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Effects of Choline Administration on In Vivo Release and Biosynthesis of Acetylcholine in the Rat Striatum as Studied by In Vivo Brain Microdialysis

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Abstract: The purpose of the present study is to clarify the effects of the administration of choline on the in vivo release and biosynthesis of acetylcholine (ACh) in the brain. For this purpose, the changes in the extracellular concentration of choline and ACh in the rat striatum following intracerebroventricular administration of choline were determined using brain microdialysis. We also determined changes in the tissue content of choline and ACh. When the striatum was dialyzed with Ringer solution containing 10 μM physostigmine, ACh levels in dialysates rapidly and dose dependently increased following administration of various doses of choline and reached a maximum within 20 min. In contrast, choline levels in dialysates increased after a lag period of 20 min following the administration. When the striatum was dialyzed with physostigmine-free Ringer solution, ACh could not be detected in dialysates both before and even after choline administration. After addition of hemicholinium-3 to the perfusion fluid, the choline-induced increase in ACh levels in dialysates was abolished. Following administration of choline, the tissue content of choline and ACh increased within 20 min. These results suggest that administered choline is rapidly taken up into the intracellular compartment of the cholinergic neurons, where it enhances both the release and the biosynthesis of ACh. **Key Words:** Acetylcholine—Choline—Brain microdialysis—Biosynthesis—Striatum. **Koshimura K. et al.** Effects of choline administration on in vivo release and biosynthesis of acetylcholine in the rat striatum as studied by in vivo brain microdialysis. *J. Neurochem.* **54,** 533–539 (1990).

Recently, the cholinergic system in the brain has been highlighted, since dysfunction of this system may be involved in the pathogenesis of Alzheimer-type dementia (Davies and Maloney, 1976; Whitehouse et al., 1982; Koshimura et al., 1986, 1987, 1988). Biosynthesis of acetylcholine (ACh) from choline and acetyl-CoA in the brain is catalyzed by an enzyme, choline acetyltransferase (ChAT; acetyl-CoA:choline *O*-acetyltransferase; EC 2.3.1.6), which is thought to be present mainly in the cytosol of the cholinergic neurons (Tuček, 1984, 1985). Choline utilized for ACh biosynthesis is considered to be taken up from the extracellular fluid into the cholinergic neurons by a high-affinity choline uptake system (Guyenet et al., 1973; Haga and Noda, 1973; Yamamura and Snyder, 1973; Barker and Mittag, 1975; Simon et al., 1976). Since the extracellular and intracellular concentrations of choline are estimated to be low compared with K_m values of the high-affinity choline uptake system and ChAT, respectively, both the uptake system and ChAT are considered to be unsaturated with choline (Tuček, 1984, 1985). In fact, ACh content in the brain is reported to increase following peripheral (Cohen and Wurtman, 1975; Haubrich et al., 1975) or intracerebroventricular (i.c.v.) (Haubrich et al., 1975; Haubrich and Chippendale, 1977) administration of choline. Furthermore, using deuterated choline, several investigators have shown that a sustained elevation of choline levels in the plasma

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Abbreviations used: ACh, acetylcholine; AChE, acetylcholinesterase; ChAT, choline acetyltransferase; i.c.v., intracerebroventricular.

increased the incorporation of deuterated choline into ACh in the brain (Choi et al., 1975; Racagni et al., 1975). However, these data do not necessarily indicate that choline administration increases ACh biosynthesis, since the increase in the ACh content in the brain could result from either an increase in the ACh concentration in the extracellular compartment subsequent to inhibition of acetylcholinesterase (AChE; acetylcholine acetylhydrolase; EC 3.1.1.7) or an increase in the ACh concentration in the intracellular compartment subsequent to inhibition of ACh release. In this context, to precisely evaluate the effects of choline administration on the in vivo release and biosynthesis of ACh, it is of paramount importance to know the changes in the ACh concentration in the extracellular compartment following choline administration. In the present study, using an in vivo brain microdialysis method to monitor the changes in the extracellular concentration of ACh, we investigated the effects of choline administration on the in vivo release and biosynthesis of ACh.

MATERIALS AND METHODS

Brain microdialysis

Male Wistar rats, weighing 250–300 g, were anesthetized with diethylether and placed in a stereotaxic frame (incisor bar 5 mm above the auricular horizontal plane). The skull was exposed by a midline skin incision and a burr hole of 3-mm diameter was drilled. A dialysis probe (BDP 21-03; EICOM, Kyoto, Japan) was implanted in the right striatum [coordinates taken from bregma and the sagittal suture with the skull flat, anterior, 2.0 mm, lateral, 3.0 mm, ventral, −5.5 mm, from the atlas of Pellegrino et al. (1979)] and was fixed in place with cranioplastic cement. The location of the probe was confirmed by visual examination of the brain at the end of each experiment. The dialysis probe was connected to the perfusion pump and to the sample loop of the automated sample injector (model 10; EICOM) by means of polyethylene tubing. The dialysis probe was continually perfused at a flow rate of 4.8 μl/min with Ringer solution (147 m*M* NaCl, 3.4 m*M* $CaCl_2$, and 4 m*M* KCl, pH 6.1) and dialysates were collected every 20 min in the sample loop of the automated sample injector, which was on line with an HPLC system. Since analysis of choline and ACh was finished within 20 min, the sample loop was set to be held in the load position during 20 min and was automatically switched to the injection position for 20 s, after which the cycle was repeated.

After rats recovered from ether anesthesia, brain microdialysis was carried out under a free-moving condition. Usually, four or five 20-min control dialysates were collected before i.c.v. injection of either a choline chloride or a saline solution. ACh measurements were then continued for an additional 2 h.

i.c.v. injection

At the time of implantation of a microdialysis probe, a stainless-steel cannula (external diameter, 0.5 mm) for i.c.v. injection of choline was stereotaxically implanted into the left cerebral ventricle (anterior, 0.0; lateral, 1.7; ventral, −3.5) and was fixed in place with cranioplastic cement. The cannula was connected to a microsyringe by means of polyethylene tubing, and 5 μl of a choline chloride (10, 25, or 50 μmol) solution or a physiological saline solution for a control group was injected into the cerebral ventricle. In another control experiment, in which we examined the effects of changes in osmotic pressure or ionic strength on the amount of choline and ACh in dialysates, we intracerebroventricularly injected 50 μmol of NaCl that was dissolved in 10 μl of distilled water because of its insolubility.

Extraction of choline and ACh in brain

In a series of experiments separate from the in vivo release measurement, the tissue levels of choline and ACh in the striatum were determined following i.c.v. injection of 50 μmol of choline chloride. Choline and ACh in the striatum were extracted according to the method of Budai et al. (1986) with a slight modification. At various times following i.c.v. injection of a choline chloride or a saline solution, rats were killed by microwave irradiation (2,450 Hz, 4.5 kW, 1.2 s; Toshiba model 6402-A, Tokyo, Japan). Immediately thereafter, the rats were decapitated and their heads were placed in ice. The striatum was removed, weighed, and homogenized in 2 ml of 0.1 *M* perchloric acid containing 10 nmol/ml *n,n*-dimethyl(*n*-isopropyl)-3-amino-1-propanol (isopropylhomocholine) as an internal standard. The homogenate was kept in ice for 20 min; then the pH was adjusted to 6–7 by adding 50 μl of 4 *M* K_2CO_3 and the mixture was centrifuged at 18,000 *g* for 20 min at 4°C. A 300-μl aliquot of the supernatant was applied onto a column (0.6 × 1.0 cm) of Amberlite CG-50 (Na^+ form), which had been equilibrated with 0.1 *M* phosphate buffer (pH 6.1). After washing with 2 ml of distilled water, choline and ACh were eluted with 1.2 ml of 1 *M* perchloric acid. The pH of the eluate was adjusted to 6–7 by adding 300 μl of 4 *M* K_2CO_3 and the mixture was centrifuged. The resulting supernatant was analyzed for choline and ACh.

Assay of choline and ACh

Choline and ACh in the dialysates and in the extracts of the striatum were measured using HPLC combined with an immobilized enzyme reactor and an electrochemical detector according to the method of Damsma et al. (1985). In brief, a column (Eicompak AC-Gel, 6 × 150 mm; EICOM) was used for separation. An enzymatic reactor containing AChE and choline oxidase (choline: oxygen 1-oxidoreductase; EC 1.1.3.17), covalently attached to aminopropyl-controlled pore glass, converted ACh and choline to hydrogen peroxide, which was detected by an electrochemical detector with a platinum electrode (model 100; EICOM) at 450 mV. The mobile phase was delivered by a Hitachi 655A pump (Hitachi, Tokyo, Japan) at 1.0 ml/min and contained 0.1 *M* sodium phosphate buffer (pH 8.0), 300 mg/L sodium 1-decanesulfonate, and 65 mg/L tetramethylammonium chloride. Quantification of collected choline and ACh was performed by comparison with peak heights of authentic standards.

Materials

Drugs were obtained from the following sources: ACh bromide, choline chloride, physostigmine sulfate, and atropine sulfate from Wako Pure Chemical Industries, Osaka, Japan; sodium 1-decanesulfonate from Tokyo Kasei Kogyo, Tokyo, Japan; AChE (type VI-S) and choline oxidase from Sigma Chemical, St. Louis, MO, U.S.A.; hemicholinium-3 from Aldrich Chemical Co., Milwaukee, WI, U.S.A.; aminopropyl-controlled pore glass from Electro-Nucleonics Inc., Fairfield,

NJ, U.S.A. Isopropylhomocholine was a generous gift from EICOM, Kyoto, Japan.

Statistical analysis

All results were expressed as means ± SEM. The data were subjected to a two-way analysis of variance, and when significant F values were encountered, the Newman-Keuls multiple-range test was used to test for significant differences between treatment means (Steel and Torrie, 1960). A probability level of $p < 0.05$ was considered statistically significant.

RESULTS

Before implantation of a dialysis probe in the striatum for determination of in vivo ACh release, we attempted to determine the recovery of choline and ACh through the dialysis membrane. For this purpose, the probes were placed in Ringer solution containing various concentrations of choline and ACh (1, 10, and 100 μM) and were perfused with Ringer solution at 4.8 μl/min. The concentrations of choline and ACh in the dialysates collected for 20 min were compared with the concentrations outside the dialysis tube to express the recoveries of choline and ACh through the dialysis membrane as concentration in dialysates/concentration in surrounding fluid × 100%. The recoveries of choline and ACh were 16.8 ± 1.0 and 14.4 ± 1.0%, respectively, at 1 μM, 15.0 ± 0.9 and 15.4 ± 0.8% at 10 μM, and 11.3 ± 1.3 and 10.8 ± 1.1% at 100 μM (n = 4). The data in the present study were presented without correction for recovery.

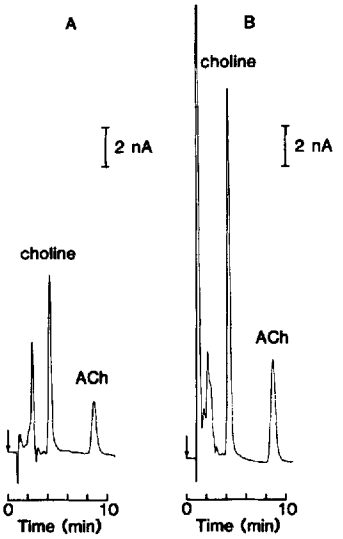

FIG. 1. Chromatograms of authentic standards (5 pmol) of choline and ACh (**A**) and of the striatal dialysate (**B**). A dialysis probe was stereotaxically implanted in the striatum and perfused at a flow rate of 4.8 μl/min with Ringer solution containing 10 μM physostigmine. The dialysate was collected every 20 min in the sample loop of the automated sample injector and was automatically injected into an HPLC combined with an enzyme reactor and an electrochemical detector as indicated by the arrows. A standard solution containing 5 pmol of choline and ACh was also injected into the HPLC using the automated sample injector.

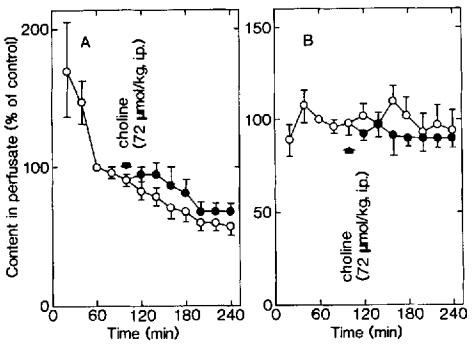

FIG. 2. Time course of the basal levels of choline and ACh in the striatal dialysates in the presence of 10 μM physostigmine and the effects of 72 μmol/kg of choline given intraperitoneally on the amount of choline and ACh collected in the dialysates. The striatum was dialyzed as described in the legend to Fig. 1. Immediately after the fifth fraction had been collected, rats were intraperitoneally injected with a physiological saline solution (○) or a choline chloride solution (72 μmol/kg; ●) as indicated by arrows. Levels of choline (**A**) and ACh (**B**) in each fraction were expressed as percentages of the control values in the third fraction (choline, 9.2 ± 0.7 pmol/20 min, n = 4; ACh, 8.6 ± 1.2 pmol/20 min, n = 4). Each value is the mean ± SEM of four determinations.

Figure 1 shows a typical chromatogram of the striatal dialysate in the presence of 10 μM physostigmine. It appears that ~10 pmol of choline and ACh was present in the dialysate collected during 20 min in the steady state.

Figure 2 shows the time course of the basal levels of choline and ACh in the striatal dialysates in the presence of 10 μM physostigmine and the effects of 72 μmol/kg of choline given intraperitoneally on the amount of choline and ACh collected in the dialysates. The amount of choline collected in the dialysate per 20-min period rapidly decreased during the initial 40 min after the beginning of brain microdialysis, and thereafter the rate of the decrease became slower. The gradual decrease was observed up to 240 min (Fig. 2A). The amount of ACh collected in the dialysates per 20-min period was constant over a period of 300 min (there were no significant differences between the various 20-min fractions as determined by analysis of variance) (Fig. 2B).

Since the content of ACh in the brain is reported to increase following intraperitoneal injection of choline (Cohen and Wurtman, 1975; Haubrich et al., 1975), we first examined the effects of choline administered intraperitoneally on the release of ACh using in vivo brain microdialysis. As shown in Fig. 2, the amount of both choline and ACh collected in the dialysates was unchanged following intraperitoneal administration of 72 μmol/kg of choline, the dose that is reported to increase the content of both choline and ACh in the brain (Haubrich et al., 1975). Then we determined the content of choline and ACh in the striatum 20 min fol-

lowing intraperitoneal administration of choline. The content of choline in the striatum slightly increased: The values of the control and the choline-administered groups were 64.8 ± 4.5 (n = 6) and 83.6 ± 3.9 (n = 6) nmol/g, respectively. In contrast, the contents of ACh in the striatum were unchanged: The values of the control and the choline-administered groups were 68.1 ± 2.5 (n = 6) and 72.8 ± 3.2 (n = 6) nmol/g, respectively. From these results, we wondered if the amount of choline reaching the brain was not sufficient to enhance release and biosynthesis of ACh, when choline was administered peripherally. Therefore, we then attempted to administer choline directly into the brain by i.c.v. injection.

Figure 3 shows the effects of various doses (10, 25, and 50 μmol) of choline administered intracerebroventricularly on the amount of choline and ACh in the striatal dialysates. Choline levels in the dialysates were elevated after a lag period of 20 min following administration of various doses of choline and reached a maximum in the third fraction collected between 40 and 60 min after the injection. The elevation in choline levels was observed up to 140 min after the injection (Fig. 3A). In contrast, ACh levels in the dialysates were elevated immediately following choline administration and reached a maximum within 20 min. The maximum elevation and the duration of the elevation of ACh levels were dependent on the doses of administered choline (Fig. 3B). The choline-induced increase in the amount of ACh in the dialysates does not seem to be a result of changes in the osmotic pressure or ionic strength, since i.c.v. administration of 50 μmol

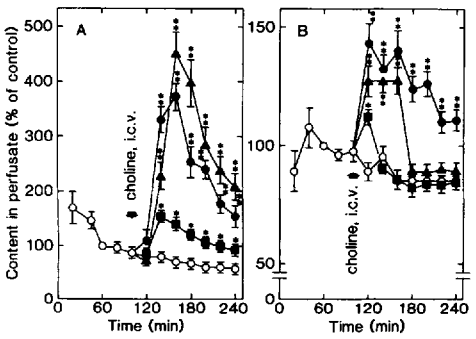

FIG. 3. Effects of various doses of choline administered intracerebroventricularly on levels of choline (**A**) and ACh (**B**) in the dialysates. The striatum was dialyzed as described in the legend to Fig. 1. Immediately after the fifth fraction had been collected, 5 μl of a physiological saline solution (○) or a test solution containing various doses of choline chloride (10 μmol, ■; 25 μmol, ▲; 50 μmol, ●) was administered intracerebroventricularly (indicated by arrows). Levels of choline and ACh collected in each fraction were expressed as percentages of the control values in the third fraction (choline, 9.2 ± 0.7 pmol/20 min, n = 4; ACh, 8.6 ± 1.2 pmol/20 min, n = 4). Each value is the mean ± SEM of four determinations. $^{*}p < 0.05$, $^{**}p < 0.01$, significantly different from the values in the corresponding fraction of the control group that was injected with a physiological saline solution.

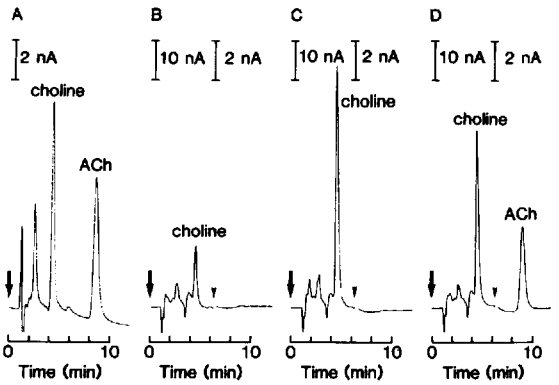

FIG. 4. Effects of i.c.v. administration of choline on the levels of ACh in the striatal dialysates without addition of physostigmine to the perfusion fluid. The striatum was dialyzed as described in the legend to Fig. 1 except that physostigmine was not added to the perfusion fluid. **A:** A chromatogram of authentic standards of choline (5 pmol) and ACh (15 pmol); **B:** a chromatogram of the striatal dialysate before i.c.v. administration of choline; **C:** a chromatogram of the dialysate collected between 40 and 60 min after i.c.v. administration of 50 μmol of choline; **D:** a chromatogram of the dialysate collected after choline administration followed by addition of physostigmine to the perfusion fluid. Eighty minutes after choline administration, the perfusion fluid was replaced by the one containing 10 μ*M* physostigmine and the dialysates continued to be collected. In this figure is shown the chromatogram of the dialysate collected between 20 and 40 min after addition of physostigmine. Arrows represent the time when the sample was injected onto the HPLC. Arrowheads represent the time when the sensitivity of the electrochemical detector was changed.

of NaCl had little effect on the amount of choline and ACh collected in the dialysates (data not shown).

To confirm that the choline-induced increase in the amount of ACh in the striatal dialysates was not due to an increase in the extracellular concentration of ACh subsequent to inhibition of AChE, we dialyzed the striatum with physostigmine-free Ringer solution and examined the effects of choline on the amount of ACh in the striatal dialysates (Fig. 4). When the striatum was dialyzed with physostigmine-free Ringer solution, ACh could not be detected in the dialysates both before (Fig. 4B) and even after (Fig. 4C) i.c.v. administration of 50 μmol of choline. When physostigmine was added to the perfusion fluid at a final concentration of 10 μ*M* 80 min after choline administration, ACh became detectable (Fig. 4D).

We then attempted to determine whether exogenously administered choline directly stimulated ACh release from the outside of the cholinergic neurons without being taken up into the cholinergic neurons or enhanced ACh release after it had been taken up into the neurons. For this purpose, we examined the effects of i.c.v. administration of choline on the amount of ACh collected in the dialysates after addition of hemicholinium-3, an inhibitor of the choline uptake mechanism, to the perfusion fluid at a final concentration of 87 m*M*. In the presence of hemicholinium-

3, i.c.v. administration of 50 μmol of choline had little effect on the amount of ACh in the dialysates (Fig. 5).

To clarify the relationship between ACh release and the intracellular concentration of choline or ACh, we attempted to determine the changes in the intracellular concentration of choline and ACh following i.c.v. administration of various doses of choline. To obtain the intracellular concentration of choline and ACh, we measured their tissue levels in the striatum (Fig. 6), since the intracellular concentration of choline and ACh can be estimated from both tissue levels of these substances and their concentrations in the dialysates. Following i.c.v. administration of choline, tissue levels of choline in the striatum, different from the amount in the dialysates, rapidly increased and reached a maximal level within 20 min. The maximum level was dependent on the dose of administered choline. Thereafter, choline levels gradually decreased with a similar rate constant, but even 140 min after the administration, it was significantly higher than the control value (Fig. 6A). Tissue levels of ACh in the striatum also increased within 20 min following i.c.v. administration of various doses of choline. At 10 μmol of choline, ACh levels in the striatum returned to the control value within 40 min, but at 25 and 50 μmol of choline, ACh levels continued to increase and reached the maximum level within 60 min. At 25 μmol, ACh levels returned to the control value within the next 20 min, but at 50 μmol of choline, the increase in ACh levels in the striatum was observed even 140 min after the injection.

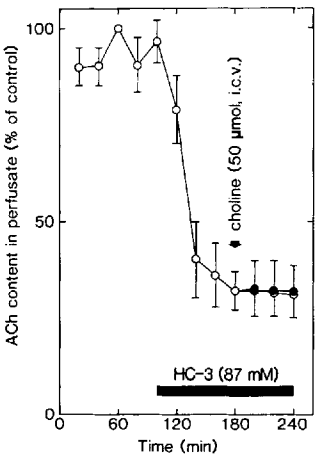

FIG. 5. Effects of hemicholinium-3 on choline-induced increase in ACh levels in the dialysates. The striatum was dialyzed as described in the legend to Fig. 1. Immediately after the fifth fraction had been collected, hemicholinium-3 was added to the perfusion fluid at a final concentration of 87 m*M* as indicated by the bar. After ACh levels in the dialysates reached a steady state, 5 μl of a physiological saline solution (○) or a choline chloride solution (50 μmol; ●) was intracerebroventricularly administered as indicated by the arrow. ACh levels in each fraction were expressed as percentages of the control values in the third fraction (8.6 ± 1.2 pmol/20 min). Each point represents the mean ± SEM of four determinations.

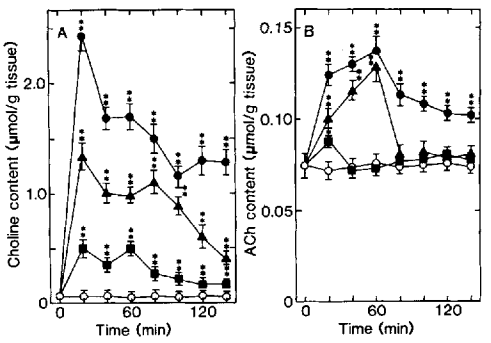

FIG. 6. Effects of i.c.v. administration of choline on levels of choline (**A**) and ACh (**B**) in the striatum. At various time after i.c.v. administration of 5 μl of either a physiological saline solution (○) or a choline chloride solution (10 μmol, ■; 25 μmol, ▲; 50 μmol, ●), rats were killed by microwave irradiation and levels of choline and ACh in the striatum were measured as described in Materials and Methods. Each value is the mean ± SEM of five determinations. $^{**}p < 0.01$, significantly different from the values of the control group that was injected with a physiological saline solution.

Thus, the maximum level attained and the duration of the increase in ACh levels were dependent on doses of administered choline (Fig. 6B). The changes in the tissue levels of ACh correlated well with those in the amount of ACh in the dialysates as shown in Fig. 4B.

DISCUSSION

When brain microdialysis was performed in the presence of physostigmine, the amount of ACh collected in the striatal dialysates increased dose dependently following i.c.v. administration of various doses of choline (Fig. 3B). It is generally accepted that after ACh is released from the cholinergic nerve terminals into the synaptic clefts, it is rapidly degraded by AChE (MacIntosh, 1981). Therefore, the increase in the amount of ACh in the dialysates is considered to result from either an increase in ACh release or an increase in the extracellular concentration of ACh subsequent to inhibition of AChE by administered choline.

When brain microdialysis was performed without addition of physostigmine, an inhibitor of AChE, to the perfusion fluid, ACh could not be detected in the dialysates both before and after administration of choline (Fig. 4B and C). This suggests that the choline administered intracerebroventricularly had little effect on the activity of AChE. Thus, these results taken together strongly indicate that the increase in the amount of ACh collected in the dialysates is not the result of an increase in the extracellular concentration of ACh subsequent to inhibition of AChE by administered choline, but the result of an actual increase in ACh release from the cholinergic nerve terminals.

The increase in in vivo ACh release is not the result of changes in osmotic pressure or ionic strength induced by administration of choline chloride, since so-

dium chloride solution had no effect on in vivo ACh release (data not shown).

After pretreatment with hemicholinium-3, the choline-induced increase in in vivo ACh release was completely abolished (Fig. 5). Furthermore, within 20 min following i.c.v. administration of choline, the concentration of choline in the extracellular compartment in the striatum monitored by brain microdialysis was unchanged (Fig. 4A), whereas tissue levels of choline in the striatum increased maximally (Fig. 6A), suggesting that most of exogenously administered choline was rapidly taken up into the intracellular compartment. These results taken together strongly suggest that exogenously administered choline does not stimulate ACh release from the outside of the cholinergic neurons but that it is rapidly taken up into the neurons to enhance ACh release.

Two types of choline uptake mechanisms are reported: high affinity and low affinity (Haga and Noda, 1973; Yamamura and Snyder, 1973). It is considered that the high-affinity type is specific for the cholinergic neurons and that it is involved in ACh biosynthesis in the neurons (Guyenet et al., 1973; Barker and Mittag, 1975; Simon et al., 1976). In contrast, the low-affinity type is considered to be present not only in the cholinergic neurons but also in other cells such as glial cells (Massarelli et al., 1974), and it is reported not to be involved in ACh biosynthesis (Haga and Noda, 1973; Yamamura and Snyder, 1973). Since administered choline was taken up very rapidly without a change in the extracellular concentration of choline (Figs. 3A and 6A), we cannot know whether it was taken up only by the high-affinity choline uptake mechanism or by both of mechanisms. Thus, it is totally unknown whether the increase in the intracellular choline concentration, which is represented by tissue levels of choline, reflects either an increase in the choline concentration only in the cholinergic neurons or an increase in the cholinergic neurons and glial cells.

Following administration of choline, tissue levels of ACh increased (Fig. 6B). Because ACh could not be detected in the extracellular fluid both before and after administration of choline when brain microdialysis was performed with physostigmine-free Ringer solution (Fig. 4B and C), tissue levels of ACh are considered to exclusively reflect the concentration of ACh in the intracellular compartment.

It is generally accepted that the change in the intracellular ACh concentration (reflected by tissue levels of ACh in the present study) is determined by the difference between the rate of ACh biosynthesis and the rate of ACh release from nerve terminals. In the present study, both the intracellular ACh concentration and the ACh release monitored by brain microdialysis increased following administration of choline (Figs. 3B and 6B). These results strongly suggest that choline administration increased ACh biosynthesis in the cholinergic neurons. Thus, the present study confirmed the notion that both ChAT and a high-affinity choline uptake mechanism in the cholinergic neurons are not saturated with its substrate, choline (Tuček, 1984, 1985). However, since ACh is reported to be synthesized in muscle fibers by enzymes other than ChAT (Miledi et al., 1982), it remains the possibility that ACh synthesis in noncholinergic neurons by enzymes other than ChAT was pushed by high intracellular concentration of choline. Considering that the time course and the extent of the increase in ACh biosynthesis [which can be represented by the sum of the increase in the intracellular ACh concentration (Fig. 6B) and the increase in ACh release (Fig. 3B)] correlated well with the increase in ACh release, it seems that the increase in ACh release following administration of choline is the result of the increase in ACh biosynthesis, although the precise mechanism of the increase in ACh release is unknown.

Thus, the present study showed that when choline was intracerebroventricularly administered, it was rapidly taken up into the cholinergic neurons, where it enhanced the release and biosynthesis of ACh.

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