Brain Acetylcholine: Control by Dietary Choline

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Abstract. Acetylcholine concentrations in whole rat brain or in various brain regions and free choline concentrations in blood serum and brain vary with dietary choline consumption. The increases in brain acetylcholine after treatment with physostigmine (an inhibitor of acetylcholinesterase) or after consumption of a diet high in choline are additive, suggesting that choline acts by increasing acetylcholine synthesis.

The availability of amino acid precursors is an important factor controlling the rates at which neurons in rat brain synthesize such monoamine neurotransmitters as serotonin (1) and the catecholamines (2). We have shown that the administration of a single intraperitoneal dose of choline can cause sequential elevations in brain choline and acetylcholine (ACh) concentrations in rats (3). Haubrich et al. failed to detect increased brain ACh in rats given choline intravenously or intraperitoneally (4). However, in a subsequent study they did observe that brain ACh increased in guinea pigs receiving choline by intracarotid perfusion (4). Moreover, Nagler et al. have reported significant decreases of ACh content in the brains of weanling rats deprived of choline for 5 days (5). These observations have been taken as evidence that pharmacologic or pathologic changes in choline availability can also influence the synthesis of its neurotransmitter product; ACh, in rat brain (3). A significant fraction of plasma and, therefore, brain choline is of dietary origin (6). We show that physiologic variations of choline content in the diet (that is, variations within the range that omnivorous animals or humans might consume from day to day) are associated with parallel changes in brain ACh concentrations, especially within the caudate nucleus. These observations suggest that the amounts of ACh stored in terminals of central cholinergic neurons may not be constant. Thus, the number of ACh molecules released when these neurons fire may vary with nutritional status and may be altered by administering or withholding choline.

Adult male Sprague-Dawley rats were given free access to food and water and maintained in group cages in a room kept at 20° to 22°C, light (Vita-Lite, 33 μW/cm²) was furnished between 8 a.m. and 8 p.m. daily. The animals were killed between 9 and 11 a.m. at the end of the experimental period. Food and water consumption was measured daily. In experiments requiring injections, animals received 1 ml per kilogram of body weight intraperitoneally, control and treated animals were injected and killed alternately to minimize the effects of possible rhythms (7). Animals whose brains were assayed for choline and ACh were killed by a 3.5 second exposure of the head to microwave radiation in the waveguide of a modified Litton microwave oven (Medical Engineering Consultants) to protect brain choline and ACh from enzyme-mediated postmortem changes (8). Brains were excised and frozen until assayed. Choline and ACh were extracted (3) and assayed (9). Animals whose sera were assayed to determine choline concentrations were killed by decapitation; blood was collected from the cervical wound. Data were analyzed according to Student's t-test, linear regression analysis, or two-way analysis of variance.

In initial studies (Fig. 1) groups of eight to ten rats consumed the diet deficient in choline (Nutritional Biochemicals) with or without choline (0.183 or 1.83 percent) for 11 days (10). Five to seven rats from each group were killed by microwave irradiation, and their brains were dissected into various regions and assayed for choline and ACh; the remaining animals were killed by decapitation and their sera were assayed for choline. Serum choline concentrations were proportional to mean dietary choline intake (r = .91); the average serum choline concentrations for rats consuming an average of 0, 20, or 129 mg of choline daily were: 8.0, 15.3, and 32.6 nmole/ml, respectively. The ACh concentration in the caudate nucleus was 28 percent greater in rats consuming 20 mg of choline per day and 45 percent greater in rats consuming 129 mg of choline per day than in animals deprived of choline. The ACh concentration also was significantly increased in other brain regions of rats ingesting the larger amount of choline. Similar results were obtained by adding choline to the drinking water (11). In general, the consumption of large doses of choline depressed food intake (Fig. 1 and Table I); however, the lower choline dose, which also elevated caudate ACh concentrations, had no effect on food consumption.

To determine whether the choline-induced rise in brain ACh reflected accelerated synthesis or decreased breakdown of the neurotransmitter, the effects of dietary choline were compared in animals that were or were not also treated with physostigmine, an inhibitor of brain acetylcholinesterase. Groups of rats consuming a diet deficient in choline (Bio-Serv, Inc.) and drank distilled water with or without added choline chloride [1.5 or 15 mg/ml (Table 1, cerebrum animals); 15 mg/ml (Table 1, caudate animals)] for 7 days. At the end of this period, half of the animals in each group were injected with physostigmine salicylate (1 mg/ml intraperitoneally, dissolved in saline); the other half received only saline. Animals were killed by microwave irradiation 20 minutes after injection,

Fig. 1. Effect of dietary choline content on choline and ACh concentrations in various brain regions. Rats (weighing 90 g) consumed diets having an average of 0, 20, or 129 mg of choline per day for 11 days, respectively, 121 mg of choline per day, respectively, All groups consumed 17 to 19 ml of water per day. Bars represent mean concentrations, and vertical lines represent the standard error of the mean. "Rest of brain" refers to the cerebrum minus the caudate nucleus. Differences from corresponding concentrations in rats consuming no choline are indicated by *, P < .05, **, P < .01; ***, P < .001.
Table 1. Effects of varying choline concentration and administering physostigmine on cerebral and caudate choline and ACh concentrations. Groups of 10 (cerebrum) or 16 (caudate) rats (150 g) consumed a diet deficient in choline and drank distilled water, with or without added choline, freely for 7 days. On the final day of the experiment, half of the rats in each group received an intraperitoneal injection of physostigmine salicylate (1 mg per kilogram of body weight in 0.9 percent NaCl) or of saline alone, and were killed by microwave irradiation of the head 20 minutes later. Cerebrrums and caudate nuclei were assayed for choline and ACh. Because physostigmine administration did not modify choline concentrations in the brains of animals consuming any of the choline doses, data on choline concentrations in the brain of rats consumed in vivo acetyltransferase activity does not lower the concentration of ACh in the brain. The mechanism by which the brain takes up circulating choline may allow a low-affinity system that would be unsaturated in vivo (20); this would allow an increase in serum choline to raise the concentration of choline in the brain and, consequently, of ACh in the brain (7).

Choline is present in some foods normally consumed by man (for example, egg yolks, 1.7 percent by weight; meat, 0.6 percent by weight; fish, 0.2 percent by weight; cereals and cereal products, 0.1 percent by weight; and legumes, 0.2 to 0.35 percent by weight), but largely absent (from others (for example, most fruits and soft vegetables) (21, 22). Day-to-day or long-term variations in food intake can thus cause marked variations in the amounts of choline ingested. Hence, the six- to eightfold range in daily choline consumption examined in our studies may reflect variations normally encountered in human diets (23).

The observation that choline ingestion can stimulate synthesis of ACh in the brain may provide a useful approach for modifying the functional activity of cholinergic neurons. If intranuclear ACh content is indeed coupled to ACh release, in fact, on the basis of earlier work from our laboratory a patient with tardive dyskinesia, a disorder thought to result from relatively deficient cholinergic activity in the striatum, was treated successfully with large doses of choline (24). Furthermore, our observations raise the possibility that the
terior, and their cerebrrums or caudate nuclei (Table 1) were assayed for choline and ACh. The increases in cerebral or caudate ACh produced by either choline or physostigmine alone were enhanced by treatment with the other compound. In fact, the diet-induced elevations in brain ACh content were additive to the increase in ACh caused by injection of cholinesterase. These observations suggest that the mechanism by which choline intake affects ACh concentrations in the brain involves accelerated synthesis of the neurotransmitter.

Choline acetyltransferase catalyzes the synthesis of ACh from choline and acetyl coenzyme A (12). Several factors have been proposed as controlling the rate of this reaction in cholinergic neurons, for example, (i) feedback inhibition of choline acetyltransferase by its end product, ACh (13); (ii) mass action regulation of choline acetyltransferase (14); and (iii) variation of the high-affinity uptake of choline by presynaptic membranes (15). We propose that the availability of circulating choline (which depends on nutritional state) constitutes an additional factor controlling synthesis of brain ACh.

The choline acetyltransferase activity in rat brain reportedly has an in vitro $K_m$ of 18 $\mu$M for acetyl coenzyme A and 400 $\mu$M for choline (16). The concentration of free choline in plasma is approximately 10 to 50 $\mu$M (17). The concentrations of acetyl coenzyme A (18) and choline (7) (Table 1) in rat brain are approximately 7 to 11 $\mu$M and 35 to 100 $\mu$M, respectively. Therefore, choline acetyltransferase is probably unsaturated with either of its substrates in vivo. (Of course, the intracellular concentra-
tions of a number of drugs thought to act at cholinergic synapses may vary significantly as a function of nutritional state.

References and Notes

11. Our usual laboratory diet, Charles River Rat Mouse Hamster Maintenance Formula, contains 0.183 percent choline.
12. Groups of five or six rats (125 g) ate a diet deficient in choline (Nutritional Biochemicals) and drank distilled water with or without added choline chloride (1.5 or 15 mg/ml) for 11 days; the total amount of choline thus consumed averaged 0.2, or 11 mg, per rat. Both brains (minus cerebellum and brainstem) choline and ACh concentrations varied significantly with the amount of choline ingested; both choline and brain ACh significantly, by the larger 23 percent (P < 0.001).
24. For example, it is estimated that an average mixed diet for adults in the United States contains 0.5 to 0.9 g of choline or betaine per day (22). However, on a particular day, a person consuming six egg yolks in his food (for example, a sponge cake), a pound of meat, and half a pound of potatoes ingests 5 g of choline, which is already about six to 10 times the average intake.
26. We thank Miss M. Ung and Miss N. Verechek for technical assistance, and Dr. D. J. Jenden of UCLA for making helpful suggestions about this manuscript. Supported in part by grants from the Health Sciences Fund and the National Aeronautics and Space Administration and by an NIH trainingees (GM 01337) to E.L.C.
27. October 1975, revised 18 November 1975