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Acetylcholine Concentrations and Turnover in Rat Brain Structures during Anesthesia with Halothane, Enflurane, and Ketamine

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Acetylcholine and choline concentrations in brain structures of rats during anesthesia with halothane (0.7–1.0 per cent inspired), enflurane (2.7–3.0 per cent, inspired) and ketamine (40 mg/kg, iv) were measured by gas chromatography. The turnover rate (biosynthesis) of acetylcholine *in vivo* was estimated by infusing phosphoryl(Me-^{14}C)choline intravenously, determining specific activities of choline and acetylcholine, and applying principles of steady-state kinetics to compute the fractional rate constant of acetylcholine. Acetylcholine concentrations in brain structures did not change during anesthesia. Halothane decreased the acetylcholine turnover rates in all parts of the brain. Enflurane decreased the acetylcholine turnover rate in the cerebral cortex only, but not in the caudate nucleus, the hippocampus, and the hypothalamic and thalamic regions. During anesthesia with ketamine, acetylcholine turnover rates were reduced in the caudate nucleus and the hippocampus, but not in the cerebral cortex and the hypothalamic and thalamic regions. The results suggest that acetylcholine turnover rate and utilization are related to anesthetic-induced electrophysiologic changes in cortical and subcortical structures. (Key words: Anesthetics, volatile, halothane; Anesthetics, volatile, enflurane; Anesthetics, intravenous, ketamine; Acetylcholine, concentrations, brain; Acetylcholine, biosynthesis, brain.).

THE UBIQUITOUS PRESENCE of acetylcholine in the brain suggests its role as a neurotransmitter essential for cerebral function. Changes in neural activity induced by anesthesia are expected to alter the release of acetylcholine. Earlier studies have shown that sleep and anesthesia significantly increase the acetylcholine concentrations in the rat brain.[1–3] Schmidt concluded that neuronal depression during halothane anesthesia would decrease the release of acetylcholine with a consequent increase in its concentration in the brain.[3] Anesthetics could also alter the rate of acetylcholine biosynthesis. Several groups have reported that pentobarbital decreases the turnover rate of acetylcholine in the brains of mice and rats.[4–6] Trabucchi *et al.* observed that pentobarbital increased brain acetylcholine concentrations by approximately twofold, and at the same time, markedly decreased acetylcholine turnover rates, in the whole brain of the mouse and in the cerebral cortex of the rat.[6]

We measured acetylcholine and choline concentrations in different regions of rat brain during anesthesia with halothane, enflurane, and ketamine, using gas chromatography. The turnover rate of acetylcholine was estimated by intravenous infusion of phosphoryl(Me-^{14}C)choline during anesthesia. Phosphorylcholine is converted to choline and subsequently incorporated into acetylcholine. Applying the principles of steady-state kinetics, the fractional rate constant and turnover rate of acetylcholine can be calculated.[7,8]

We chose halothane as a representative anesthetic that depresses central nervous system activity uniformly.[9] Enflurane induces electrocortical seizures under certain conditions,[10,11] whereas ketamine, although inducing a state resembling anesthesia, actually increases multiple unit activity in the brain stem and limbic structures with intermittent or continuous hypersynchronous activity in the cerebral cortex and subcortical structures.[9,12,13] The question is: are the various electrophysiologic activities in regions of brain induced by these anesthetics reflected in changes in the concentration and turnover rate of acetylcholine?

Methods

Male Sprague-Dawley rats (Zivic Miller, Pittsburgh) weighing 150–200 g were placed in plastic cylinders. Halothane or enflurane vaporized with oxygen, 40 per cent–nitrogen, 60 per cent using a calibrated Fluotec or Ethranetec vaporizer (Cyprane, North America, Inc.) was delivered to the cylinders. For induction of anesthesia, we used halothane, 3 per cent, or enflurane, 4 per cent, initially. Upon loss

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of response to tail pinching, anesthetic concentration was reduced gradually to maintain light anesthesia. With halothane, an inspired concentration of 0.7 to 1.0 per cent was adequate to prevent movement on tail pinching; with enflurane, the maintenance concentrations ranged from 2.7 to 3 per cent, inspired. Ketamine HCl, 40 mg/kg, was injected intravenously. Loss of righting reflex lasted 10–15 minutes. During anesthesia, rectal temperature was monitored with thermocouples and a Yellow Springs telethermometer. Body temperature was maintained at 37–38 C with an infrared lamp.

Fifteen, 30 and 45 minutes after induction of anesthesia with halothane or enflurane, groups of rats (five or six in each group) were killed by microwave irradiation (2.5 kw at 2.45 GHz) focused to the head for two seconds to inactivate all enzymes.[14] Following ketamine injection, groups of rats were killed at 6 and 11 minutes. For each anesthetic a control group of unanesthetized rats was killed in the same manner.

Brain parts, cerebral cortex, caudate nucleus, hippocampus, and hypothalamic and thalamic regions, were dissected and placed immediately into ice-cold 0.4 N perchloric acid and homogenized. After centrifugation (35,000 $\times g$, 20 min at 0 C) the supernatants containing acetylcholine and choline were processed according to the methods described by Hanin *et al.*[15,16] and Cheney *et al.*[17] for measurement of acetylcholine and choline concentrations, using gas chromatography. (See Appendix for details.) Five nmoles of butyrylcholine chloride were added to each sample as internal standard. Protein concentrations in the sediment were determined by the method of Lowry.[18]

For the estimation of acetylcholine turnover rate we infused intravenously phosphoryl(Me-^{14}C)choline (44 μCi/μmol, New England Nuclear Corp.) at a rate of 30 μCi/kg/min for 6 min, total volume, 1.2 ml, starting 30 min after induction of inhalational anesthesia, or 5 min after iv injection of ketamine. For each experimental group (n = 6), control unanesthetized rats received the same infusion of phosphoryl(Me-^{14}C)choline. At the end of infusion rats were killed by focused microwave irradiation, and brains were dissected and processed for gas chromatography as described above. Five nmoles of ^{14}C-butyrylcholine were added to each sample as internal standard. In this series, the effluent gas from the chromatographic column was split, with one tenth directed to the flame ionization detector and nine tenths diverted to a fraction collector with tubes containing trapping medium (molecular sieve 5A, 45–60 mesh, Hewlett-Packard, Avondale, Pa.), timed to collect those fractions of effluent gas containing acetylcholine, choline and butyrylcholine. Radioactivity of trapped gas was measured by transferring the molecular sieve in these tubes to counting vials containing 20 ml of scintillation fluid. A Beckman scintillation spectrophotometer was used. For each rat, acetylcholine and choline concentrations (nmol/mg protein) and their respective specific activities (dpm/nmol) were used to calculate the fractional rate constants of acetylcholine (k_B) according to the method of Racagni *et al.*, using a mathematical model of steady-state kinetics.[7] The turnover rate is the product of fractional rate constant, k_B, and acetylcholine concentration, [ACh]. Derivation of equations for the model is appended.

Experimental data were compared with respective controls using the Student t test. *P* values less than 0.05 are considered significant.

In additional groups of rats, biparietal electroencephalograms were recorded during anesthesia, using stainless steel needle electrodes (26 ga) and a Beckman-Offner Dynograph. Arterial blood samples were obtained through an indwelling catheter placed in the femoral artery, and analyzed for *p*H and

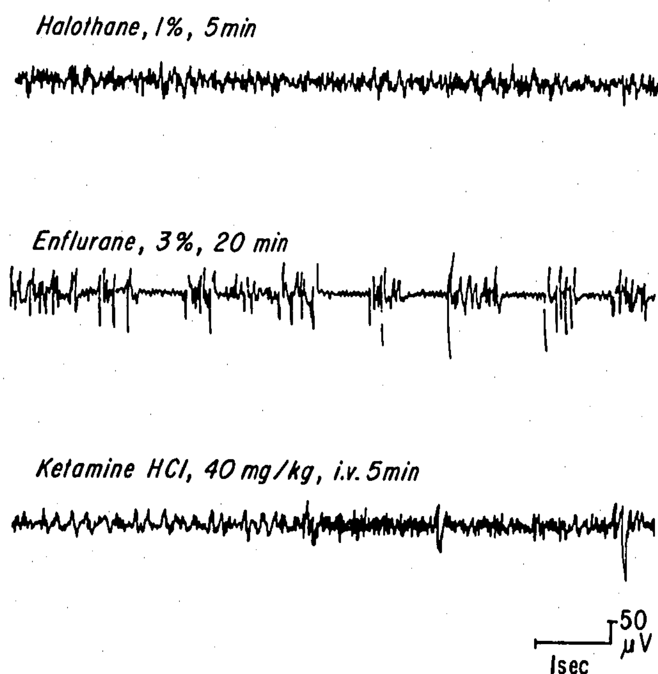

FIG. 1. Electroencephalograms during anesthesia with halothane, enflurane, and ketamine in rats, taken at the times indicated after induction of anesthesia. Note the hypersynchronous discharges with enflurane.

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TABLE 1. Effects of Halothane on Acetylcholine Concentrations and Turnover Rates in Rate Brain Structures

	Acetylcholine (ACh) (nmol/mg Protein)	Choline (Ch) (nmol/mg Protein)	SA_{ACh} (dpm/nmol)	SA_{Ch} (dpm/nmol)	m (SA_{Ch}/SA_{ACh})	k_H (hr^{-1})	TR_{ACh} (nmol/mg Protein/hr)
Cortex							
Control	0.18 ± 0.035	0.38 ± 0.071	458 ± 58	1297 ± 183	3.3 ± 0.95	16.0 ± 5.0	2.7 ± 1.1
Halothane	0.18 ± 0.020	0.29 ± 0.028	162 ± 23*	2075 ± 421	14.2 ± 2.3*	2.3 ± 0.5*	0.3 ± 0.066*
Caudate nucleus							
Control	0.55 ± 0.028	0.43 ± 0.065	264 ± 63	420 ± 34	2.0 ± 0.31	25.0 ± 4.8	14.0 ± 2.5
Halothane	0.53 ± 0.076	0.31 ± 0.020	229 ± 25	935 ± 275	4.6 ± 0.84*	8.7 ± 2.5*	4.8 ± 1.1*
Hippocampus							
Control	0.24 ± 0.047	0.45 ± 0.031	231 ± 44	406 ± 49	1.9 ± 0.20	24.0 ± 3.7	7.3 ± 2.0
Halothane	0.19 ± 0.026	0.38 ± 0.057	102 ± 26	537 ± 96	6.4 ± 1.6	8.4 ± 4.6	1.6 ± 0.81*
Hypothalamic and thalamic regions							
Control	0.26 ± 0.045	0.30 ± 0.031	122 ± 19	666 ± 80	6.1 ± 0.79	4.9 ± 1.1	1.1 ± 0.11
Halothane	0.24 ± 0.022	0.25 ± 0.020	134 ± 24	1167 ± 76*	10.2 ± 2.1	2.9 ± 0.54	0.65 ± 0.027*

Phosphoryl (Me-^{14}C) choline was infused iv (30 μCi/kg/min) for 6 min during anesthesia, inspired halothane concentration, 0.7–1.0 per cent. Values are means ± SE from six rats.

* $P < 0.05$.
SA = specific activity; k_H = fractional rate constant of ACh; TR_{ACh} = turnover rate of ACh.

P_{CO_2} using an Instrumentation Laboratory Model 313 electrode system.

Results

During light halothane anesthesia with the rats breathing spontaneously, the arterial blood *p*H was 7.40 ± .01 and P_{CO_2}, 42 ± 1 torr (mean ± SE, n = 8); with enflurane the respective values were 7.37 ± .02 and 40 ± 2 torr (n = 8). In three rats 5–10 minutes following iv injection of ketamine, arterial blood *p*H's averaged 7.41 and P_{CO_2}'s, 40 torr.

Electroencephalographic (EEG) patterns during halothane, enflurane and ketamine anesthesia are shown in figure 1. Halothane, 1 per cent inspired, produced a low-amplitude fast activity. The EEG during enflurane anesthesia was characterized by frequent hypersynchronous high-amplitude discharges. Ketamine, in the dose used, produced a low-amplitude fast activity mixed with slow rhythmic discharges at 4–6 Hz. The occasional high-amplitude activities were movement artifacts.

Concentrations of acetylcholine and choline, their respective specific activities (SA_{ACh} and SA_{Ch}), ratios

TABLE 2. Effects of Enflurane on Acetylcholine Concentrations and Turnover Rates in Rat Brain Structures

	Acetylcholine (ACh) (nmol/mg protein)	Choline (Ch) (nmol/mg Protein)	SA_{ACh} (dpm/nmol)	SA_{Ch} (dpm/nmol)	m (SA_{Ch}/SA_{ACh})	k_H (hr^{-1})	TR_{ACh} (nmol/mg Protein/hr)
Cortex							
Control	0.16 ± 0.014	0.29 ± 0.026	395 ± 48	1208 ± 214	3.4 ± 1.0	17.6 ± 6.8	2.5 ± 0.83
Enflurane	0.18 ± 0.014	0.18 ± 0.010	178 ± 27*	1743 ± 179	10.8 ± 2.3*	3.3 ± 0.84*	0.58 ± 0.15*
Caudate nucleus							
Control	0.74 ± 0.011	0.41 ± 0.50	243 ± 84	465 ± 80	2.7 ± 0.56	26.0 ± 12	15 ± 3.7
Enflurane	0.84 ± 0.064	0.36 ± 0.033	181 ± 42	553 ± 90	2.8 ± 0.46	19.3 ± 6.6	16 ± 4.9
Hippocampus							
Control	0.24 ± 0.026	0.42 ± 0.030	283 ± 42	514 ± 38	2.3 ± 0.34	19 ± 3.9	5.0 ± 1.6
Enflurane	0.27 ± 0.033	0.32 ± 0.028	277 ± 61	600 ± 105	3.0 ± 0.70	20 ± 8.3	5.6 ± 2.1
Hypothalamic and thalamic regions							
Control	0.31 ± 0.017	0.26 ± 0.001	101 ± 21	735 ± 61	8.4 ± 2.1	3.9 ± 0.97	1.1 ± 0.28
Enflurane	0.34 ± 0.041	0.24 ± 0.026	80 ± 12	891 ± 117	9.9 ± 1.9	3.6 ± 1.2	1.3 ± 0.39

Phosphoryl (Me-^{14}C) choline was infused iv (30 μCi/kg/min) for 6 min during anesthesia, inspired enflurane concentration, 2.7–3 per cent. Values are means ± SE from five or six rats.

* $P < 0.05$.
SA = specific activity; k_H = fractional rate constant of ACh; TR_{ACh} = turnover rate of ACh.

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TABLE 3. Effects of Ketamine on Acetylcholine Concentrations and Turnover Rates in Rat Brain Structures

	Acetylcholine (ACh) (nmol/mg Protein)	Choline (Ch) (nmol/mg Protein)	SA_{ACh} (dpm/nmol)	SA_{Ch} (dpm/nmol)	m (SA_{Ch}/SA_{ACh})	k_B (hr^{-1})	TR_{ACh} (nmol/mg Protein/hr)
Cortex							
Control	0.12 ± 0.015	0.37 ± 0.035	661 ± 134	1563 ± 158	3.0 ± 0.80	17.1 ± 4.2	1.8 ± 0.44
Ketamine	0.12 ± 0.020	0.48 ± 0.083	402 ± 69	1504 ± 168	4.2 ± 1.0	10.5 ± 3.1	1.2 ± 0.29
Caudate nucleus							
Control	0.51 ± 0.078	0.41 ± 0.033	295 ± 65	497 ± 47	1.9 ± 0.28	30 ± 9.9	14 ± 3.8
Ketamine	0.49 ± 0.045	0.55 ± 0.049	227 ± 43	651 ± 123	3.2 ± 0.36*	11 ± 2.2*	5.6 ± 0.70*
Hippocampus							
Control	0.21 ± 0.017	0.40 ± 0.010	250 ± 37	500 ± 67	2.0 ± 0.16	20 ± 2.1	4.3 ± 0.53
Ketamine	0.26 ± 0.020	0.67 ± 0.088*	130 ± 37	504 ± 102	5.6 ± 2.1	7.7 ± 2.2*	2.1 ± 0.67*
Hypothalamic and thalamic regions							
Control	0.40 ± 0.020	0.34 ± 0.024	109 ± 13	1031 ± 88	10 ± 1.3	2.7 ± 0.38	1.1 ± 0.16
Ketamine	0.42 ± 0.027	0.50 ± 0.072	180 ± 35	1610 ± 206	10 ± 2.0	3.7 ± 1.1	1.0 ± 0.23

Ketamine HCl, 40 mg/kg, iv, given 5 min prior to phosphoryl (Me-^{14}C) choline infusion (30 μCi/kg/min) for 6 min. Values are means ± SE from six rats.

* $P < 0.05$.
SA = specific activity; k_B = fractional rate constant of ACh; TR_{ACh} = turnover rate of ACh.

(m) of SA_{Ch}/SA_{ACh}, which indicate the incorporation of labeled Ch into labeled ACh, fractional rate constants, and turnover rates of acetylcholine (TR_{ACh}) in four regions of the brain are presented in tables 1–3. Data from experimental (anesthetized) groups are tabulated immediately below those from control (unanesthetized) groups for each of the three anesthetics.

Halothane and enflurane did not change acetylcholine and choline concentrations in the cerebral cortex, the caudate nucleus, the hippocampus, and the hypothalamic and thalamic regions. Ketamine increased the choline concentration in the hippocampus only. Halothane increased the ratios of SA_{Ch}/SA_{ACh} (m) in all regions of the brain, the increases being significant in the cortex and the caudate nucleus. In the cortex, the fractional rate constant of acetylcholine decreased from 16 to 2.3 per hour and the turnover rate from 2.7 to 0.3 nmol/mg protein/hr. Fractional rate constants and turnover rates of acetylcholine in the other three brain regions also decreased, although not to the same extent (table 1).

Enflurane increased the SA_{Ch}/SA_{ACh} ratio, and decreased the fractional rate constant and turnover rate of acetylcholine, only in the cortex, without significant change in these variables in the other brain regions (table 2).

Ketamine, in the dose used, decreased the fractional rate constant and turnover rate of acetylcholine in the caudate nucleus and the hippocampus but not in the cortex and the hypothalamic and thalamic regions. In the cortex, SA_{ACh}, fractional rate constant and turnover rate of acetylcholine appeared to decrease, but these changes were not significant (table 3).

Discussion

The study of acetylcholine turnover rate *in vivo* using the radioactive precursor choline has been made possible only recently. Several methodologic and conceptual developments are essential. The dose of radioactive precursor infused intravenously must not change the size of the choline pool in the plasma or the brain. We infused phosphoryl(Me-^{14}C) choline at a rate of 30 μCi/kg/min, equivalent to 0.7 μmol/kg/min. As the plasma and tissue phosphorylcholine pool is relatively large (0.4 μmol/g)[19] and phosphorylcholine is rapidly converted to choline, the plasma and brain concentrations of choline are not expected to change. Cheney *et al.* injected 250 μCi/kg (equivalent to 5.7 μmol/kg) of phosphoryl(Me-^{14}C)choline intravenously into mice and found no significant change in brain choline and acetylcholine concentrations.[17]

Another problem in the study of the turnover rate of acetylcholine *in vivo* is the rapid increase in brain choline concentration after death. Dross and Kewitz reported that following decapitation, choline concentrations in the rat brain increased at a rate of about 20.5 nmol/g/min.[20] This postmortem increase in choline concentration would invalidate calculations of turnover rate that take into account choline concentration and its specific activity. Rapid immersion of the brain in liquid nitrogen does not solve this problem because of the time required to freeze the whole brain. The freeze–blow method used by

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Veech *et al.*[21] does not allow the measurement of substances in discrete brain regions. Microwave irradiation focused to the brain inactivates enzymes in 1–2 seconds. The brain choline concentration remains unchanged for at least 10 minutes.[14,17] Brain structures are preserved so that brain regions can be easily dissected.

The development of radio gas chromatography has made it possible to assay tissue choline and acetylcholine and their respective specific activities simultaneously in one sample.[16,17]

In the absence of change in choline and acetylcholine concentrations, it is possible to estimate the turnover rate of acetylcholine *in vivo* according to principles of steady-state kinetics. Certain assumptions concerning the metabolic pathways of choline and acetylcholine in the brain were made.[7,8] Not all the choline in the brain is converted into acetylcholine, but the compartmentation of brain choline and acetylcholine is poorly understood. We assumed that the conversion of labeled choline into acetylcholine during the infusion proceeds at approximately the same rate as disappearance of labeled choline from the brain. As acetylcholine is hydrolyzed to choline and acetate, choline is recycled by neuronal uptake for acetylcholine synthesis. The extent of recycling of labeled choline during the short period of infusion, 6 min, is assumed to be small. The mathematical model for the estimation of acetylcholine turnover rate has been reported by Racagni *et al.*[7] and Cheney *et al.*[8] (see also Appendix).

We found that in rats the brain concentrations of choline and acetylcholine did not change during anesthesia with halothane, enflurane, and ketamine. The absence of change in acetylcholine concentration in the brain during halothane anesthesia differs from the results of Schmidt, who reported an increase in the cortex and the brainstem during light halothane anesthesia, with some further increase during deep anesthesia.[3] Schmidt killed the rats by immersion in liquid nitrogen and used bioassay for acetylcholine concentration. He also applied paired t tests for statistical analysis. One wonders whether the increases reported would have reached levels of significance if the data had been evaluated by the unpaired t test, as he obtained brain samples from different groups of animals.

Halothane decreased the turnover rate of acetylcholine in all regions of the brain examined. The change was greatest in the cortex, where the turnover rate was about 10 per cent of that of the unanesthetized rats. The decreased turnover rate of acetylcholine suggests that the release or utilization of this neurotransmitter was inhibited by halothane. This would agree with the concept proposed by Winter *et al.* that halothane is a "pure" central nervous system depressant devoid of excitatory action behaviorally and electrophysiologically.[22]

Levitt and Leslie have reported that in synaptosomes prepared from rat cerebral cortex, halothane decreased the rate of acetylcholine synthesis in a dose-dependent manner.§ However, the decrease induced by 0.8–1.7 per cent halothane was small, to 88–93 per cent of control, the control rate being 0.66 nmol/mg protein/hr. The present study *in vivo* showed that the mean turnover rates of acetylcholine in the cerebral cortices of conscious rats ranged from 1.8 to 2.7 nmol/mg protein/hr (tables 1–3). Halothane reduced the turnover rate from 2.7 to 0.3 nmol/mg protein/hr (table 1). The different results of *in vitro* and *in vivo* studies could be accounted for by the cerebral activity and the halothane-induced depression in intact rats. One presumes that synaptosomes are at a resting state *in vitro*. Levitt and Leslie raised the possibility that halothane may interfere with choline transport or inhibit choline acetyltransferase. The smallness of the change in the rate of acetylcholine synthesis observed *in vitro* suggests that these mechanisms are not operative *in vivo*. However, the question in respect to neuronal uptake of choline and choline acetyltransferase as affected by halothane remains open for further studies. These studies are necessary because halothane decreased the turnover rate of acetylcholine uniformly in all regions of the brain.

Enflurane has been shown to induce cortical hypersynchronous seizure-like activities in man,[10,23] dogs,[23] and cats.[11,24] In man, the occurrence of seizure-like activities is associated with deep enflurane anesthesia and/or hypocarbia.[25] In cats, bursts of high-amplitude spike activities in cortical and subcortical structures occurred when enflurane was administered in a concentration (5.5 per cent) to abolish withdrawal from painful stimuli.[24] The electrocorticogram in the cat has a pattern similar to that recorded from the rat (fig. 1), except for longer periods of interictal silence. The hypersynchronous discharges seemed to originate in the limbic system (amygdala and hippocampus) but were recorded from all brain structures upon loss of the withdrawal response to painful stimuli.[24] It would appear that this enflurane-induced hyperactivity of the brain is reflected in the estimated turnover rate of acetylcholine reported here. In contrast to halothane, enflurane did not affect the acetylcholine turnover

§ Levitt JD, Leslie MH: The effect of halothane on acetylcholine synthesis in synaptosomes isolated from rat brain. Abstracts of Scientific Papers, Annual Meeting of the American Society of Anesthesiologists, October 1974, pp 265–266.

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rate in subcortical structures. Nevertheless, the turnover rate of acetylcholine in the cortex did decrease, perhaps related to a reduced overall activity compared with that of restrained, conscious rats.

Ketamine, a so-called dissociative anesthetic, is considered a central nervous system stimulant by Winters *et al.*[9] It induces a catatonic state in cats, with intermittent or continuous hypersynchronous discharges from the cortex, limbic structures and the medial geniculate nucleus.[9] Wong and Jenkins also recorded hypersynchronous activities in the cortex, the caudate nucleus, the thalamus and the brainstem reticular formation in cats.[13] Tamasy *et al.* showed in cats that along with the hypersynchronous discharges in the cerebral cortex, multiple unit activities in the hippocampus and the brainstem reticular formation actually increased.[12] We found that following ketamine injection, during the period of loss of righting reflex, the turnover rate of acetylcholine decreased in the caudate nucleus and the hippocampus. Thus, in the case of ketamine, turnover of acetylcholine decreased in the presence of hypersynchronous discharges in these structures. Sung *et al.* showed the ketamine (40 mg/kg) decreased the turnover rate of serotonin and increased the turnover rate of dopamine in rats.[26] However, these changes were observed 4–6 hours following intravenous injection of ketamine. Ketamine-induced behavioral changes were apparent for only 2–3 hours. Biebuyck *et al.* reported an increase in cyclic AMP and a decrease in glutamate concentrations in rat brain during ketamine anesthesia.[27] Miletich *et al.* showed that ketamine interferes with neuronal reuptake of norepinephrine in the rat heart.[28] Indeed, the electrophysiologic and neurochemical changes induced by ketamine are complex. We cannot at present speculate on the interrelationships among the myriad of physiologic and biochemical changes induced by ketamine, which has been considered as a dissociative anesthetic, a central nervous system stimulant,[9] a hallucinogen,[29] and a drug that can induce epileptiform activity in susceptible individuals.[30]

Of interest is the finding that ketamine did not decrease the turnover rate of acetylcholine in the cortex and the thalamic region. This is in contrast to the effects of halothane, which decreased the turnover rate in all brain regions, and enflurane, which decreased acetylcholine turnover rate in the cortex only.

Results presented here suggest that the decreased turnover rate of acetylcholine, an index of acetylcholine release or utilization, is in some manner related to the electrophysiologic effects of the anesthetics studied. Our results also emphasize that different anesthetics do not affect the central nervous system in the same manner, behaviorally, electrophysiologically, and in respect to neurochemical changes.

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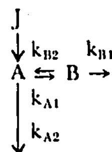
APPENDIX

1. Gas chromatographic conditions: Nitrogen (15 ml/min, 70 psi) was used as carrier gas, with air (200 ml/min, 20 psi) and hydrogen (40 ml/min, 14 psi) comprising the flame gases. Column temperature was maintained at 185 C, and injector and detector temperatures were 200 and 270 C, respectively.

Silane-treated glass columns, 1.83 meters, with an inner diameter of 2.5 mm, were packed with 28 per cent Pennwalt 223 plus 4 per cent KOH on Gas Chrome R (80–100 mesh, Applied Science Laboratories, Inc, State College, Pa).

2. The calculation of TR_{ACh} in various brain areas was performed using the following kinetic model:



where

$$k_{A1} + k_{A2} = k_A \text{ and } k_{B1} + k_{B2} = k_B$$

A is the compartment of free brain Ch and B is the compartment of brain ACh. The rate of entrance of radioactive Ch into compartment A is indicated by J, while k_A and k_B are the fractional rate constants for the efflux of products from A and B compartments.

Therefore, the SA_{Ch} in compartment A and the SA_{ACh} in compartment B would be expected to change with time as shown in equations 1 and 2:

$$\frac{dSA_{Ch}}{dt} = J - k_A SA_{Ch} \quad (1)$$

$$\frac{dSA_{ACh}}{dt} = k_B(SA_{Ch} - SA_{ACh}) \quad (2)$$

Imposing the condition that $SA_{Ch} = 0$ at $t = 0$ where t is the duration of infusion, equation 1 can be integrated:

$$SA_{Ch(t)} = \frac{J}{k_A}(1 - e^{-k_A t}) \quad (3)$$

$$k_A = \frac{-1}{t_2} \ln\left[1 - \frac{SA_{Ch(t_2)}}{SA_{Ch(t_1)}}(1 - e^{-k_A t_1})\right] \quad (4)$$

under the condition where $SA_{ACh} = 0$ at $t = 0$, integration of equation 2 yields:

$$SA_{ACh(t)} = \frac{J}{k_A}\left(1 - \frac{k_B e^{-k_A t}}{k_B - k_A} + \frac{k_A e^{-k_B t}}{k_B - k_A}\right) \quad (5)$$

Since J can be controlled and SA_{Ch} and SA_{ACh} can be experimentally measured, k_A can be calculated from equation 4. Following the calculation of k_A, k_B can be determined from equation 5. A solution can be found to equation 5 so that TR_{ACh} can be evaluated using a single time point of PCh infusion. However, the k_A must first be determined from multiple points. Equation 3 is solved for J/k_A, substituted into equation 5, and the natural log taken. Thus

$$k_B = \frac{-1}{t_1} \ln\left[1 - \frac{k_B}{k_A} + \frac{k_B}{k_A} e^{-k_A t_1} + \frac{1}{m}\left(\frac{k_B}{k_A} - 1\right)(1 - e^{-k_A t_1})\right] \quad (6)$$

where $m = SA_{Ch(t_1)}/SA_{ACh(t_1)}$. Since t_1, $SA_{Ch(t_1)}$ and $SA_{ACh(t_1)}$ can be experimentally determined, equation 4 can be solved for k_A and equation 6 can be solved for k_B.

TR_{ACh} was determined by multiplying k_B by the ACh concentration:

$$TR_{ACh} = k_B(\text{ACh}) \quad (7)$$

This approach was used to determine the k_B following drug treatment. Since we have found that the value of k_A of each brain area is highly reproducible under identical experimental conditions, we have assumed that when drug treatments did alter the k_A this change would not cause a change in the relationship existing between SA_{Ch} and k_A. Based on these assumptions the following equation was derived:

$$k_{A'} = \frac{k_A(1 - e^{-k_{A'} t_1})}{R_s(1 - e^{-k_A t_1})} \quad (8)$$

where $k_{A'}$ is the fractional rate constant for the Ch compartment under study in the drug-treated rats and R_s is the ratio of SA_{Ch} in treated rats to SA_{Ch} in control rats.[7,8]

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