Labeled Choline and Phosphorylcholine: Body Distribution and Brain Autoradiography: Concise Communication

Robert P. Friedland, Chester A. Mathis, Thomas F. Budinger, Brian R. Moyer, and Mark Rosen

Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, and Veterans Administration Medical Center, Martinez, California, Department of Neurology, University of California, Davis

Following intravenous injection of labeled choline or phosphorylcholine in rats and mice, the brain uptake as percent injected dose was less than 0.2% with 6–12% going to kidney and 3–6% to liver. A study of [^{14}C]choline autoradiography in a stump-tailed macaque demonstrated a five- to sixfold greater uptake in gray matter than in white matter. Dynamic positron imaging of [^{11}C]choline in a rhesus monkey demonstrated rapid brain uptake followed by rapid washout, with heavy late uptake in muscle. The use of labeled choline and choline analogs as imaging agents in human studies is constrained by the low brain uptake relative to extracerebral tissues.

J Nucl Med 24: 812–815, 1983

The ability of positron emission tomography (PET) to measure the distribution of labeled molecules prompted an investigation of the short-term distribution of intravenously injected choline and phosphorylcholine. High-affinity choline uptake (HACU) is a crucial step in acetylcholine synthesis (*1–3*). The high-affinity system is rate-limiting and linearly correlated with acetylcholine turnover rate (*1–3*). A low-affinity uptake system in the blood-brain barrier has been demonstrated for choline (*4,5*). Extensive studies showing the influence of dietary choline and serum choline levels on CNS acetylcholine synthesis have been presented (1,6,7). Phosphorylcholine, a precursor of brain choline, has been used previously in studies of central acetylcholine turnover (*8–11*), but quantification of the brain uptake and organ distribution of phosphorylcholine have not been reported. In order to evaluate the feasibility of using labeled choline or phosphorylcholine in human studies, it is necessary to determine their distribution in the body after intravenous administration. Accordingly we carried out studies on the organ distribution of intravenously administered C-14 choline, C-11 choline, and C-14 phosphorylcholine in mice and rats. The distributions of C-14 choline and C-11 choline in monkeys, studied by autoradiography and PET, are also presented as a baseline for future PET imaging studies.

Received Oct. 18, 1982; revision accepted Apr. 15, 1983.

For reprints contact: Robert P. Friedland, University of California, Veterans Administration Medical Center (127), 150 Muir Rd., Martinez, CA 94553.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 140–160 g, female B6/JAX mice, a stump-tailed macaque monkey, and a rhesus monkey had free access to food and water before administration of the tracers.

DRUGS AND INJECTIONS

Distribution studies. Carbon-11-labeled choline was prepared by the reaction of $^{11}CH_3I$ with N,N-dimethylaminoethanol in acetone at 0°C (unpublished method). The radiopure (>99%) product (*12*) had a specific activity of ~500 Ci/mmol and was made neutral and isotonic before injection. Choline [methyl-^{14}C]choline*

TABLE 1. ORGAN DISTRIBUTION OF C-14 CHOLINE, C-11 CHOLINE, AND C-14 PHOSPHORYLCHOLINE

	Percent of injected dose/organ*			
	C-14 choline†	C-11 choline‡	C-14 phosphoryl-choline§	C-14 phosphoryl-choline¶
Brain	0.08	0.05	0.17	0.09
	(0.07, 0.09)	(0.07, 0.05, 0.03)	(0.17, 0.18)	(0.09, 0.12, 0.06)
Heart	0.32	0.30	0.34	0.23
	(0.28, 0.37)	(0.34, 0.20, 0.34)	(0.44, 0.25)	(0.17, 0.33, 0.20)
Lung	—	1.2	1.4	0.74
		(1.3, 0.77, 1.5)	(1.8, 1.1)	(0.86, 0.72, 0.64)
Liver	5.1	2.9	6.1	3.2
	(6.1, 4.1)	(1.9, 4.5, 2.3)	(6.0, 6.2)	(4.4, 2.6, 4.3)
Kidney	7.2	6.9	12.0	5.7
	(6.2, 8.3)	(7.6, 6.8, 6.3)	(12.6, 11.0)	(3.0, 9.5, 4.7)

* Mean (individual values).
† C-14 choline distribution 10 min after injection in mice (N = 2).
‡ C-11 choline distribution 15 min after injection in rats (N = 3).
§ C-14 phosphorylcholine distribution in rats 5 min after injection (N = 2).
¶ C-14 phosphorylcholine distribution in rats at 5 min after a 5-min infusion (N = 3).

in a 2% ethanol solution has a specific activity of 58 mCi/mmol. Phosphorylcholine [methyl-^{14}C]choline† was obtained in a 70% ethanol solution with a specific activity of 55 mCi/mmol). The ethanol solvent was evaporated under a stream of nitrogen and the choline and phosphorylcholine taken up in normal saline solutions. The precursor ethanolamine was present in the C-11 compound but not in the C-14 compounds.

Injections were made into the tail veins of unanesthetized rats and mice restrained in plastic holders. Bolus injections (0.2 ml in mice and 0.5 ml in rats) of 20–25 μCi of C-14- and 100–200 μCi of C-11-labeled drugs were made and the animals killed by decapitation. Constant-infusion injections (0.6–0.9 ml) of 16–22 μCi of C-14 phosphorylcholine were made over a 5-min period and the animals were killed 5 min after the end of injection.

Autoradiographic study. Choline [methyl-^{14}C]choline in 95% ethanol had a specific activity of 46 mCi/mmole. A bolus of 500 μCi of C-14 choline chloride was injected into the saphenous vein of an adult macaque monkey lightly anesthetized with 8 ml of a 2% sodium thiamylal. The animal was killed 10 min after injection by immersion in a dry-ice and acetone bath, and was then decapitated.

ASSAY

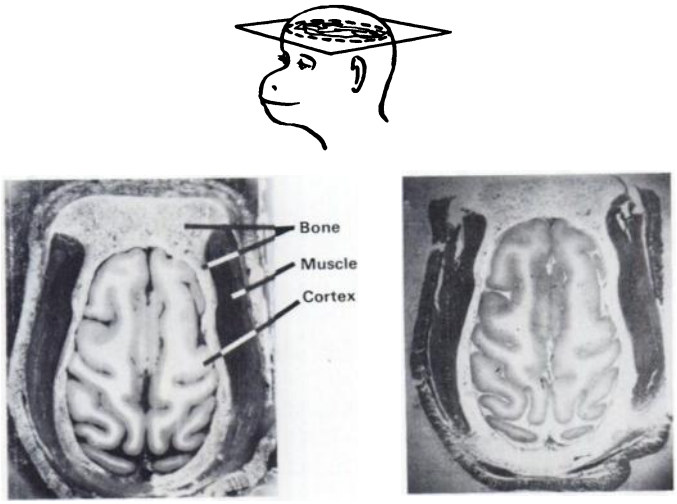
Distribution studies. The brain, liver, kidney, heart, and lungs were removed immediately after death. The C-11 activity in the organs and the carcass was assayed in a gamma well counter. The organs from the C-14 studies were homogenized in ground-glass tissue grinders, precipitated with trichloroacetic acid, and centrifuged. The supernatant activity was counted in a solution of Aquasol† using a scintillation counter. The precipitate was digested in Protosol† for 3 days at 55°C and assayed for C-14 activity in a solution of Aquasol. An aliquot of the C-14 solution used for injection was counted and used as a standard for the calculations of percent injected dose.

Autoradiographic study. The frozen monkey head was embedded in carboxymethyl cellulose frozen to −20°C and sliced into serial 20-μm sections on a Jung cryomicrotome, then dessicated by sublimation overnight on cellulose acetate tape. Sections were planted on Kodak SB-5 film for ten days and removed before development of the film. Film images were digitized with an image digitizer. Relative optical densities in selected regions of interest were determined using an image display system and computer.

Dynamic PET study. An adult rhesus monkey was anesthetized with methoxyflurane and studied using the Donner 280-crystal tomograph (resolution 8 mm FWHM). Intravenous bolus injection of 1.2 mCi C-11 choline was followed by sampling of tomographic data at 5-sec intervals for 20 min from a slice 7 mm thick taken through the brain. Correction for attenuation was performed through the use of transmission data previously obtained at the same level.

RESULTS

Table 1 shows the organ distribution data for choline and phosphorylcholine. The radiolabeled choline activity



PHOTOGRAPH

^{14}C-choline
AUTORADIOGRAPH

FIG. 1. C-14 choline autoradiography in stump-tailed macaque.

in the brain at 10 min was 0.08% of the injected amount, and at 15 min it was 0.05%. Five minutes after bolus injection of C-14-labeled phosphorylcholine there was 0.17% uptake of the injected activity in the brain.

Figure 1 shows a photograph and corresponding autoradiogram of a transverse section of the monkey's head. The ratio of activities for caudate nucleus to corona radiata, determined by the image digitizer, was 6.5; for parietal cortex to corona radiata it was 4.8, and for masticatory muscle to corona radiata 9.9.

Figure 2 shows the sum images for 0–1 and 1–20 min of C-11 choline distribution in the monkey head. Rapid brain uptake in the early image is followed by rapid washout, with heavy uptake in extracerebral tissues in the later image. The monkey's left ear is visible at the top of the figure.

DISCUSSION

The low relative uptake of choline and phosphorylcholine by brain relative to other organs indicates severe limitations for human studies because excessive radiation doses to kidney and liver will be sustained if sufficient activity for a brain image is injected. Thus these compounds could not be used intravenously in tracer studies of brain function in human subjects. Brain choline uptake of 0.45% in mice at 15 sec has been reported (*13*). This is higher than our values for five or more minutes after injection. These higher values probably reflect label in the blood pool. Low brain extraction of administered choline has also been reported in the anesthetized guinea pig (*14*). Gardiner and Paton (*15*) report a brain (cortex) choline uptake that is 41% of kidney uptake and 38% of liver uptake following a 3-hr i.v. infusion of C-14 choline in cats. Such a prolonged infusion could not be performed using radiolabeled C-11 choline in human studies.

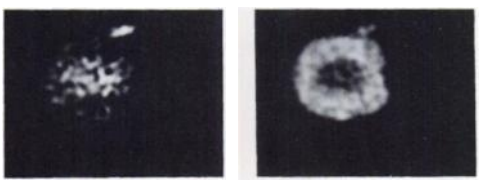


FIG. 2. C-11 choline distribution in monkey head, 0–1 and 1–20 min after injection.

It has been found that after common-carotid injection, 60–70% of the infused choline is removed by the tissues as determined by arteriovenous differences (*16*). This may be a falsely high figure, in that a substantial portion of the choline taken up was most likely removed by tissues perfused by the external carotid artery. The high density of radionuclide concentration in the extracranial musculature seen in the C-11 PET image and the C-14 autoradiograph (Figs. 1 and 2) is the basis for this interpretation. Oldendorf and Braun (*17*) found the extraction to be ~6.3% following single-pass intracarotid injection of C-14 choline. Their data suggest that about 0.9% of the intravenous dose should accumulate in the brain in the first few seconds. This agrees with the data of Schuberth (*13*) for 15 sec after injection. The initial accumulation of about 1% of the injected dose is a transient due to uptake and washout from the intravascular and interstitial fluid spaces. Our data, using dynamic tomography in monkey, confirm the fact that the 1% uptake is a transient phenomenon. Most of the choline tracer that clears from the blood during the first pass

re-enters the circulation and is distributed to other organs.

Carbon-14 choline autoradiography in the stump-tailed macaque demonstrated a higher uptake in gray matter than in white throughout the brain. It is unlikely that this is related to label in the blood because of the rapid turnover of choline (1.3 min in rat) (*18,19*). However, at this time we do not have an independent measure of the contribution from the blood pool. Also, the gray-to-white activity ratio is higher than what would be expected from cerebral blood volume differences alone (*20*). This study demonstrates the feasibility of using labeled choline for CNS autoradiographic studies. Autoradiography with labeled choline has been used in the mouse with the in vitro phrenic-nerve hemidiaphragm preparation to demonstrate the sodium-dependent high-affinity choline uptake system (*21*).

While the radiation dose to the liver and kidney might limit human studies using the intravenous route, animal studies of cholinergic systems using this route are promising. The rapid labeling of brain choline by i.v. injection of labeled choline has been extensively documented (*19,22,23*). Choline-containing phospholipids are also labeled, but this does not reach a maximum until several hours after injection (*4,10,19,22,23*). Choline distribution at short intervals after injection may be more reflective of HACU in the CNS than that after long intervals, and, if administered through the intracarotid route, it has some promise for investigative studies where the medical benefits may warrant this approach.

FOOTNOTES

* Amersham.

† New England Nuclear.

ACKNOWLEDGMENT

This research was supported by the Medical Research Service of the Veterans Administration and by the director Office of Energy Research, Office of Health and Environmental Research of the U.S. Dept. of Energy under Contract No. DE-AC03-76SF00098.

REFERENCES

1. JOPE RS: High affinity choline transport and acetylCoA production in brain and their roles in the regulation of acetylcholine synthesis. *Brain Res Rev* 1:313-344, 1979
2. MURRIN LC: High-affinity transport of choline in neuronal tissue. *Pharmacology* 21:132-140, 1980
3. KUHAR MJ, MURRIN LC: Sodium-dependent, high affinity choline uptake. *J Neurochem* 30:15-21, 1978
4. CORNFORD EM, BRAUN LD, OLDENDORF WH: Carrier mediated blood-brain barrier transport of choline and certain choline analogs. *J Neurochem* 30:299-308, 1978
5. PARDRIDGE WM, OLDENDORF WH: Transport of metabolic substrates through the blood-brain barrier. *J Neurochem* 28:5-12, 1977
6. JENDEN DJ: Regulation of acetylcholine synthesis and release. In *Psychopharmacology and Biochemistry of Neurotransmitter Receptors.* Yamamura, Olsen, Usdin, Eds. Elsevier North Holland, pp 3-15, 1980
7. ZEISEL SH: Dietary choline: biochemistry, physiology, and pharmacology. *Annual Review of Nutrition Nutr* 95:121, 1981
8. CHENEY DC, COSTA E, HANIN I, et al.: Application of principles of steady-state kinetics to the *in vivo* estimation of acetylcholine turnover rate in mouse brain. *J Pharmacol Exp Ther* 192:288-296, 1975
9. RACAGNI G, CHENEY DL, TRABUCCHI M, et al: Measurement of acetylcholine turnover rate in discrete areas of rat brain. *Life Sci* 15, 1961-1975, 1975
10. CHENEY DL, COSTA E: Pharmacological implications of brain acetylcholine turnover measurements in rat brain nuclei. *Ann Rev Pharmacol Toxicol* 17:369-386, 1977
11. ZSILLA G, RACAGNI G, CHENEY DL, et al: Constant rate infusion of deuterated phosphorylcholine to measure the effects of morphine on acetylcholine turnover rate in specific nuclei of rat brain. *Neuropharmacology* 16:25-30, 1977
12. COMAR D, CARTRON JC, MAZIERE M, et al: Labelling and metabolism of methionine-methyl-^{11}C. *Eur J Nucl Med* 1: 11-14, 1976
13. SCHUBERTH J, SPARF B, SUNDWALL A: A technique for the study of acetylcholine turnover in mouse brain *in vivo*. *J Neurochem* 16:965-700, 1969
14. HAUBRICH DR, WANG PFL, WEDEKING PW: Distribution and metabolism of intravenously administered choline [methyl-^{3}H] and synthesis *in vivo* of acetylcholine in various tissues of guinea pigs. *J Pharmacol Exp Ther* 193:246-255, 1975
15. GARDINER JE, PATON WDM: The control of the plasma choline concentration in the cat. *J Physiol* 227:71-86, 1972
16. GARDINER JE: The infusion of choline into the carotid circulation of the cat. *Arch Int Pharmacodyn Ther* 209:150-161, 1974
17. OLDENDORF WH, BRAUN LD: [^{3}H] Tryptamine and ^{3}H-water as diffusible internal standards for measuring brain extraction of radio-labeled substances following carotid injection. *Brain Res* 113:219-224, 1976
18. FREEMAN JJ, JENDEN DJ: The source of choline for acetylcholine synthesis in brain. *Life Sci* 19:949-961, 1976
19. DROSS K, KEWITZ H: Concentration and origin of choline in the rat brain. *Naunyn Schmiedebergs Arch Pharmacol* 274, 91-106, 1972
20. PHELPS ME, HUANG SC, HOFFMAN EJ, et al: Validation of tomographic measurement of cerebral blood volume with C-11-labeled carboxyhemoglobin. *J Nucl Med* 20:328-334, 1979
21. RUCH GA, KOELLE GB, SANVILLE UJ: Autoradiographic demonstration of the sodium-dependent high-affinity choline uptake system. *Proc Nat Acad Sci USA* 79:2714-2716, 1982
22. ANSELL GB, SPANNER S: The metabolism of [Me^{14}C]-choline in the brain of the rat *in vivo*. *Biochem J* 110:201-206, 1968
23. GROTH DP, BAIN JA, PFEIFFER CC: The comparative distribution of ^{14}C-labeled 2-dimethylaminoethanol and choline in the mouse. *J Pharmacol Exp Ther* 124:290-295, 1958