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KINETICS OF [^{3}H]CHOLINE AND [^{3}H]ACETYLCHOLINE METABOLISM IN SEVERAL REGIONS OF RAT BRAIN FOLLOWING INTRACEREBROVENTRICULAR INJECTION OF [^{3}H]CHOLINE

EFFECTS OF HALOPERIDOL

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Abstract—Intracerebroventricular (icv.) injection of [^{3}H]choline in conscious rats produced a rapid, efficient labeling of brain choline and acetylcholine (ACh) stores without altering steady-state levels of endogenous ACh. The kinetics of [^{3}H]choline and [^{3}H]ACh metabolism were measured in seven brain regions for up to 10 min following icv. administration of [^{3}H]choline. The initial rate of formation of [^{3}H]ACh varied in different brain areas, being greatest in the striatum and least in the hypothalamus. In contrast, the rate of [^{3}H]choline metabolism was similar in all regions of the brain. Pretreatment of rats with haloperidol resulted in an increase in the apparent synthesis rate of ACh only in the striatum and rostral hypothalamus, pointing to possible dopaminergic–cholinergic interaction in these regions.

Isotopic and nonisotopic methods to biochemically estimate the rate of central cholinergic activity in experimental animals have been described. In nonisotopic approaches, the rate of acetylcholine (ACh) utilization usually is determined by measuring the change in ACh levels as a function of time after synthesis is prevented (with hemicholinium) [1–3] or hydrolysis is prevented (with cholinesterase inhibitors) [4]. This approach has found wide applicability; however, hemicholinium and cholinesterase inhibitors are potent pharmacological agents which may produce behavioral or physiological changes in the experimental animal. Such effects may interfere with the action of a drug or response which is being evaluated in terms of its effects on ACh turnover rate. Under such circumstances, isotopic methods of turnover analysis would be more appropriate. These involve the injection of radioisotopic or stable isotopic tracers to label precursor and ACh pools in the brain [5–9]. In both isotopic approaches, labeled precursor is pulse injected or slowly infused into the systemic circulation of experimental animals, and it then equilibrates with brain choline metabolites. A major drawback to methods involving systemic injection of labeled choline is that choline transport from blood to brain tissue may be altered in certain disease states or in response to various drug treatments. Altered kinetics of precursor transport into the brain could lead to measurement of changes in labeled ACh kinetics unrelated to neuronal activity. A second limitation of this technique is the large amount of isotope that must be administered to significantly label brain stores in the adult rat. Cost considerations often may preclude large turnover studies in this experimental animal.

In this study [^{3}H]choline was introduced directly into the cerebrospinal fluid of conscious rats through previously implanted guide cannulae. This route of administration produces a consistent and efficient labeling of endogenous brain choline and ACh. The utility of this technique was established in studies of ACh biosynthesis in seven discrete regions of rat brain. Using this approach, the effects of haloperidol, a neuroleptic agent which has been reported to increase the rate of metabolism of ACh in the striatum of experimental animals [10–13], were examined.

MATERIALS AND METHODS

Male, Wistar rats (220–320 g) were obtained from Taconic Farms, Inc., Germantown, NY. They were housed in our animal care facilities in small groups and maintained on Wayne Lab Blocks and tap water *ad lib.*, with a 12-hr light, 12-hr dark cycle for 2 weeks prior to surgery. At this time rats were anesthetized with ketamine hydrochloride (150 mg/kg, i.p.) and placed in a stereotaxic frame according to Konig and Klippel [14]. A 22 gauge × 12 mm, Teflon cannula guide (Plastic Products, Inc., Roanoke, VA) was inserted through a burr hole in the skull and implanted over the left lateral cerebral ventricle at 1.5 mm lateral to the bregma and 2 mm below the cortical surface. The entire cannula assembly, including anchoring screws, was made of Teflon or nylon, these materials being transparent to microwave irradiation (see below). Following surgery, the rats were placed in individual cages and allowed to recover for 1 week before the experiment.

Labeling of endogenous brain choline and acetylcholine stores. A stainless steel injection cannula (28 gauge) was connected to a 50-μl Hamilton syringe via a 15-cm length of polyethylene tubing. The cannula and tubing were filled with a saline solution containing 20 μCi of [^{3}H]choline (80 Ci/mmole) and were connected to a syringe which was placed in a

Harvard pump. Rats were gently restrained by hand, and the injection cannula was then inserted through the guide cannula to a depth of 3.5 mm below cortex. [^{3}H]Choline was injected into the cerebral ventricles at a rate of 2 μCi·μl^{-1}·sec^{-1} for 10 sec. This was followed immediately by an additional 5 μl of saline to flush the cannula. The rats were killed 1, 2, 5 or 10 min following the termination of the injection by a beam of microwave irradiation (2450 MHz, 3.8 kW for 2.2 to 2.3 sec) focused on the head using a Gerling–Moore model 4104 Metabostat (Santa Clara, CA). Rats were placed in the microwave restraining chamber no longer than 45 sec before exposure to minimize any effects of prolonged immobilization stress. Experiments were always performed at the same time of day, such that the animals were killed 3–5 hr after resumption of lighting in the animal rooms.

Brains were cooled on ice and dissected into cerebral cortex, striatum, hippocampus, midbrain-pons, medulla oblongata, and hypothalamus which was bisected coronally into rostral and caudal segments. The tissues were placed on dry ice, weighed, and homogenized in 1 N formic acid–acetone (15:85) in glass–Teflon homogenizers at a concentration of about 100 mg tissue/ml (except the hypothalamic segments which were at 50 mg/ml); extraction was 95–100% complete. Homogenates were centrifuged (200 *g*, 20 min), and 0.5-ml aliquots of the resulting supernatant fractions were dried under a stream of dry air at 50°. At this point the samples were stored, dessicated, and frozen (−20°) until analyzed (within 3 weeks).

Separation of ^{3}H*-labeled choline metabolites.* The dried extracts were reconstituted in 0.5 ml of 10 mM phosphate-buffered saline (pH 7.0) and centrifuged at 12,000 *g* for 15 min. Aliquots (5 μl) of the supernatant fraction were taken for measurement of total radioactivity. Cellulose thin-layer chromatography (TLC) plates (Eastman) were pre-spotted at 1.3-cm intervals with 5 μl of a solution containing 2 mg/ml of unlabeled ACh iodide and choline chloride in absolute ethanol. Extract (5 μl) from each of the seven brain regions was spotted directly over the pre-spot. The absolute values for radioactivity ranged from 1000 dpm (cortex) to 7000 dpm (rostral hypothalamus) per 5-μl sample. The TLC plates were placed in pre-equilibrated Gelman chromatography chambers containing *n*-butanol–water–ethanol–acetic acid (8:3:2:1) and developed for 2.5 hr. Areas of the plates containing choline and ACh were visualized by exposure to iodine vapors. R_f values for choline and ACh were, respectively, 0.5 and 0.56. The spots were marked and the iodine was removed by steaming the plates. Spots were cut out and placed in scintillation vials. The radioactivity was eluted quantitatively by adding 1 ml of 0.01 N HCl–ethanol (30:70) to each vial and shaking them gently in a 50° water bath for 1 hr. Radioactivity was measured by adding 10 ml of scintillation mixture (Riafluor, New England Nuclear, Boston, MA) and counting in a Beckman scintillation spectrometer. Sample cpm were corrected for blank and quench characteristics. Overlap of the radioactivity associated with ACh and choline spots was less than 1%. [^{3}H]ACh and [^{3}H]choline "contents" of tissues were expressed as percentages of the total extracted radioactivity. This eliminated variability between animals due to differences in the total amount of label reaching each brain area. The specific activity of ACh was defined as % [^{3}H]ACh/(nmoles ACh per g brain tissue) as suggested by Nordberg and Sundwall [15]. Samples were taken in duplicate for all determinations.

Measurement of endogenous acetylcholine. Endogenous ACh was determined from 10 μl of reconstituted brain extracts by a radioimmunoassay procedure [16] previously described, except that [^{14}C]ACh was used as the labeled ligand in place of [^{3}H]ACh. This modification was necessary because [^{3}H] present in the aliquots from the tracer experiments could interfere with the radioimmunoassay. Working solutions were made up in phosphate-buffered saline (PBS) containing 2×10^{-5} M physostigmine just before use. Serial dilutions of the standard (1.6 to 100 pmoles) or of 10-μl aliquots of the samples were incubated with 100 μl of antiserum (final dilution 1:375) in PBS containing 0.125% bovine gamma globulin, 0.5% bovine serum albumin both from the Sigma Chemical Co. (St. Louis, MO), and 100 μl [^{14}C]ACh, 60 mCi/mmole (final dilution 1:3750), in a total volume of 500 μl. Antibody bound label was precipitated by addition of an equal volume of saturated ammonium sulfate. Following centrifugation, the pellet was dissolved in 5 ml of Riafluor. The limit of sensitivity of the assay under these conditions was 3 pmoles of ACh.

Statistical analysis. Values are presented as means ± S.E. The difference between means of two groups was estimated using Student's *t*-test for unpaired data and was considered significant when $P < 0.05$. Comparisons between means of several populations were performed using analysis of variance.

RESULTS

Intracerebroventricular injection of [^{3}H]*choline.* Intracerebroventricular (icv.) injection of 20 μCi (0.25 nmole) of [^{3}H]choline resulted in a rapid labeling of brain ACh and choline stores (Figs. 1 and 2). However, steady-state levels of ACh were unaltered by this tracer amount of choline for 10 min following injection (Table 1). Total formic acid–acetone extractable radioactivity (^{3}H-tot) was distributed unevenly in the brain, with the cerebral cortex having the least, and the hypothalamic regions having the greatest accumulations (Table 2). The amount of radioactivity for an individual brain region did not change throughout the course of the study (1–10 min), indicating that distribution of label throughout the brain was essentially complete within 1 min of the injection (Table 2).

The time course of [^{3}H]ACh formation in brain regions following icv. injection of [^{3}H]choline is shown in Fig. 1. [^{3}H]ACh content increased rapidly for 5 min and decreased between 5 and 10 min after injection. The initial rate of formation of [^{3}H]ACh varied in different brain areas. [^{3}H]ACh content 2 min after injection was lowest in the rostral hypothalamus (3.7%) and greatest in the striatum (18.1%). The low percentage of [^{3}H]ACh measured in the hypothalamus was not related to the relatively large accumulation of label in that area, since

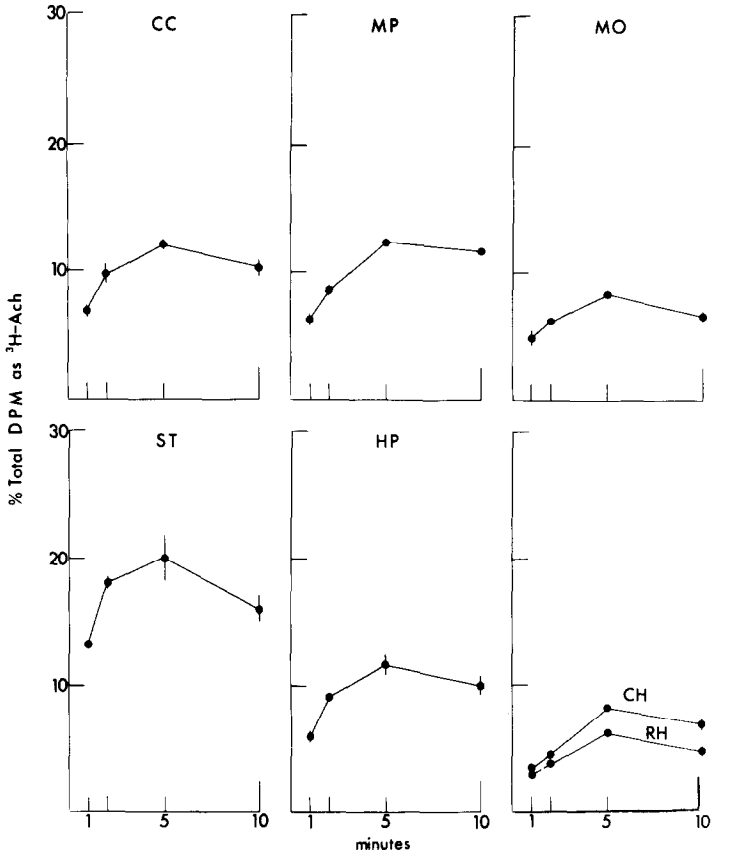

Fig. 1. Time course of [^{3}H]ACh formation in several brain regions following icv. injection of 20 μCi of [^{3}H]choline. Time points refer to intervals between the end of injection and exposure to microwave irradiation. The vertical lines indicate S.E., which in many cases were smaller than the diameter of the symbols. Each point is the mean of four to five experiments. Abbreviations: CC, cerebral cortex; MP, midbrain-pons; MO, medulla oblongata; ST, striatum, HP, hippocampus, RH, rostral hypothalamus; and CH, caudal hypothalamus. The ordinate is the percentage of total extractable radioactivity (^{3}H-tot) found as [^{3}H]ACh.

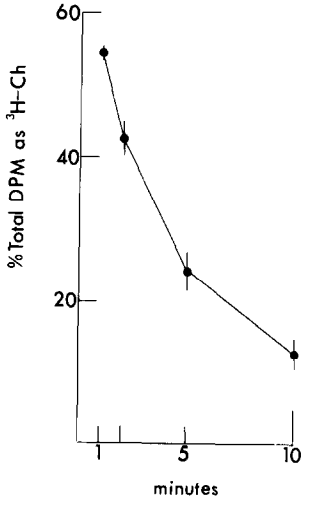

Fig. 2. Time course of [^{3}H]choline ([^{3}H]Ch) in the cerebral cortex following icv. injection of 20 μCi of [^{3}H]choline. The vertical lines indicate S.E. and each point is the mean of four to five experiments.

[^{3}H]ACh content and the total radioactivity in brain regions were poorly correlated ($r = 0.49$).

[^{3}H]Choline declined exponentially from 1 to 10 min after injection in all brain areas. In contrast to the regional variation in the rate of [^{3}H]ACh formation, [^{3}H]choline declined at a similar rate in all brain regions (data not shown). The $T_{\frac{1}{2}}$ values for [^{3}H]choline decline ranged from 3.0 to 4.4 min (average 3.8 ± 0.19 min). As an example, the decline in [^{3}H]choline content of the cerebral cortex is depicted in Fig. 2.

Effect of haloperidol on the biosynthesis of [^{3}H]*ACh.* It has been well established by several other laboratories that certain neuroleptic agents including haloperidol produce an increase in the rate of activity of striatal cholinergic neurons [10–13]. The purpose of the following experiment was to examine the effects of haloperidol on the rate of regional [^{3}H]ACh biosynthesis following icv. injection of [^{3}H]choline. Rats were injected subcutaneously with 1 mg/kg of haloperidol 1 hr before receiving an icv. injection of [^{3}H]choline as described above. These animals were then subjected to micro-

Table 1. Regional brain ACh content before and after icv. injection of 250 pmoles (20 μCi) [^{3}H]choline*

Brain region	ACh content (nmoles/g)				
	Time following injection (min)				
	0	1	2	5	10
Cerebral cortex	22.7 ± 3.1	18.7 ± 2.0	23.3 ± 1.1	18.8 ± 1.8	18.6 ± 1.2
Midbrain-pons	31.4 ± 1.3	29.2 ± 1.7	31.9 ± 1.3	27.7 ± 1.5	28.2 ± 0.69
Medulla	23.2 ± 1.7	24.0 ± 1.7	26.4 ± 1.3	23.2 ± 2.1	22.0 ± 1.3
Striatum	60.2 ± 4.1	57.5 ± 3.2	62.7 ± 6.1	54.8 ± 2.6	56.0 ± 3.0
Hippocampus	25.4 ± 3.1	24.0 ± 2.4	26.3 ± 1.3	23.9 ± 3.2	21.6 ± 1.9
Rostral hypothalamus	20.8 ± 0.6	22.8 ± 0.69	24.1 ± 2.0	18.0 ± 1.9	20.5 ± 1.8
Caudal hypothalamus	28.1 ± 2.8	27.2 ± 2.2	27.8 ± 1.9	23.8 ± 1.1	24.2 ± 1.9

* Each mean ±S.E.M. is the average of four to five experiments.

wave irradiation at 2 min following [^{3}H]choline injection. The results from these experiments are summarized in Table 3. Haloperidol significantly reduced the striatal ACH content but did not affect the [^{3}H]ACh content. Therefore, ACh specific activity was increased significantly by 37%. Haloperidol did not alter the level of [^{3}H]choline in any brain region. The neuroleptic also produced a significant 19% increase in the level of [^{3}H]ACh in the rostral hypothalamus and a 31% increase in specific activity.

Calculation of ACh turnover rate. While determination of ACh biosynthesis at one early time point following icv. injection of [^{3}H]choline can measure changes in cholinergic neuronal activity, the data from multiple time points also may be employed to calculate the apparent turnover rate of ACh (TR_{ACh}). The time course of ACh specific activity in the various brain regions following icv. injection of [^{3}H]choline is shown in Fig. 3. The highest levels (measured after 5 min) were reached in the cerebral cortex and hippocampus. Since it is not possible to directly measure the specific activity of choline serving as precursor for ACh synthesis, the turnover rate of ACh was calculated employing only the values of ACh specific activity as suggested by Zilversmit [7].

Since [^{3}H]choline declined approximately exponentially, the assumption was made that the specific activity of brain choline serving as precursor for ACh synthesis decreases exponentially with time following icv. injection. The model also assumes a single, open metabolic pool of brain ACh where random metabolic breakdown prevails. Under these conditions, the fractional rate constant for ACh (fractional turnover rate, k) can be obtained by decomposing the curve of the semilog plot of ACh specific activity as a function of time into two single exponentials. This was done by drawing a tangent to the descending portion of the curve, subtracting values for the early ascending portion of the curve, and replotting the differences. The curve with the steeper slope was obtained by linear regression ($r < 0.95$ in all cases) and the $T_{\frac{1}{2}}$ gave k (min^{-1}) by the equation $1/k = 1.44T_{\frac{1}{2}}$. The turnover rate (in nmoles·g^{-1}·min^{-1}) of ACh is the product of k and the ACh content (nmoles/g). The calculated values of k can only be considered approximations because of the small number of time points measured. The values calculated for k and TR_{ACh} for each brain region are listed in Table 4. The relative degrees of cholinergic activity in these regions predicted by calculation of TR_{ACh} are consistent with the relative rates of [^{3}H]ACh accumulation, i.e. greatest in the striatum and least in rostral hypothalamus.

DISCUSSION

The method of introduction of labeled precursor directly into the cerebrospinal fluid of rats has been applied to the analysis of regional brain catecholamine [18] and serotonin [19] turnover. Using a similar approach, the dynamics of [^{3}H]choline and [^{3}H]ACh metabolism in seven regions of rat brain following icv. injection of a tracer amount (0.25 nmole) of [^{3}H]choline have been studied.

Table 2. Total extractable radioactivity at several times following icv. injection of 20 μCi of [^{3}H]choline*

Brain region	Radioactivity (dpm·(mg, tissue ×10^3)$^{-1}$)			
	Time following injection (min)			
	1	2	5	10
Cerebral cortex	3.2 ± 0.4	2.7 ± 0.3	3.3 ± 0.3	3.1 ± 0.5
Midbrain-pons	11.7 ± 1.7	12.8 ± 0.7	12.9 ± 0.4	11.3 ± 0.5
Medulla	10.4 ± 1.1	9.2 ± 1.3	12.1 ± 0.1	10.4 ± 1.6
Striatum	9.9 ± 1.0	10.6 ± 1.4	10.1 ± 0.6	9.9 ± 0.6
Hippocampus	8.4 ± 2.1	7.9 ± 0.6	7.5 ± 0.5	7.1 ± 1.1
Rostral hypothalamus	27.3 ± 3.1	24.2 ± 2.5	26.6 ± 1.1	22.5 ± 0.4
Caudal hypothalamus	17.5 ± 4.4	15.1 ± 0.5	16.9 ± 0.2	18.5 ± 0.8

* Total dose of [^{3}H]choline, 250 pmoles. Each mean is the average of four to five experiments.

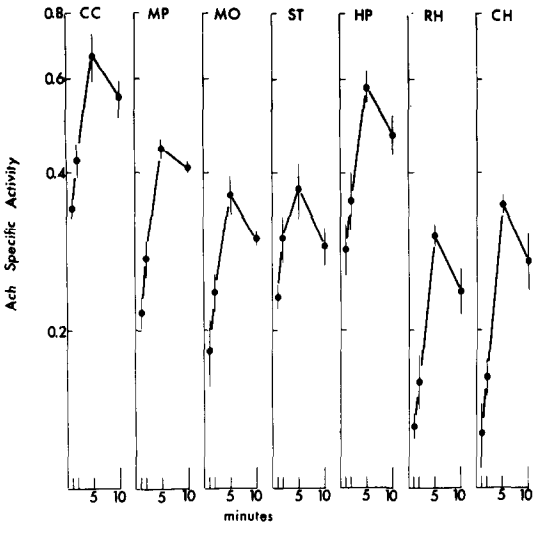

Fig. 3. Time course of ACh specific activity in several brain regions following icv. injection of 20 μCi of [^{3}H]choline. The vertical lines indicate S.E. and each point is the mean of four to five experiments. Abbreviations as in the legend of Fig. 1.

Radioactivity was unevenly distributed in the brain areas by 1 min after injection of [^{3}H]choline. These regional differences might reflect different degrees of diffusion throughout various parts of the brain, although it is difficult to attribute the marked differences in label accumulation in rostral and caudal hypothalamic segments purely to diffusional differences. Despite these differences, sufficient label was incorporated in all areas of the brain following injection of only 20 μCi of [^{3}H]choline to permit the detection of [^{3}H]choline and [^{3}H]ACh. While the amounts of tritium in each brain region remained constant for 10 min, the levels of [^{3}H]choline and [^{3}H]ACh changed rapidly. One minute after injection, [^{3}H]choline accounted for between 50 and 56% of the total radioactivity present in each brain region. [^{3}H]Choline content then declined approximately exponentially at a similar rate in all brain regions. In contrast, the kinetics of [^{3}H]ACh synthesis following icv. injection of [^{3}H]choline varied in different brain areas.

Atweh and Kuhar [20] have provided evidence that the rate of [^{3}H]ACh formation in the brain following a pulse intravenous injection of [^{3}H]choline reflects the actual rate of cholinergic activity. They have demonstrated that measurement of hippocampal [^{3}H]ACh formation at an early time interval following pulse intravenous injection of [^{3}H]choline is the best indicator of changes in neuronal cholinergic activity in that region. Their attempts to include rates of [^{3}H]choline metabolism in the calculation of hippocampal ACh synthesis rate often led to erroneous conclusions concerning measured changes in cholinergic activity. Free choline and [^{3}H]choline levels were not useful indicators of cholinergic activity. The present findings of similar rates of [^{3}H]choline metabolism in brain regions showing wide variations in [^{3}H]ACh metabolism, along with the fact that haloperidol had no effect on striatal choline or [^{3}H]choline levels, support this concept. Since the only other brain metabolite of choline known to be labeled significantly during 30 min following injection of labeled choline is phosphorylcholine [21], the rate of decline of [^{3}H]choline in brain may reflect its incorporation into the much larger pool of phosphorylcholine. For this reason it may not be appropriate to consider, as others have previously [5–9], the rate of change of choline specific activity in calculating the fractional turnover rate of ACh.

While the initial rate of [^{3}H]ACh accumulation

Table 3. Effect of haloperidol on the biosynthesis of regional brain acetylcholine at 2 min following icv. injection of [^{3}H]choline*

Brain region	Treatment†	% Total dpm as [^{3}H]choline	% Total dpm as [^{3}H]ACh	ACh content (nmoles/g)	ACh sp. act.‡
Cerebral cortex	C	42.6 ± 2.4	9.8 ± 0.8	23.2 ± 1.1	0.42 ± 0.93
	H	46.4 ± 2.0	8.9 ± 0.6	21.4 ± 1.7	0.42 ± 0.03
Midbrain-pons	C	42.8 ± 2.9	8.6 ± 0.8	31.9 ± 1.3	0.27 ± 0.02
	H	47.2 ± 1.9	8.2 ± 0.3	31.5 ± 1.8	0.26 ± 0.02
					0.25 ± 0.2
Medulla oblongata	C	43.9 ± 5.0	6.2 ± 0.3	25.4 ± 1.3	0.25 ±
	H	45.6 ± 1.8	6.7 ± 0.2	27.2 ± 1.0	0.01
Striatum	C	43.9 ± 2.8	18.1 ± 0.5	62.7 ± 6.1	0.30 ± 0.03
	H	41.0 ± 5.1	17.1 ± 1.3	41.2 ± 2.2§	0.41 ± 0.02§
Hippocampus	C	43.9 ± 1.7	9.2 ± 1.0	26.3 ± 1.3	0.35 ± 0.04
	H	44.0 ± 4.5	9.4 ± 0.3	23.7 ± 1.3	0.40 ± 0.03
Rostral hypothalamus	C	45.3 ± 2.9	3.7 ± 0.2	24.1 ± 2.0	0.16 ± 0.01
	H	40.8 ± 4.4	4.4 ± 0.2§	21.4 ± 1.5	0.21 ± 0.21‖
Caudal hypothalamus	C	45.4 ± 2.9	4.4 ± 0.1	27.8 ± 1.9	0.16 ± 0.01
	H	45.3 ± 3.7	5.1 ± 0.3	28.2 ± 2.5	0.19 ± 0.02

* Each mean is the average of five experiments.
† C = controls, H = haloperidol (1 mg/kg, s.c.; 1 hr before [^{3}H]choline).
‡ Specific activity of ACh (% [^{3}H]ACh formed/ACh content).
§ $P < 0.05$, significantly different compared to control value.
‖ $P < 0.10$, significantly different compared to control value.

Table 4. Fractional rate constant (k) and turnover rate (TR_{ACh}) of regional brain acetylcholine

Brain region	k (min^{-1})	TR_{ACh}* ($nmoles \cdot g^{-1} \cdot min^{-1}$)
Cerebral cortex	0.55	12.6 ± 1.7
Midbrain-pons	0.49	15.2 ± 0.6
Medulla	0.46	10.6 ± 0.8
Striatum	0.56	34.2 ± 2.3
Hippocampus	0.51	12.9 ± 1.6
Rostral hypothalamus	0.29	6.0 ± 0.2
Caudal hypothalamus	0.27	7.6 ± 0.8

* $TR_{ACh} = k$ (min^{-1}) × ACh content (nmoles/g).

following [^{3}H]choline injection may be a useful indicator of cholinergic neuronal activity, measurement of ACh specific activity is necessary to fully appreciate the neurochemical changes which take place when the steady state of ACh is perturbed. The haloperidol experiment provides an excellent example of this concept. Haloperidol produced a decrease in the striatal content of ACh without altering the rate of [^{3}H]ACh synthesis (Table 3), thus producing a 37% increase in the ACh specific activity. It has been suggested that haloperidol and other dopamine blocking drugs enhance the activity of cholinergic neurons in the striatum by blocking a tonic inhibitory, dopaminergic input to striatal cholinergic neurons (for reviews see Refs. 22–24). However, the rate of synthesis of ACh is relatively unaffected while the release of ACh is enhanced [25, 26]. Thus, a new, lower steady-state level is gradually attained. Using a nonisotopic method, we reported a similar effect of reserpine on ACh metabolism in the striatum [3]. The present study demonstrated that haloperidol also increased the synthesis of [^{3}H]ACh in rostal hypothalamus but was without effect in the caudal hypothalamus. Both cholinergic [27–30] and dopaminergic [31–33] pathways exist in this brain region, and it is possible that an interaction between cholinergic and dopaminergic neurons occurs in one or more nuclei of the rostral hypothalamus.

Employing a single, open compartment model of rapidly mixing pools of precursor and product, the apparent fractional rate constant for ACh, k, was calculated by assuming that choline specific activity declined exponentially with time [17]. Values for k and TR_{ACh} are presented in Table 4 and appear to be similar to values for analogous brain regions in mice and rats reported by other investigators using different kinetic approaches [7, 8, 25, 34, 35]. More detailed methods of kinetic analysis involving measurement of precursor and product specific activity over multiple time intervals are time consuming, expensive, and offer little more information than measurements of [^{3}H]ACh biosynthesis and ACh specific activity at one early time point following icv. injection of [^{3}H]choline. Based on the present findings with haloperidol, it is clear that this method can detect drug-induced changes in regional brain cholinergic activity.

The icv. route of administration of labeled choline has an important advantage over peripheral injection, however, when drug treatments or disease states are known to alter the transport of precursor from blood to brain tissue. For example, Nordberg [36] has demonstrated that the kinetics of [^{3}H]choline metabolism in the brain following an intravenous pulse injection are altered by drug treatments which produce peripheral hemodynamic changes. Therefore, any drug that affects the state of the cardiovascular system conceivably could alter the kinetics of labeled choline or ACh formed in the brain following peripheral administration of labeled precursor. Depending upon the method employed, such changes may lead to measured changes in TR_{ACh} which are unrelated to central cholinergic neuronal activity.

In summary, the present study (1) describes the use of a rapid, sensitive and economic method to estimate the rate of ACh biosynthesis in discrete regions of rat brain, (2) supports the possibility that the free choline and the labeled choline content of brain tissue after injection of labeled choline do not reflect the choline pool serving as precursor to ACh synthesis, and (3) indicates the possibility of a cholinergic–dopaminergic interaction in the rostral hypothalamus. Many of the published methods for separation of choline and ACh, and measurement of endogenous ACh, may be used in conjunction with direct icv. injection of labeled choline. The present method, which uses TLC for separating [^{3}H]choline and [^{3}H]ACh coupled with the radioimmunoassay procedure for measurement of ACh, requires little sample preparation and no internal standards since recovery at all steps is quantitative. Forty reconstituted samples can be processed in 1 day and analyses may be performed on as little as 3 mg (wet weight) of brain tissue.

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