Life Sciences Vol. 16, pp. 1095-1102 Pergamon Press
Printed in the U.S.A.

BRAIN ACETYLCHOLINE: INCREASE AFTER SYSTEMIC CHOLINE ADMINISTRATION

Edith L. Cohen and Richard J. Wurtman

Laboratory of Neuroendocrine Regulation
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

(Received in final form March 3, 1975)

Summary

Administration of choline chloride i.p. to rats causes a dose-dependent increase in the brain concentration of the neurotransmitter, acetylcholine (ACh). This increase is maximal (22% after a 60-mg/kg dose) 40 minutes after injection. These observations suggest that precursor availability may influence brain ACh synthesis, just as brain tryptophan and tyrosine levels have previously been shown to control the syntheses of brain serotonin and catecholamines.

Acetylcholine (ACh) is synthesized from choline and acetyl-CoA in a reaction catalyzed by choline acetyltransferase (CAT) (1,2). Mammalian brain, unlike liver, may not be capable of *de novo* choline synthesis by methylation of phosphatidyl-ethanolamines (3); thus, central cholinergic neurons may largely depend on the blood for their supply of the ACh precursor (4). Precursor availability has previously been shown to influence the rates at which central neurons synthesize such monoamine neurotransmitters as serotonin (5) and the catecholamines (6). Our observations indicate that experimentally induced changes in brain choline level caused by its intraperitoneal administration can similarly elevate brain ACh levels.

Methods

Male Sprague Dawley rats weighing 150-200 g were kept at 20-22°C in a room lit between 8 AM and 8 PM by "Vita-Lite"

(50 to 75 foot-candles; Duro-Test Corp., North Bergen, N.J.), and were given *ad libitum* access to food (Charles River Rat-Mouse-Hamster Maintenance Formula) and water for 5 days prior to the experiments. In both experiments an injection volume of 1 ml/kg was administered i.p. to each rat beginning at 9 AM. In the time-course study, rats were injected with choline chloride (60 mg/kg) dissolved in saline (0.9% NaCl), and killed 20, 40, 60, or 80 minutes after injection. In the dose-response study, rats were injected with various doses of choline chloride, and killed 40 minutes later. Control animals, injected with 1 ml/kg of saline, were killed alternately with choline-treated rats in both studies.

The reported levels of choline and acetylcholine in rat brain vary widely from one group of investigators to another, depending on the methods used to kill animals, and to extract and assay the choline and ACh. Microwave heating inactivates acetylcholinesterase and other catabolic enzymes; this may protect brain choline and ACh levels from postmortem enzyme-mediated changes (7-9). In the present experiments animals were killed by 3.5-second microwave irradiation of the head in the wave-guide of a modified Litton microwave oven (10). Brains were excised and frozen until assayed. Choline and ACh were assayed by a sensitive radioenzymatic method (11) after tissue homogenization in 15% 1N formic acid/acetone solution, pellet re-extraction with 70% of the above solution in acetone, and organic extraction of the supernatants with heptane:isoamyl alcohol (8:1 v/v). This killing and assaying procedure, as used in our laboratory, normally gives total brain choline values of 26.8 $\pm$ 0.9 nmoles/g brain, and total ACh values of 27.3 $\pm$ 0.4 nmoles/g brain in 200-g unstressed male rats.

These values conform closely with those reported elsewhere by investigators using similar methods (7-9). Data were analyzed either by Student's *t*-test or linear regression analysis.

Results

Twenty minutes after choline chloride injection (60 mg/kg, i.p.), brain choline rose significantly to 223 ± 7% of control values ($p < 0.001$); it subsequently declined, reaching control values 60 minutes after injection (Fig. 1). Brain ACh concentration rose significantly ($p < 0.001$) to 122 ± 2% of control values after 40 minutes, and returned to basal levels by 80 minutes. The time course of the changes in brain choline and acetylcholine concentrations exhibited a precursor-product type of relationship. The increase in brain ACh concentration 40 minutes after i.p. choline chloride was dose-dependent between 15 mg/kg and 60 mg/kg (Fig. 2); greater doses of choline did not cause greater increases in ACh concentration.

Discussion

These studies may provide the first evidence that the systemic administration of choline can, by raising brain choline concentrations, increase brain acetylcholine levels *in vivo*. In studies on pentobarbital-anesthetized guinea pigs, Haubrich *et al.* (12) failed to detect a significant increase in brain ACh 2 min after animals received 400 μmoles/kg of choline intravenously. (Animals given an intracarotid perfusion of choline [10 μmoles/kg/min] for 15 min did display increased brain ACh.) The disparity between these findings and our own may reflect the difference in methods used to kill the animals (decapitation, which is associated with higher choline, and lower ACh levels (13) than microwave), species differences, the effect of the anesthetic, or the short time interval after

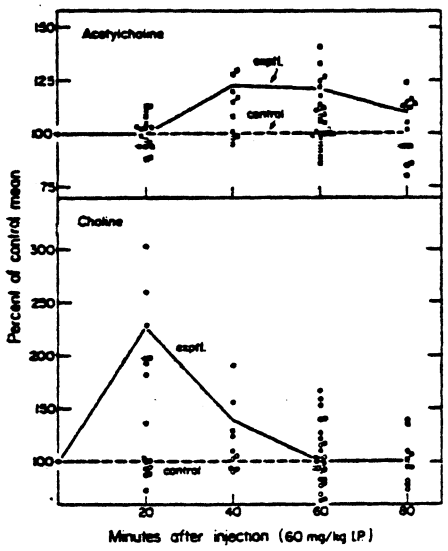

FIG. 1

150-200-gram male rats received choline chloride (60 mg/kg i.p.) in saline (0.9% NaCl) (closed squares and circles), or the diluent alone (open squares and circles). The animals were killed at various times after injection by microwave irradiation of the head. Brain choline and ACh concentrations were measured by a radioenzymatic assay (11). Data are expressed as percents of control means; each point represents an individual animal.

choline administration at which brains were taken.

The regional distributions of choline and acetylcholine in brain are similar (14). This indicates that a major store of free choline for acetylcholine biosynthesis may lie within cholinergic neurons. These neurons apparently take up choline by a specific, high-affinity uptake mechanism (15-18). Our observations suggest that the mechanism by which the brain takes up circulating choline is not saturated *in vivo* (Fig. 2); This mechanism may or may not be the same as the one operating in cholinergic terminals (19). *In vitro* experiments show a strong correlation between choline penetration into brain synaptosomes, as affected by various manipulations, and ACh synthesis rate (20).

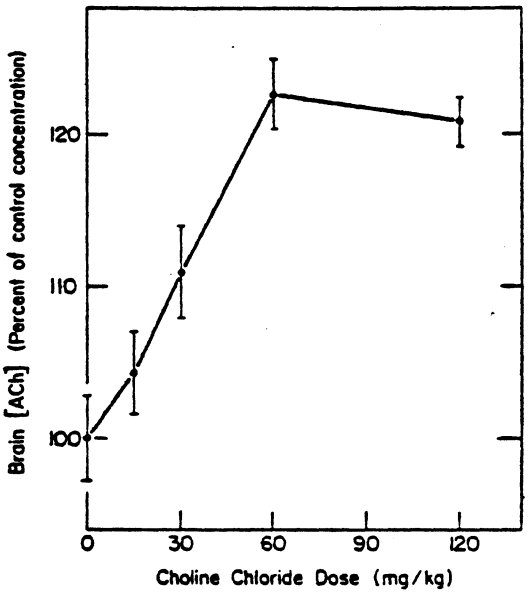

FIG. 2

Groups of 5-9 male rats weighing 150-200 grams received various doses i.p. of either choline chloride in saline (0.9% NaCl) or the diluent, and were killed 40 minutes later by microwave irradiation of the head. A radioenzymatic method was used to assay choline and ACh concentrations in whole brain (11). Data were analyzed by linear regression analysis. Brain ACh levels correlated highly with dose of choline administered for doses up to 60 mg/kg (r = 0.72; the slope is significantly different from zero at $p < 0.001$).

Rat brain CAT has an *in vitro* Km of 18 μM for acetyl-CoA, and 0.4 mM for choline (21). Rat brain contains 5 to 8 nmoles/g (7 to 11 μM) acetyl-CoA (22), and about 25 nmoles/g (37 μM) choline (8). Therefore, even assuming that both substrates are distributed throughout the brain in a single compartment fully available to CAT, the enzyme probably is not saturated with respect to either of its two substrates *in vivo*. The presence of excess enzyme in the mammalian brain is further supported by the fact that partial *in vivo* inhibition of CAT does not lower brain ACh levels (23, 24).

Since CAT, which synthesizes ACh from choline and acetyl-CoA, probably is not saturated *in vivo*, and since the uptake mechanism for choline uptake into brain neurons may also be unsaturated, it seemed reasonable to hypothesize that increased plasma choline concentration would raise the intraneuronal choline concentration in brain, and as a result, accelerate the synthesis of ACh; this in turn could elevate brain ACh levels. These preliminary studies indicate that brain choline and ACh levels do increase after i.p. choline chloride injection (which presumably raises blood choline concentrations). The most likely explanation for the rise in brain ACh is that its synthesis was accelerated by the induced rise in brain choline concentration. Hence, precursor availability may influence the synthesis of brain ACh *in vivo*, just as it controls the synthesis of brain serotonin (5) and brain catecholamines (6). Studies are now underway to determine the physiological significance of choline-induced changes in brain acetylcholine.

Acknowledgment

We thank Ms. Mee-Ling Kwok for excellent technical assistance. This work was supported in part by grants from the United States Public Health Service (AM-11237, AM-14228). Ms. Cohen has an NIH Traineeship (GM 01337).

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