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Comparative Studies on the Metabolism of β-Dimethylaminoethanol in the Mouse Brain and Liver Following Administration of β-Dimethylaminoethanol and Its *p*-Chlorophenoxyacetate, Meclofenoxate[1)]

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1) After intravenous treatments of mice with equimolar doses of ^{14}C-labeled β-dimethylaminoethanol (DMAE*) or its *p*-chlorophenoxyacetate [MF(DMAE*)], brain levels of DMAE* were found to be much higher in the latter treatment, due to the better penetration of the ester derivative, followed by hydrolysis.

2) DMAE* in the brain administered in either form of drugs was gradually phosphorylated to yield DMAE*-P, which was in turn converted to ptd-DMAE*, seemingly the end metabolite of DMAE* in the brain.

3) Acid-soluble and lipid cholines derived from DMAE* and MF (DMAE*) were also found in the brain. However, methylations of DMAE or its ester were attributed to occur in organs other than the brain, such as the liver, as revealed by relative incorporations of a labeled methyl group into lipid choline in the brain and liver after the treatment of methionine-methyl-^{14}C.

β-Dimethylaminoethanol (DMAE)[3)] and its *p*-chlorophenoxyacetic acid ester (MF) are known as a brain stimulator.

In the previous autoradiographic studies[4)] we found that MF, when administered intravenously to mice, rapidly penetrated into the central nervous systems. Significant amounts of DMAE molecule derived from MF were retained in the brain, whereas the acid moiety of the drug rapidly disappeared from the central nervous systems. *p*-Chlorophenoxyacetic acid was excreted without metabolic conversion,[5)] however, DMAE is likely to be involved in the endogenous metabolism.

The purpose of the present study is to elucidate the metabolism of DMAE in the brain following MF administration in comparison with free DMAE administration. Such information would be essential for understanding the mechanism and difference of the central stimulations by DMAE and MF. Further, it is of particular biochemical interest to elucidate the metabolism of DMAE, as a precursor of choline base, in the brain because there have been conflicting reports concerning the ability[6,7)] of the brain to synthesize choline *de novo*. Therefore, the metabolism in the liver was also examined since it is well known that hepatic tissue is capable of synthesizing choline *de novo*.

1) This work was presented at the 94th annual meeting of the Pharmaceutical Society of Japan at Sendai in 1974.
2) Location: *33–94, Enoki-cho, Suita, Osaka.*
3) The following abbreviations are used in this paper: DMAE*; β-dimethylaminoethanol-1,2-^{14}C, MF; meclofenoxate hydrochloride, MF(DMAE*); MF labeled by DMAE*, MF (PCPA*); MF labeled by *p*-chlorophenoxyacetate-carboxyl-^{14}C and MF (DMAE*, PCPA*); MF labeled by both DMAE* and *p*-chlorophenoxyacetate-carboxyl-^{14}C. Abbreviations without asterisk mean the non-labeled compounds. Ptd-; phosphatidyl-, DMAE-P; phosphoryl DMAE. CDP-; cytidine diphosphate-.
4) H. Miyazaki, A. Kagemoto, M. Ishi-i, Y. Minaki, and K. Nakamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 1681 (1971).
5) Y. Mitta, M. Kishi, and Y. Minaki, *Radioisotopes*, **16**, 29 (1967).
6) K. Dross and H. Kewitz, *Arch. Pharmacol.*, **274**, 91 (1972).
7) G.B. Ansell and S. Spanner, *J. Neurochem.*, **14**, 873 (1967).

Experimental

Labeled Compounds——DMAE* was the product of New England Nuclear Co. The preparations of labeled MF were described before[4,5] and MF (DMAE*, PCPA*) was the mixture of MF (DMAE*) and MF (PCPA*) in the known proportions. Specific activities of these compounds were adjusted to 500 μCi/mmole by the addition of non-labeled compound. Methionine-methyl-^{14}C(54 mCi/mmole) was purchased from Radiochemical Centre.

Chemicals——Ptd-DMAE and DMAE-P were prepared by the method of Baer and Pavanaram,[8] and Ansell and Spanner,[9] respectively. Ptd-choline, phosphorylcholine and alkaline phosphatase (Type I) were purchased from Sigma Chemical Co., Ltd. All other chemicals were of reagent grade.

Procedures——Male dd mice weighing about 30 g were used. Drugs were given intravenously in a dosage 0.4 mmoles equivalent/kg. After the drug administration, mice were decapitated. In one experiment with MF (DMAE*, PCPA*), the head was immediately immersed into dry ice-ether and brain was dissected out under the frozen state. In other experiments, brain and liver were excised as rapidly as possible at room temperature and frozen on dry ice.

Labeled methionine was given intraperitoneally in a dosage of 270 μCi/kg 1 hr after intravenous administration of MF, DMAE or vehicle (0.9% sodium chloride).

Extraction——Unless otherwise stated, liver and brain were extracted by homogenization with 10—20 volumes of ice-cold 5% perchloric acid. After centrifugation the residue was washed twice with the cold acid. The acid extracts (acid-soluble fraction) were combined, neutralized with 12N potassium hydroxide, chilled and filtered to remove potassium perchlorate.

The acid-insoluble residue was successively extracted with 20 volumes of 1% potassium acetate in ethanol, twice with 30 volumes of ethanol–ether mixture (2: 1) and finally with 20 volumes of ether. The combined extracts, the lipid fraction, were processed further as described below.

The lipid-free acid-insoluble residue after these extractions was no longer radioactive, when processed by Sample Oxidizer (Packard Instruments).

Analyses——Radioactivity was determined in Packard 3380 liquid scintillation spectrometer in Bray's solution.[10] Paper and thin-layer chromatograms were scanned by Nuclear Chicago Actigraph III.

Free DMAE* in the acid soluble fraction was collected by using the Thunberg apparatus as follows: 0.1 ml of the fraction was mixed with 0.1 ml of saturated sodium carbonate in the lower tube. Upper trap of the apparatus contained 1.0 ml of 1N sulfuric acid. Whole apparatus was then evacuated and closed. The sample solution in the lower tube was heated in a boiling water bath and the upper trap was air-cooled by a fan for 1 hr. Under these conditions, known amounts of DMAE* were trapped in the sulfuric acid in 90—100% recovery. For the collection of DMAE-P, the acid soluble fraction was pre-treated with alkaline phosphatase and processed similarly.

The acid soluble total cholines were collected as free choline by ion exchange chromatography as follows: the fraction was treated with alkaline phosphatase, concentrated at 35°, filtered to remove precipitates and to the filtrate concentrated hydrochloric acid was added to make the acid concentration of 0.01N. The solution was then passed through the column of Dowex 50W (H) and choline was eluted as described previously.[11] Choline isolated was estimated by the method of Appleton, *et al.*[12]

The lipid fraction was once evaporated and the residue was dissolved in a small amount of chloroform and filtered. The filtrate was chromatographed on silica gel plates (Silica gel G) with chloroform: methanol: water=95: 35: 2 and chloroform: methanol: acetic acid: water=25: 15: 4: 2. The relative amounts in the radioactivity of DMAE* metabolites were estimated from the peak area on the resulting radiochromatogram of the developed plate.

For the isolation of lipid cholines, the lipid fraction dissolved in chloroform was completely evaporated and hydrolyzed in 1N hydrochloric acid by boiling in a hot oil bath. The mixture was washed several times with chloroform. Aqueous phase was evaporated at 35° and the residue was dissolved in a small amount of 0.01N hydrochloric acid. The solution was chromatographed on Dowex 50W (H) as described above.

Identification of Metabolites——Metabolites of DMAE* examined in the experiments were all identified by co-chromatography with the authentic substances.

8) E. Baer and S.K. Pavanaram, *J. Biol. Chem.*, **236**, 2410 (1961).
9) G.B. Ansell and S. Spanner, *J. Neurochem.*, **9**, 253 (1962).
10) G.A. Bray, *Anal. Biochem.*, **1**, 279 (1960).
11) R. Mehta, S.A. Wagle, and B.C. Johnson, *Biochim. Biophys. Acta*, **35**, 287 (1959).
12) H.D. Appleton, B.N. Ladu, Jr., B.B. Levy, J.M. Steele, and B.B. Brodie, *J. Biol. Chem.*, **205**, 803 (1953).

Results

Penetration of MF into the Brain

MF (DMAE*, PCPA*) was given intravenously. Forty seconds after injection, mice were decapitated. Brain was excised at room temperature from mouse 1 and 2, and under the frozen state from 3 and 4, and homogenized with ethanol containing 5% MF. A radiochromatogram of the extract from each animal is shown in Fig. 1a, where the peaks corresponding to DMAE* (Rf=0.2), MF* (Rf=0.6) and p-chlorophenoxyacetic acid-^{14}C (Rf=0.95) are evident. To the extract was added 2 volumes of ether to precipitate MF. The precipitates were recrystallized four times and the resultant crystals were chromatographed. Its radiochromatogram (Fig. 1b) gave one peak corresponding to MF in Rf value. The radioactive material was proved to be the intact MF as follows: the crystals were hydrolyzed with 2N hydrochloric acid in 67% ethanol for two days at room temperature and the solution was developed on paper. The radiochromatograms (Fig. 1c) showed two peaks, corresponding to DMAE* and p-chlorophenoxyacetic acid-^{14}C with their initial radioactivity ratio in MF. The penetration of the intact ester into the brain was thus demonstrated by these experiments.

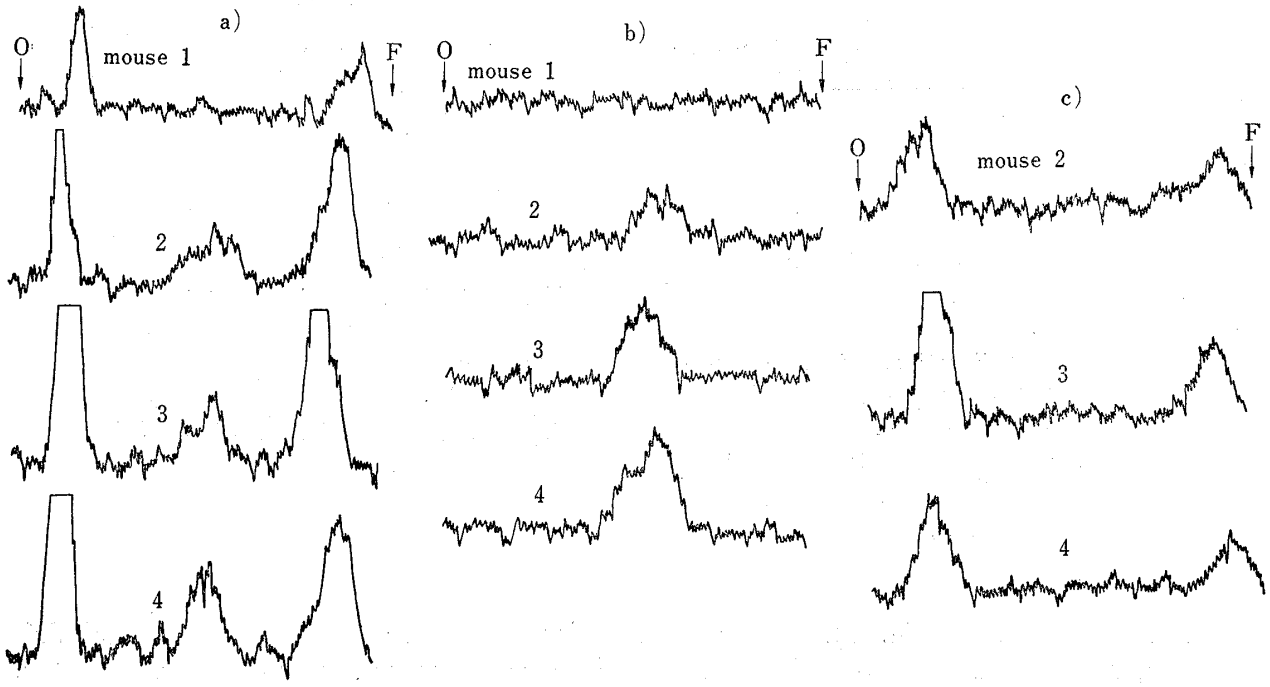

Fig. 1. Radiochromatogram of Cerebral Radioactive Materials after *i.v.* Administration of MF (DMAE*, PCPA*)

The ratio of DMAE*/PCPA* in MF was 1/1 in mouse 1 and 2, and 1/0.8 in mouse 3 and 4, respectively. The solvent system used for the paper chromatography was the upper phase of BuOH: AcOH: water=8: 1: 10.
a) radioactive materials in crude brain extract
b) radioactive material after several recrystallizations with non-labeled MF carrier
c) radioactive materials in hydrolysate of the crystals

Acid-soluble DMAE* and DMAE*-P

Levels of radioactivity in the cerebral and hepatic acid-soluble fractions after administration of MF (DMAE*) and DMAE*, are illustrated in Fig. 2a and 2b, respectively.

Shortly after administration of MF (DMAE*) radioactivity of DMAE* accounted almost entirely for the radioactivity in the cerebral acid-soluble fraction (Fig. 2a). Thereafter, DMAE* gradually decreased, and correspondingly its phosphate increased. The radioactivity of DMAE*-P reached the maximal level at about 20 min after dosing. In agreement with the results in the previous report,[5] levels of the radioactivity in the brain were far lower when

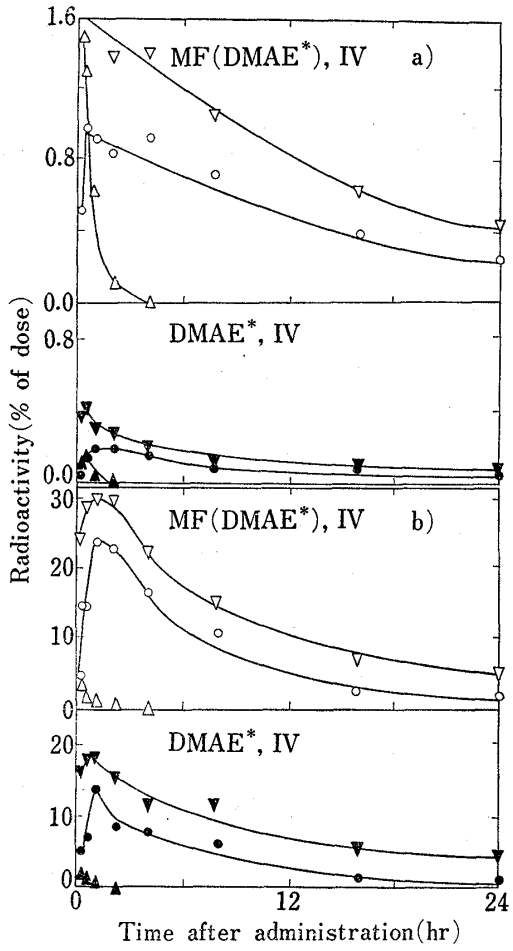

Fig. 2. Radioactivity in the Acid-Soluble Fraction of Brain (a) and Liver (b), Respectively

Open and closed symbols indicate the data obtained from MF (DMAE*) and DMAE* administration, respectively. ▽▼ : total acid-soluble fraction, ○● : DMAE*, △▲ : DMAE*-P

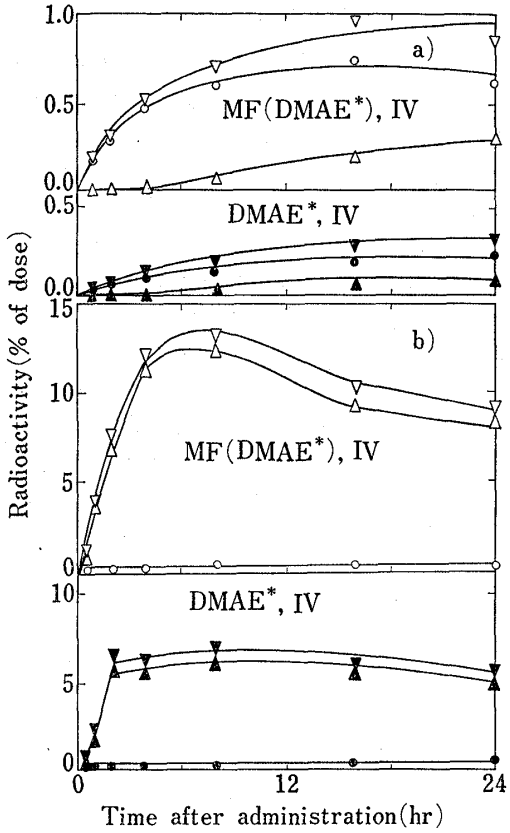

Fig. 3. Radioactivity in the Lipid Fraction of Brain (a) and Liver (b), Respectively

Open and closed symbols indicate the data obtained from MF (DMAE*) and DMAE* administration, respectively. ▽▼ : total lipid fraction, ○● : ptd-DMAE*, △▲ : ptd-choline

free DMAE* was given (Fig. 2a). However, after either free DMAE* or MF (DMAE*) administration, decrease in DMAE levels accompanied concomitant increase in DMAE*-P levels.

In contrast to the brain, DMAE* in the liver was converted much more rapidly to DMAE*-P, since DMAE*-P accounted mostly for the radioactivity in the hepatic acid-soluble fraction even shortly after administration as shown in Fig. 2b. Levels of radioactivity in DMAE*- or MF (DMAE*)-treated mouse liver were found about two fold higher in the latter, suggesting exceeding permeability of the ester to the hepatic cell membrane.

Lipid Fraction

Levels of radioactivity in the lipid fraction were found to increase with concomitant decrease in the acid-soluble fraction, as shown in Fig. 3.

Radioactivity of ptd-DMAE* in the brain gradually increased and reached a plateau about 4 hr after administration either of MF (DMAE*) or DMAE* (Fig. 3a). Interestingly, the appearance of ptd-choline* in the brain much delayed as compared to that of ptd-DMAE*, in either treatments with DMAE* or MF (DMAE*).

In contrast to the brain, the liver synthesized ptd-choline more rapidly (Fig. 3b), and ptd-DMAE* was only in very small amounts throughout the experimental period.

Table I. Specific Activity of Choline Base after Intravenous Administration of MF (DMAE*) and DMAE*

Drug administered	Mouse	Time after administration (hr)	Liver (10^{-3} dpm/μmole)		Brain (10^{-3} dpm/μmole)	
			Acid-Soluble Fraction	Lipid Fraction	Acid-Soluble Fraction	Lipid Fraction
DMAE*	1	4	37	22	0.55	0.07
	2		38	17	1.1	0.33
MF(DMAE*)	1	4	39	35	2.0	0.24
	2		—a)	45	1.2	0.43
DMAE*	1	24	19	14	3.9	1.2
	2		31	16	3.6	0.96
MF(DMAE*)	1	24	41	25	9.9	3.4
	2		43	28	—	—

a) not determined

Table II. Specific Activity of Lipid Choline Following Administration of ^{14}C-Methyl-Methionine

Drug	Liver (dpm/μg)	P	Brain (dpm/μg)	P	n
None	543±33		5.30±0.51		4
DMAE	562±36	NS(C)	4.82±0.13	NS(C)	3
MF	409±32	<0.05(C)	4.39±0.23	NS(C)	4
		<0.05(D)		NS(D)	

Values are means±S.E.
NS: not significant, C: compared with control and D: compared with DMAE n: number of animals

Incorporation of DMAE* into Choline

Incorporation of DMAE* into acid-soluble and lipid cholines was examined, and the results are listed in Table I.

The hepatic acid-soluble total cholines showed higher specific radioactivity than lipid cholines in both animals given DMAE* and its ester. Similarly, the specific radioactivity of cerebral choline was higher in acid-soluble fraction than in lipid fraction. These results are essentially the same as those reported by Ansell and Spanner.[13] The specific activities of both acid-soluble and lipid cholines in the brain were not so much affected as might be expected from a marked difference in radioactivity levels of DMAE* derivatives between DMAE*- and MF (DMAE*)-treated animals.

Incorporation of Methionine-methyl-^{14}C into Choline

The specific radioactivities of lipid choline are compared in Table II among control, DMAE- and MF-treated mice after the administration of methionine-methyl-^{14}C.

In control animals, the methylation of ptd-ethanolamine derivatives would result in the labeling of lipid choline along the ptd-choline biosynthetic pathway. In DMAE-treated animals the incorporation of ^{14}C-methyl group into hepatic lipid choline was of similar magnitude to the control mice. On the other hand, slight decrease was observed in mice treated with MF, indicating that the amount of DMAE molecule derived from MF was so sufficient that endogenous methylation of ptd-ethanolamine could be affected.

Incorporation of ^{14}C-methyl group into cerebral lipid choline was of 1% level of that incorporated into hepatic choline in each animal. Despite the several times higher abundance of DMAE derivatives in the MF-treated brain (Fig. 3a), no significant difference was found in

13) G.B. Ansell and S. Spanner, *Biochem. J.*, **122**, 741 (1971).

the specific activity of the lipid choline between control or DMAE-treated mice and MF-treated animals. Accordingly, it seems that appearance of ptd-choline in the brain is independent of DMAE derivative levels in this tissue. These results imply that the lipid choline in the brain does not originate from DMAE derivatives in the brain itself.

Discussion

After intravenous administration of MF to mice, it was demonstrated that MF penetrated into brain as an intact ester, followed by hydrolysis to form the acid and DMAE. The former was known to be excreted without further biotransformation.[5] Metabolism of the latter in the brain was revealed in the present study that DMAE was phosphorylated to form DMAE-P, which was in turn converted to ptd-DMAE probably *via* CDP-DMAE. The time course of these sequential metabolism of DMAE suggests that ptd-DMAE is the end-product in the brain. The physiological level of DMAE in the acid soluble fraction is reported to be about 1 nmole/g brain of cat.[14] The amounts of DMAE penetrated into brain were calculated, based on the specific activity, to be about 1 and 0.1 μmoles/g tissue when MF and DMAE were given in the present experiment, respectively. Therefore, these administrations would elevate the actual DMAE level in brain to more than one hundred fold of the physiological level. Accordingly, levels of the DMAE-derivatives in acid-soluble and lipid fractions would be elevated in brain in a manner shown in Fig. 2a and 3a, respectively.

Thus, the present study showed that DMAE was finally incorporated into cerebral phospholipid about 4 hr after either administration of MF or DMAE. The level of DMAE-phospholipid in the brain was also demonstrated to be several fold higher in the former treatment. Effect of these two drugs on the central nervous system has been reported that MF has the central activities even after single administration (*e.g.*, ref. 15) whereas free DMAE was shown to be a brain stimulator only when administered repeatedly.[16] These observations are in good agreement with the hypothesis proposed by Groth, *et al.*[17] that at least a part of the central activity of DMAE itself is closely related to its final metabolite in the brain. As mentioned, a marked quantitative difference of DMAE derivatives in the brain may partly explain the difference in onset of the central actions of the two drugs.

The stepwise methylations of ptd-ethanolamine are believed to be the biosynthetic pathway of choline base *de novo*. The brain seemingly lacks in this methylating activity,[7] and therefore other route should be present for choline supply to this tissue. Ansell and Spanner[13] postulated the hypothesis that cerebral choline originates from the tissues other than brain and is primarily transported into brain in a lipid soluble form, probably as ptd-choline. According to their hypothesis, it is reasonable that cerebral incorporations of DMAE* into the acid-soluble and lipid choline, together with ^{14}C-methyl incorporation into the lipid choline were not so affected in animals treated with MF, DMAE and nothing (Table I and II), in contrast to marked differences in levels of cerebral DMAE derivatives. The levels of cerebral ptd-choline* were found to be much lower, despite those of DMAE derivatives, following either administration of MF (DMAE*) or DMAE*. There found seemingly a presence of the time lag (until about 4 hours) of ptd-choline appearances in the brain, followed by a gradual increase in its levels (Fig. 3a). There proposed an opposite hypothesis[6] that brain synthesizes acid-soluble choline derivatives from acid-soluble DMAE derivatives. However,

14) C.G. Honegger and R. Honegger, *Nature*, **184**, 550 (1959).
15) G. Quadbeck, B. Claver, and G. Minet, *Arzneimittel. Forsch.*, **14**, 563 (1964); Von J. Kugler, A. Doenicke, and E. Harted, *ibid.*, **21**, 82 (1973).
16) C.C. Pfeiffer, E.H. Jenney, W. Gallagher, R.P. Smith, W. Bevan, Jr., K.F. Killam, E.K. Killam, and W. Blackmore, *Science*, **126**, 610 (1957).
17) D.P. Groth, J.A. Bain, and C.C. Pfeiffer, *J. Pharmacol. Exptl. Therap.*, **124** (1958).

the time courses in cerebral levels of ptd-choline, together with ^{14}C-methyl incorporation in the present study are in much more conformity with the transport hypothesis of choline derivatives into brain, implying that the cerebral ability to form choline is negligible if any.

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