Short Communication

Steady-State Concentrations of Choline and Acetylcholine in Rat Brain Parts during a Constant Rate Infusion of Deuterated Choline

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Summary. An intravenous infusion of deuterated choline at constant rate for 6 min (5 or 25 \( \mu \)moles kg\(^{-1}\) min\(^{-1}\)) significantly increases the concentration of choline in plasma, occipital cortex and striatum. Both 5 and 25 \( \mu \)moles kg\(^{-1}\) min\(^{-1}\) increase the concentration of acetylcholine in cortex but only 25 \( \mu \)moles kg\(^{-1}\) min\(^{-1}\) increases the acetylcholine content in striatum. In contrast, 1 \( \mu \)mole kg\(^{-1}\) min\(^{-1}\) does not change the choline or acetylcholine content in cortex or striatum. A single pulse injection of choline (200 \( \mu \)moles kg\(^{-1}\)) causes a significant increase in the concentration of choline in striatum 30 sec following injection. The choline content returns to normal values within 2 min. These studies show that when a pulse injection of a non-tracer dose of radioactive choline is used to measure brain acetylcholine turnover rate the maintenance of steady state must be verified within seconds after the pulse injection of radioactive choline. When constant infusion of deuterated choline is used to measure turnover rate of acetylcholine in the brain of rats, a dose of 1 \( \mu \)mole kg\(^{-1}\) min\(^{-1}\) appears to be a maximal infusion rate.

Key words: Choline — Acetylcholine — Infusion — Striatum — Occipital Cortex — Mass Fragmentography.

Introduction

Recent studies in whole brain of guinea pigs (Haubrich et al., 1974) and mice (Jenden et al., 1974) suggested that the steady-state concentrations of choline (Ch) and acetylcholine (ACh) did not change when animals were injected intravenously with a pulse injection of Ch. However, Dross and Kewitz (1972) found that blood borne Ch penetrates easily through the blood brain barrier of rats even though the concentration gradient is from brain to blood. Since current methods to measure the turnover rate of ACh in brain tissue after injection of labeled...
Ch (Jenden et al., 1974; Saelens et al., 1973; Schuberth et al., 1970; Dross and Kewitz, 1966) assume steady state and calculate turnover rate by applying principles of steady-state kinetics to the change with time of Ch and ACh specific activity, it is important to determine whether or not the intravenous injection of Ch alters the Ch or ACh content in various regions of rat brain.

Methods

In our experiments Male Sprague-Dawley rats (Zivic-Miller, Allison Park, Pa.) weighing between 120-150 g were used. The animals were kept at constant temperature (23±1°C) with alternating light/dark periods of 14 and 10 hrs, respectively. The experiments were carried out in the morning in order to eliminate fluctuations in Ch and ACh content due to diurnal variations (Hanin et al., 1970). Compounds were dissolved in 0.9% NaCl. The deuterated compounds, Ch-2H$_4$ or Ch-D$_4$ \([N^+(CH$_3$)$_3$ C$_2$H$_4$OH Br]\), ACh-2H$_8$ or ACh-D$_8$ \([N^+(CH$_3$)$_3$ C$_2$D$_8$OC (0) CH$_3$ Br]\), Ch-3H$_9$ or Ch-D$_9$ \([N^+(CD$_3$)$_3$ C$_2$H$_4$OH Br]\), and ACh-3H$_9$ or ACh-D$_9$ \([N^+(CD$_3$)$_3$ C$_2$H$_4$OC (0) CH$_3$ Br]\) were obtained from Merck, Sharp and Dohme, Quebec, Canada.

Ch-D$_4$ (1, 5 or 25 μmoles kg$^{-1}$ min$^{-1}$) was infused through the rat tail vein for 6 min at a constant rate (0.2 ml min$^{-1}$). During the infusion, rats were not anesthetized but were restrained in plastic cages. At the end of the infusion, there was no evidence of sedation. Tim rats were then killed by exposing their heads for 2 sec to a focused beam of microwave radiation (2.0 kW; 2.45 GHz, 75 mW cm$^2$) (Stavinoha and Weinstraub, 1974; Guidotti et al., 1974; Racagni et al., 1974). Koslow et al. (1974) have shown that this killing procedure stabilizes the brain concentration of Ch and ACh and allows dissection and assay of ACh in brain nuclei. The brain regions were dissected and the ACh and Ch extracted according to the method of Hanin et al. (1973) as modified by Cheney et al. (1975a). To measure plasma concentrations of Ch, animals were decapitated without exposing them to microwave radiation in order to avoid hemolysis. Preparation of extracts for GC-MS were described previously (Cheney et al., 1975b). Concentrations of ACh, Ch, ACh-D$_4$, ACh-D$_9$ were measured with a quadrupole gas chromatograph-mass spectrometer (GC-MS) (Finnigan 3000). The conditions for gas chromatography were 30 ml/min of helium flow; flash heater 240°C; column 145°C. The derivatives were injected into an 8 foot glass column (3 mm, i.d.) packed with 28% Penwalt 223 and 4% KOH on Gas Chrom R. Ch-D$_9$ and ACh-D$_9$ were used as internal standards. Ion fragments with m/e of 58, 60 and 64 were selected for mass fragmentographic determination of Ch and ACh as described by Hanin and Schuberth (1974) and Hammer et al. (1968).

Results

The concentration of endogenous plasma Ch did not change significantly in rats infused with Ch-D$_4$ (Fig. 1). However, total Ch (endogenous Ch plus Ch-D$_4$) increased significantly when rates of either 5 μmoles kg$^{-1}$ min$^{-1}$ or 25 μmoles kg$^{-1}$ min$^{-1}$ (Fig. 1) were infused. With the infusion of 5 μmoles kg$^{-1}$ min$^{-1}$ of Ch-D$_4$ the plasma content of Ch (Ch + Ch-D$_4$) almost doubled at 2 min of infusion and this level was maintained for the following 6 min. In contrast, when 25 μmoles kg$^{-1}$ min$^{-1}$ were