This study shows that the vesicular-bound pool of ACh is capable not only of emptying but also of replenishing its contents independently of the cytoplasm. Depleted vesicular ACh is refilled with newly synthesized ACh formed from extracellular choline but not from preformed or newly synthesized cytoplasmic ACh; therefore, it appears that cholinergic vesicles may be supplied with ACh by vesicular choline acetyltransferase (E.C. 2.3.1.6) activity.

Lithium treatment reduces the level of vesicular-bound ACh by 70 percent without altering the level of the cytoplasmic ACh, which suggests that cytoplasmic ACh may not be mobilized into vesicles even when their ACh pools are expandable. This observation does not dismiss the possibility that ACh enters vesicles by diffusion, since lithium treatment could conceivably reduce the ACh content of all vesicles by 70 percent which might be inadequate to permit diffusion from the cytoplasm into the vesicles.

Accumulation of extracellular ACh by brain tissue, unlike that of extracellular choline, is believed to be nonspecific for cholinergic nerve endings (*9*). Consequently, the ratios of labeled to total ACh found in subcellular compartments after incubations with [^{14}C]ACh cannot be compared with those attained after incubations with [^{14}C]choline since more of the extracellular [^{14}C]ACh would be expected to enter noncholinergic nerve endings. That lithium pretreatment facilitates the movement of newly synthesized ACh (formed from extracellular [^{14}C]choline) but not preformed [^{14}C]ACh into vesicles suggests that newly synthesized and not preformed ACh replaces ACh that has been released from vesicles. Whether extracellular ACh enters the vesicles directly or by way of the cytoplasm and then the vesicles cannot be determined from this study.

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Choline Administration: Modification of the Central Actions of Atropine

Abstract. *The anticholinergic agent atropine decreases acetylcholine concentrations and increases high-affinity choline uptake in cortical and hippocampal regions of rat brain. Administration of choline 1 hour before atropine prevents both of these atropine-induced alterations. These findings suggest that alterations in acetylcholine precursor availability may modify the effects of centrally active anticholinergic agents.*

Recent investigations indicate that there is a positive relationship between choline availability and brain concentrations of acetylcholine (ACh) (*1*). Dose- and time-related increases in ACh concentrations have been noted after both injection and dietary administration of choline (*2, 3*). There is also a positive correlation between dietary choline administration and the concentration of nicotinic receptors in brain (*4*). To elucidate the pharmacological significance of these findings, we investigated the effects of increased choline availability on atropine-induced alterations in central cholinergic neurons. The effects of atropine on ACh concentrations and on Na^+-dependent high-affinity choline uptake in rat brain hippocampus and cortex were studied and compared in naive and choline-treated animals.

Male Sprague-Dawley rats (175 to 225 g) were maintained on a 12-hour light-dark cycle with food (Standard Purina Rat Chow, 9.5 nmole of choline per kilogram) and water freely available. Drugs were dissolved in saline and administered (0.1 ml per 100 g of body weight) between 9 and 11 a.m. Rats were injected with either saline or atropine sulfate (5 mg/kg, intraperitoneally) 1 hour after the administration of either saline or choline chloride (60 mg/kg, intraperitoneally) and killed 30 minutes after the second injection. Results in our laboratory have shown that the maximum ACh depletion is achieved 30 minutes after the administration of atropine (*5*). Furthermore, 1 hour was allowed to elapse after choline administration to minimize any transient alterations induced by this agent (*2*). Animals were killed by microwave irradiation (2.4 seconds, 2750 Mhz, 1250 watts) focused on the head for ACh and choline determinations (*6*) or by decapitation for studies of Na^+-dependent high-affinity choline uptake.

For ACh and choline analyses, brains were removed, chilled in ice-cold pentane, and dissected into discrete brain areas by using a brain slicing apparatus (*7*). Slices from the hippocampus and midbrain cortex were isolated, homogenized in 2 ml of acetonitrile (containing 2

Table 1. Effects of choline pretreatment on atropine-induced ACh depletion in rat cortex and hippocampus. Values are expressed as nanomoles of ACh or choline per gram of brain tissue ± the standard error of the mean. The number of determinations is given parentheses. The procedure and doses are described in the text.

Treatment	Cerebral cortex		Hippocampus	
	ACh (nmole/g)	Choline (nmole/g)	ACh (nmole/g)	Choline (nmole/g)
Saline (controls)	14.3 ± 0.67 (7)	14.9 ± 1.09 (7)	24.9 ± 1.20 (5)	19.5 ± 2.43 (5)
Choline	13.1 ± 1.17 (7)	15.8 ± 1.61 (7)	22.9 ± 1.82 (6)	26.1 ± 2.97 (5)
Atropine	11.4 ± 0.84*(7)	17.4 ± 1.38*(6)	19.4 ± 1.00*(7)	28.6 ± 2.57*(7)
Atropine following choline	13.0 ± 1.13 (6)	18.4 ± 2.26 (6)	22.5 ± 1.60 (6)	26.9 ± 5.36 (5)

*Significantly different from control ($P < .05$) by two-tailed Student's t-test.

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nmole of propionylcholine iodide), and prepared for the simultaneous determination of ACh and choline by pyrolysis gas chromatography (*8, 9*).

To study for Na^+-dependent high-affinity choline uptake, brains were removed and chilled in ice-cold 0.32*M* sucrose. Slices from the cortex and hippocampus were isolated as described and homogenized in 5 ml of 0.32*M* sucrose. Synaptosomes were prepared by centrifugation of the homogenate at 0°C for 10 minutes at 1000*g*, followed by recentrifugation of the supernatant at 0°C for 20 minutes at 17,500*g*. The pellet (P_2) containing the synaptosomes was resuspended in 3 ml of 0.32*M* sucrose, and 100-μl portions were incubated with [^{3}H]choline (final concentration, $3.9 \times 10^{-8}M$) for 4 minutes at 37°C in both Na^+-free and Na^+ buffer (*10*). Synaptosomal protein was determined by the method of Lowry *et al.* (*11*).

The effects of administration of atropine or choline, or both, on ACh and choline concentrations in the cortex and hippocampus are shown in Table 1. Atropine sulfate (5 mg/kg) significantly decreased ($P < .05$) the ACh content in both the cortex and hippocampus to approximately 80 percent of the control values. The choline levels in the cortex and hippocampus increased to 177 and 147 percent of the control values, respectively.

Choline administration did not alter the ACh concentration significantly in either of the brain areas studied, from 15 to 90 minutes after drug injection (*12*). When animals were treated with choline chloride 60 minutes before the administration of atropine, however, the ACh-depleting action of atropine was reduced. In both cortex and hippocampus, choline pretreatment decreased the effects of atropine so that ACh concentrations in these animals were not significantly different from the control values.

Atropine significantly ($P < .05$) increased Na^+-dependent high-affinity choline uptake into synaptosomes from the cortex and hippocampus to 142 and 127 percent of the control values, respectively (Table 2). Increases were also noted after choline administration (124 percent of the control value in the cortex and 114 percent in the hippocampus), but the values were not significantly different from control values. When animals were treated with choline 60 minutes before atropine administration, the previously observed effects of atropine were totally prevented. The Na^+-dependent high-affinity choline uptake into synaptosomes from animals treated with choline and then by atropine was not different from that in controls and was significantly less ($P < .05$) than that in atropine-treated animals.

The effects of atropine on brain levels of ACh are thought to result primarily from presynaptic interactions, although the specific membrane site of the interactions remains unknown. Atropine potentiates ACh turnover and release, resulting in a decrease in neurotransmitter content (*13–15*). Furthermore, recent studies have shown that this atropine-induced loss of ACh occurs from the vesicular fraction, while cytoplasmic pools remain unaltered (*16*), which supports the hypothesis of neurotransmitter release from storage sites. Since labeled ACh formed in nerve terminals after the administration of radiolabeled choline is bound to a high-molecular-weight component of the synaptosomes (*17*), presumably the vesicles (*18*), choline may decrease the ACh-depleting action of atropine by altering mechanisms regulating the synthesis, storage, or release of vesicular ACh.

Another indicator of cholinergic function, Na^+-dependent high-affinity choline uptake, appears to be linked to cholinergic nerve terminal activity, and the rate of impulse flow most likely regulates the rate of this uptake system (*10*). Atropine is known to enhance cholinergic activity (*13, 14*), thereby potentiating ACh turnover (*15*) and increasing Na^+-dependent high-affinity choline uptake (*19*). These results have been interpreted as indicating that choline uptake increases in proportion to the need for the precursor choline (*10*). However, if the nerve terminal has an abundant supply of choline (either free or bound in the form of phospholipids), choline uptake may not be altered by impulse traffic. Therefore, exogenous choline administration, which has been shown to supply significant amounts of precursor for ACh synthesis in the brain (*20*), may prevent the atropine-induced increase in the high-affinity choline uptake system.

Table 2. Effects of choline pretreatment on atropine-induced increase of Na^+-dependent high-affinity choline uptake in cortical and hippocampal synaptosomes. Values are expressed as picomoles of choline per 4 minutes per milligram of protein. Each value represents the mean of six determinations ± the standard error of the mean. The procedure and doses are described in the text.

Treatment	Uptake (pmole/mg per 4 minutes)	
	Cerebral cortex	Hippocampus
Saline (controls)	0.57 ± 0.04	2.0 ± 0.11
Choline	0.71 ± 0.05	2.3 ± 0.14
Atropine	0.81 ± 0.06*	2.5 ± 0.25*
Atropine following choline	0.64 ± 0.04†	2.1 ± 0.08†

*Significantly different from value for controls ($P < .05$). †Significantly different from value for atropine-treated animals ($P < .05$).

In summary, the findings presented in this report support the hypothesis that the nutritional intake of choline may be a significant factor in determining the action of drugs on cholinergic synapses (*3*). Further studies on the effects of choline availability will undoubtedly increase our knowledge of basic cholinergic mechanisms. Such studies are also essential for understanding what effects nutritional state may have in determining the efficacy and consequent usefulness of pharmacological agents which affect the cholinergic system.

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