMER, 1968), (2) from the breakdown

TABLE 2. EFFECT OF MAXIMAL ORAL Ch DOSE ON CSF LEVELS IN PATIENTS WITH HUNTINGTON'S DISEASE

Patient*	Dose (g Ch/day)	CSF Ch (nmol/ml) Before treatment	During treatment†
2	20	2.0	3.5
3	20	1.6	1.9
4	8	2.2	not measured
5	20	2.2	4.0
6	12	1.2	2.1
7	12	1.8	3.3
8	8	1.4	3.0
9	20	1.6	3.7
	Mean ± S.E.M.	1.8 ± 0.1	3.1 ± 0.3‡

* CSF was not obtained from patient No. 1.
† CSF samples were collected 1 h after a Ch dose at the same time of day the first sample was collected.
‡ $P < 0.01$, Student's t-test.

of endogenous bound brain Ch (e.g. choline phospholipids) (SCHUBERTH & JENDEN, 1975), and (3) from the Ch that is formed after ACh release and hydrolysis and not taken up into the presynaptic cholinergic terminal (i.e. the 'sink' function of the CSF) (JÖNSSON *et al.*, 1969; AQUILONIUS *et al.*, 1972). If CSF Ch does derive to a significant extent from the hydrolysis of released ACh, and if brain ACh levels are indeed reduced in patients with HD (MCGEER *et al.*, 1973; BIRD & IVERSEN, 1974; STAHL & SWANSON, 1974), it might be anticipated that CSF Ch levels would be lower in such patients than in control subjects. Such a reduction was noted by AQUILONIUS *et al.* (1972), but was not found in our subjects.

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