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Temporal dynamics and regional distribution of [^{14}C]serine uptake into mouse brain

Jan Paweł Woronczak[1], Ewa Siucińska[2], Małgorzata Kossut[2] and Jolanta Barańska[1]

[1]Department of Cellular Biochemistry and [2]Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Abstract. In order to examine the uptake of L-serine into brain structures and brain metabolic compartments, L-[U-^{14}C]serine was injected into tail vein of mice. The uptake was examined 30 min, 90 min, 3 h and 5 h after injection by both quantitative autoradiography of coronal brain sections and by biochemical analysis. Brain radioactivity was extracted and partitioned into protein associated pellets, metabolites soluble in aqueous phase and lipids soluble in the organic phase. Most of the radioactivity was found in the aqueous phase, about 10% was incorporated into lipids. Among phospholipids the highest label was found in phosphatidylserine, then in phosphatidylethanolamine and in phosphatidylcholine, it amounted to 52%, 30% and 18% of label by 90 min after injection, respectively. The brain distribution of L-serine uptake resembled that described for strychnine-insensitive [^{3}H]glycine binding, with cortical structures being preferentially labelled.

Address for correspondence:
Jolanta Barańska
Department of Cellular Biochemistry
Nencki Institute of
Experimental Biology
3 Pasteur St.
02-093 Warsaw, Poland

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INTRODUCTION

L-serine can be used in mammalian brain in a number of metabolic pathways. It can also, by a number of ways, influence the animals cognitive capacities. L-serine, itself, or converted into glycine, modulates NMDA receptor (Danysz et al. 1990, McDonald et al. 1990, Hashimoto et al. 1992), and converted into phosphatidylserine (PS) affects the central cholinergic systems (Pepeu et al. 1989), plays an important role as an activator of protein kinase C (Nishizuka 1986), and has a positive effects on the behaviour and learning of aged animals (Calderini et al. 1986). Recent interest in exogenous application of serine derivatives as pharmacological agents aimed at amelioration of mnemonic brain functions (Gelbmann and Muller 1991) called for closer examination of dynamics of serine incorporation into the brain.

It has been shown in numerous *in vivo* studies (Battistin et al. 1970, Oldendorf 1971, Yudilevich et al. 1972, Banos et al. 1975, Shershen and Lajtha 1976, Betz and Goldstein 1978), that unidirectional uptake of amino acids from the blood to the brain *via* blood-brain-barrier occurs easily for the large neutral amino acids as, e.g., L-phenylalanine, L-leucine, L-tryptophan and L-methionine, whereas there is little or no transport of small neutral amino acids, such as L-alanine, L-serine, L-proline and glycine. Amino acids belonging to the first group are essential amino acids, whereas the second group represents nonessential ones (Oldendorf 1971). It has been suggested that the slow brain uptake of nutritional amino acids of the second group may be correlated with the ability of the brain to synthesize them from a glucose substrate (Rose et al. 1948), whereas amino acids belonging to the first group are essential in that sense that most probably they cannot be synthesized by brain and must enter the brain from the circulating blood (Banos et al. 1973).

However, L-serine and L-alanine enter the brain more rapidly than glycine and such amino acids as L-glutamate and L-asparate. Uptake of glycine, L-asparate and L-glutamate to the brain in rats is negligible (Oldendorf 1971), and does not occur in mice (Battistin 1970). It has been therefore suggested that L-serine and L-alanine, although not essential for the whole body, can be essential for the brain as a source of putative precursors used by the brain to make glycine (from L-serine) and glutamate and asparate (from L-alanine) (Battistin et al. 1970). Interconversion of glycine and serine in the brain has been documented (Shank and Aprison 1970); also it has been demonstrated (Banos et al. 1975) that the brain cells synthesize glycine from serine, and not the reverse. As it is known, glycine in CNS plays a role as a central transmitter which by binding to the N-methyl-D-asparate (NMDA) receptor modulates this receptor-channel complex (Johnson and Ascher 1987, Danysz et al. 1990, McDonald et al. 1990, Hashimoto et al. 1992).

On the other hand, L-serine may be used in the brain not only for glycine production and for the synthesis of proteins, but also for the lipid synthesis. In mammals, PS is formed by Ca^{2+}-dependent and non-energy requiring exchange of L-serine with the base of preexisting phospholipids (Kanfer 1980, Barańska 1982, Czarny et al. 1993). PS formed in such reaction is easily decarboxylated into phosphatidylethanolamine (PE), which is converted into phosphatidylcholine (PC) *via* three successive methylations. These metabolic conversions may play a key role in the remodeling of preexisting phospholipids and may be a potential source for the generation of new choline molecules (Blusztajn et al. 1979).

We have previously shown (Woronczak et al. 1993) that PS synthesized from labelled serine in slices or homogenates from different brain regions was slowly metabolized to PE, and PE was converted into PC in an insignificant range. In the present study we examined brain lipid metabolism *in vivo*, including PS synthesis and turnover following intravenous injection of [^{14}C]serine in mice. We also compared the radioactivity between lipids and water-soluble metabolites and investigated regional radioactivity of the brain by autoradiographic determinations.

METHODS

Chemicals

L-[U-^{14}C]serine (160 mCi/mmol) was purchased from Amersham, UK. Aluminium TLC plates covered with Silica Gel 60 (without fluorescence indicator) and Silica Gel H for preparing TLC plates were from Merck, Darmstadt, FRG.

Animals

Male Swiss mice aged 4 weeks, body weight of about 20 g, were used. The animals were given food and water *ad lib.* until 1 h before the beginning of the experiment.

Procedures

INJECTIONS

Mice were injected into tail vein with 10 μCi of L-[U-^{14}C]serine in 150 μl of Tris-HCl buffer (pH 7.4). At appropriate times (30 min, 90 min, 3 h, or 5 h) mice were anaesthetized with overdose of Nembutal and perfused through the aorta with physiological saline. Brains were then rapidly removed, weighted, frozen in isopentane chilled to -60°C and stored at -70°C prior to cutting. The frozen brains were cut into 20 μm coronal sections on a cryostat at -20°C. Three of every ten sections were picked up on cover slips, dried rapidly at 60°C and processed for film autoradiography, whereas all remaining material was collected, weighted and used for biochemical analysis.

BIOCHEMICAL ANALYSIS

Sections collected for biochemical analysis and any other remaining material from each brain were pooled and homogenized in methanol (5 vol). From this homogenate aliquots to determine the total radioactivity were taken, then 10 vol of chloroform were added and lipids were extracted according to Folch et al. 1957. The phases were separated by centrifugation. Organic phase (representing hydrophobic compounds) and water phase (representing hydrophilic compounds) were separated from insoluble pellet (representing proteins). Pellets were solubilized in formic acid. From all three phases aliquots were taken for radioactivity counting. Samples of organic phase (about 1/4 of total amounts) were evaporated under nitrogen, dissolved in small volumes of chloroform/methanol (1:1) and lipids were separated using two-dimensional thin-layer chromatography on Silica Gel 60 plates (0.2 mm thick) or Silca Gel H plates (0.5 mm thick). The solvent in the first dimension was chloroform/methanol/25% ammonium hydroxide/water (55:33:4:2, v/v), and in the second chloroform/methanol/acetic acid/water (30:15:6:1, v/v). Spots were visualized with iodine and ninhydrin and scrapped off for radioactivity counting. Radioactivity of all samples was determined with Beckman LS6000TA scintillation counter using toluene/ethanol scintillator.

Autoradiography

Twenty-micrometers thick brain sections were mounted on coverslips, glued on a cardboard and apposed, together with sets of calibrated ^{14}C standards (American Radiochemical), against Kodak X-ray films at room temperature for 7 weeks. Films were developed with Kodak D-19 developer.

Image analysis

After development of autoradiograms, regional concentrations of radioactivity were analysed and quantified by a computer-assisted image analysis system (256 gray levels). Optical density (gray levels) was converted to ^{14}C counts using calibrated ^{14}C standards curve. Concentration of ^{14}C was measured in the following brain structures: cerebral cortex, hippocampus, thalamus, striatum, cerebellum, pons and in the cerebral ventricles. Optical density readings were taken from each section on which a given structure has been identified. Three readings were taken at each site. The results are expressed as a mean of all readings ± SD.

RESULTS

Figure 1 presents the uptake of radioactive serine into the brains of mice. The total brain radioactivity increased slowly and reached a maximum by 90 min after injection (Fig. 1A). This value amounted to approximately 0.25% of the injected L-[U-^{14}C]serine. After that time the decrease in the total radioactivity occurred, and by 5 h after injection it amounted to less than 50% of the maximal total radioactivity.

Brain radioactivity was extracted and partitioned in protein-associated pellets, metabolites soluble in aqueous phase and lipids soluble in the organic phase. As shown in Fig. 1A and B, the highest radioactivity was found in aqueous phase. The radioactivity of this phase, representing [^{14}C]serine itself and its aqueous-soluble metabolites, was maximal at 30 min (about 80% of the total brain radioactivity) and after that time decreased linearly. On the other hand, radioactivity in proteins and lipids increased up to 3 h, and reached a maximum 40% in proteins and 15% in lipids of total brain radioactivity. These data indicated that L-serine transported to the brain can be there further used for proteins and lipids production.

Preliminary experiments showed that among the lipidic organic phase obtained from brains about 70-90% of the label from [^{14}C]serine was incorporated into phospholipids. Though variable, the remaining 10-30% radioactivity was tentatively identified as free fatty acids, most probably originating from the carbon skeleton of L-[U-^{14}C]serine after its deamination, and neutral lipids which

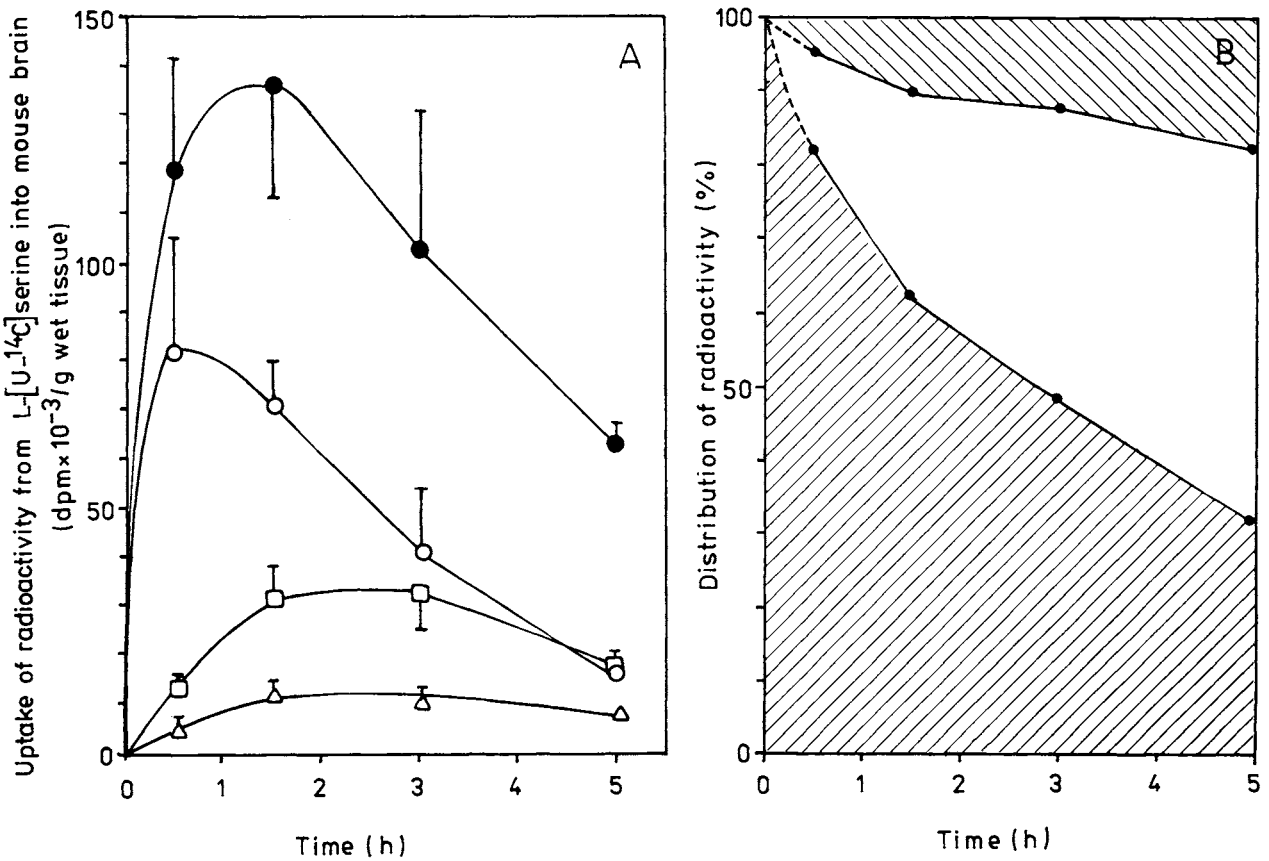

Fig. 1. A, incorporation of radioactivity from L-[U-^{14}C]serine into mouse brain (●) total, (O) water-soluble compounds, (□) proteins, (Δ) lipids. B, distribution of radioactivity from L-[U-^{14}C]serine among: water-soluble compounds (right striped area on the bottom of the figure), proteins (plain area) and lipids (left striped area on the top of the figure) in mouse brain (surface plot). The results are the mean ± SD for 4 mice.

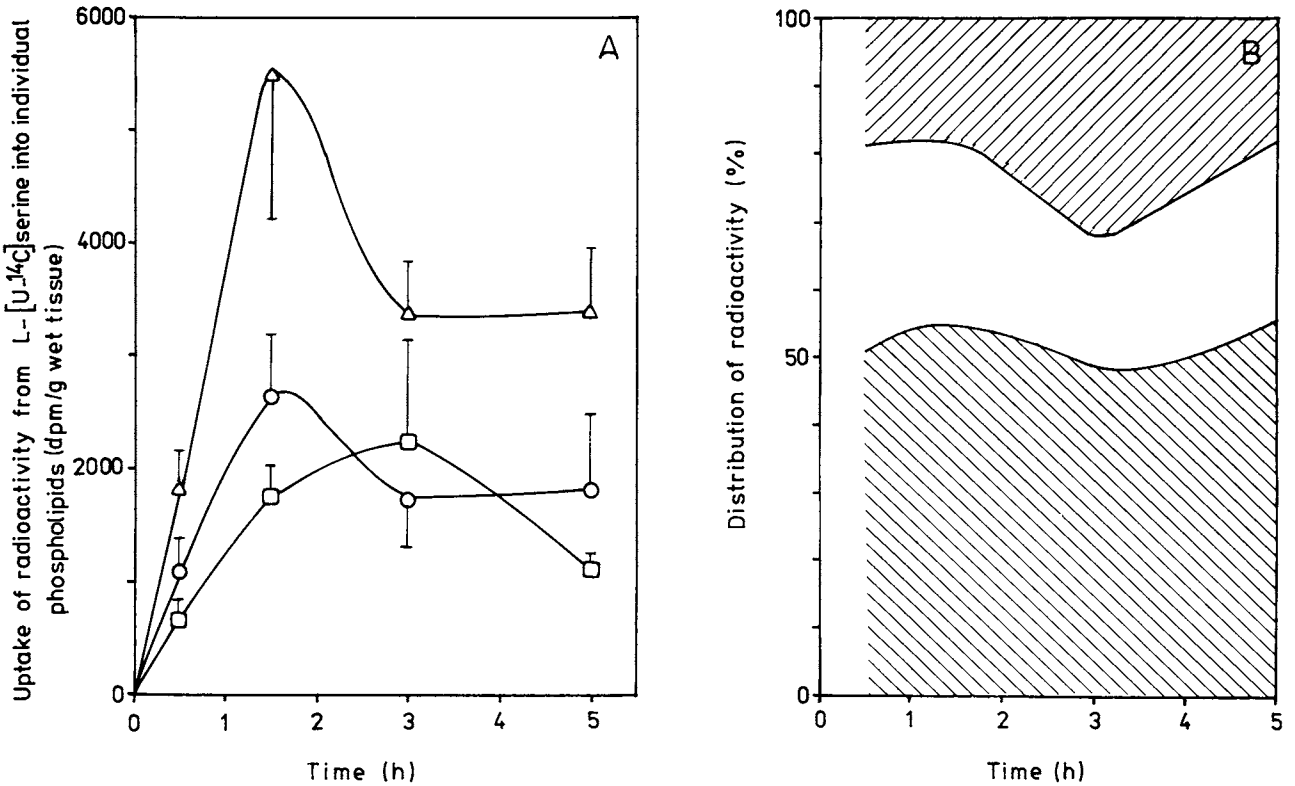

Fig. 2. A, incorporation of radioactivity from L-[U-^{14}C]serine into individual phospholipids in mouse brain (Δ) PS,(O) PE, (□) PC. B, distribution of radioactivity from L-[U-^{14}C]serine among: PS (left striped area on the bottom of the figure), PE (plain area), and PC (right striped area on the top of the figure) in mouse brain (surface plot). The results are the means ± SD for 4 animals.

radioactivity was probably a result of deacylation-reacylation reactions. Thin-layer chromatography of phospholipids, showed that PS, PE and PC constituted about 85% of the labelled phospholipids, with minor contribution from corresponding lyso-compounds and sphingomyelin. The distribution of radioactivity among PS, PE and PC is presented in Fig. 2. As is shown, PS constituted the highest radioactivity of labelled phospholipids (Fig. 2A). Newly synthesized PS was significantly decarboxylated to PE, since radioactivity in both phospholipids increased simultaneously up to approximately 90 min, then decreased with time, and after 3 h nearly reached equilibrium. In contrast, radioactivity in PC increased slowly and reached a maximum by 3 h after injection (Fig. 2A). The distribution of radioactivity between PS, PE and PC by 90 min after injection was about 52% in PS, 30% in PE and 18% in PC (Fig. 2B).

Uptake of radioactive L-serine was also measured with quantitative autoradiography at various time intervals after injection in following brain structures: cerebral cortex, hippocampus, thalamus and striatum. The analysis of autoradiograms showed that dependent on time uptake of radioactivity for each of above structures was similar (not shown) as that presented for total brain radioactivity (Fig. 1A).

The examination of brain radioactivity with quantitative autoradiography 30 min and 3 h after injection of L-[U-^{14}C]serine revealed distinct regional differences. The distribution of radioactivity showed that in both time points the highest labelling was present in the cerebral cortex (Fig. 3). High labelling was also found in hippocampus, then in cerebellum, lower in thalamus and striatum and the lowest in pons (Fig. 3).

^{14}C autoradiography of coronal brain sections, obtained 3 h after injection of [^{14}C]serine are shown

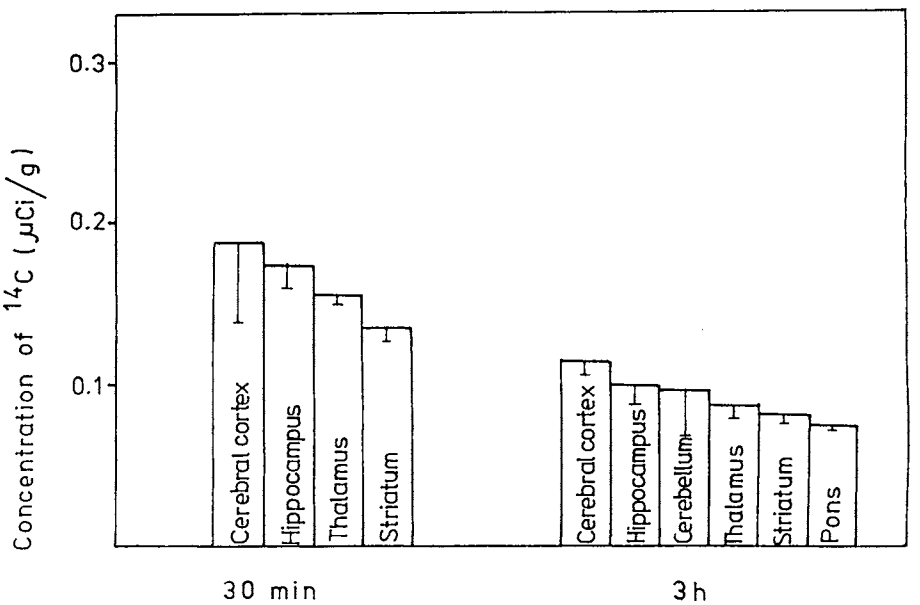

Fig. 3. Incorporation of radioactivity from L-[U-^{14}C]serine into various anatomical regions of mouse brain after 30 min and 3 h. The results are the means ± SD for 3 animals.

in Fig. 4. Pseudocolor images reveal a pattern of labelling with the heaviest density in the lumen of cerebral ventricles, in the neocortex, hippocampus and septum. Distinct lamination of label was observed in the cortex and in the hippocampus. In the cortex supragranular layers were labelled more densely than layers V and VI. In the hippocampus the heaviest label was found in field CA3. The labelling was lower and more uniform in the thalamus and striatum. Gray matter structures were labelled more heavily than white matter.

DISCUSSION

This study demonstrated that intravenously applied L-serine is taken up most readily by neo- and paleocortex, most of its radioactivity is found in the soluble phase but, with longer incorporation times, a significant proportion is found in phosphatidylcholine.

In animals, the brain is the tissue richest in PS, which constitutes about 15% of all phospholipids (Barańska 1982). The base exchange reaction is the only mechanism for the formation of this phospholipid in animals tissues, whereas different pathway exist for the synthesis of PE and PC phospholipids (Kanfer 1980, Barańska 1982, Czarny et al. 1993). PS in the brain is easily decarboxylated into PE (Butler and Morell 1983). The enzymatic system which catalyzes the methylation of PE to PC also exist in the brain, most probably in the synaptic plasma membranes (Blusztajn et al. 1979), but is much less active than in the liver (Blusztajn et al. 1979, Percy et al. 1982, Woronczak et al. 1993). It has been estimated in *in vitro* studies that only about 1% of the total brain PC originated from the phospholipid methylation (Percy et al. 1982, Woronczak et al. 1993). However, in this study we showed that radioactive serine injected intravenously into mice labelled PC significantly. Similarly, much higher synthesis of PC from 3-[^{3}H]serine injected into lateral ventricles of rat brain than that observed in *in vitro* studies has been reported by Gatti et al. 1988.

These results might suggest that successive metabolic conversion of PE to PC (from L-serine *via* PS) requires undisturbed structures of the brain. On the other hand, it seems more likely that the radioactivity found in PC molecule may originate not only from the base of PE, but also from other radioactive compounds originating from of L-[U-^{14}C]serine, e.g., free fatty acids or glycerol. The supposition that injected L-serine is metabolized in the liver (*via* PS and PE) to PC and then it is incorporated into lipoproteins of the blood and transported to the brain, although cannot be excluded, seems unlikely. A possible role of circulating phospholipids as precur-

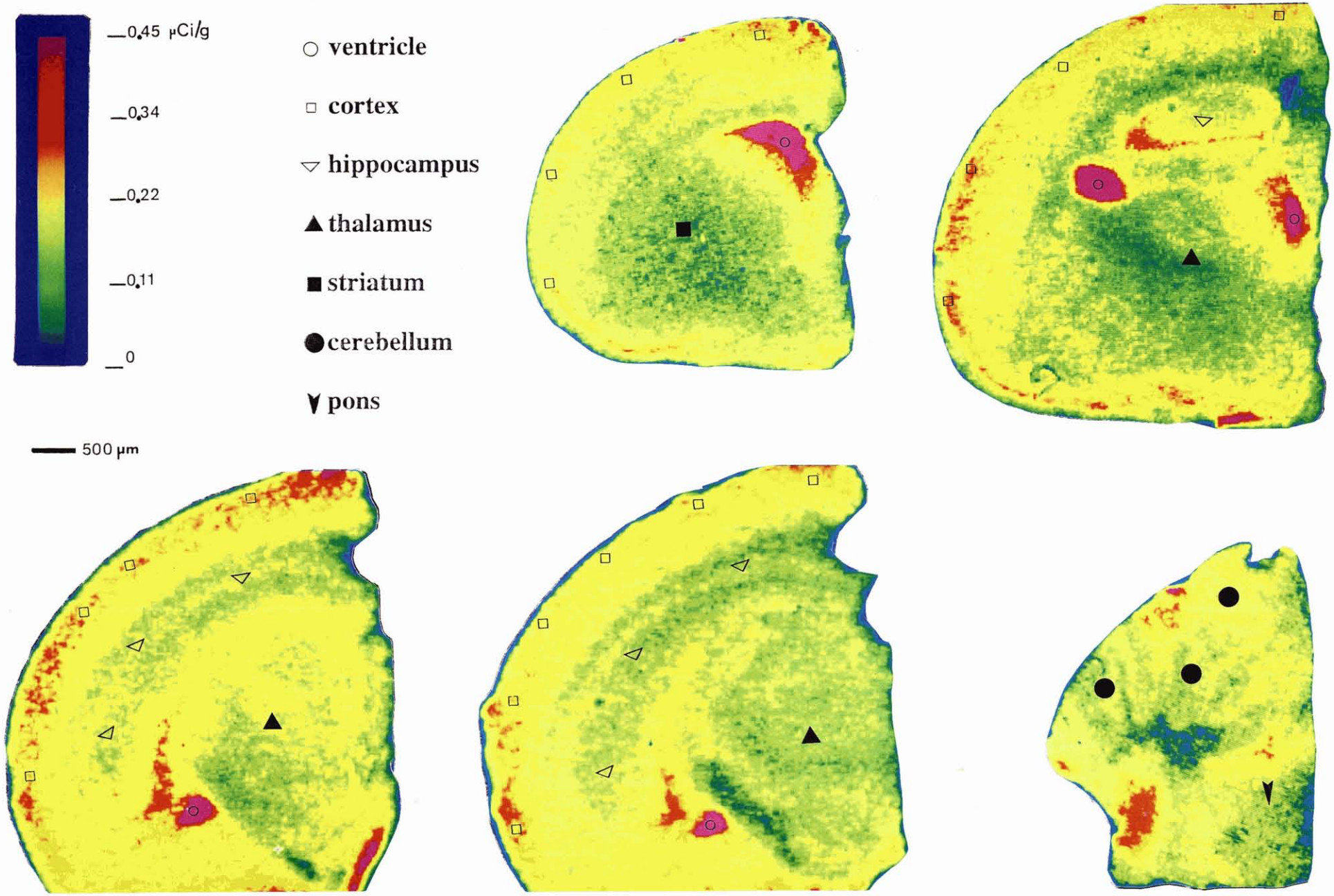

Fig. 4. Pattern of incorporation of radioactivity from L-[U-^{14}C]serine into mouse brain - pseudocolored reconstruction of autoradiograms taken from coronal sections three hours after injections.

sors of brain choline is rather doubtful since permeability of the brain-blood barrier for lipoproteins is similar as that found for nondiffusible compounds (e,g., dextran) (Loffelholz et al. 1993). It is worth noting, that our preliminary experiments with 3-[^{3}H]phosphatidylserine liposomes injected intravenously to mice showed a negligible incorporation of the radioactivity into brain structures (not shown). Nevertheless, it is clear, that L-serine transported to the brain may be actively metabolized in numerous catabolic and anabolic metabolic processes, and that the radioactivity found in different brain regions and revealed in autoradiograms is, in fact, a sum of various metabolites, originated from L-[U-^{14}C]serine.

Because glycine may be formed in brain from serine (Shank and Aprison 1970), it is tempting to compare [^{14}C]serine autoradiographic distribution found in this study with the regional distribution of [^{3}H]glycine binding sites in the brain (McDonald et al. 1990). As it is known, glycine binds to strychnine-insensitive binding site in the NMDA operated channels in the brain and potentiates NMDA-induced increase in cation conductance. This action of glycine may be displaced by D-amino acid isomer of serine and alanine, and also by L-form of serine, but in a much smaller range (McDonald et al. 1990). Glycine can also bind to strychnine-sensitive binding sites associated with glycine receptors that predominate in spinal cord and pons (Danysz et al. 1990), whereas strychnine-insensitive [^{3}H]glycine binding has been found to be highest in hippocampus and cerebral cortex (McDonald et al. 1990).

The pattern of [^{14}C]serine uptake observed in this study corresponds generally to that found for strychnine-insensitive [^{3}H]glycine binding, with cortical structures being preferentially labelled and with upper layers of the cortex having the denser label than the lower ones. In contrast to [^{3}H]glycine binding, CA1 field of the hippocampus shows lower [^{14}C]serine uptake than CA3 field. It remains to be elucidated if the label represents glycine, or for example, L-serine binding to the glycine site (Danysz et al. 1990). It may also, to some degree, reflect binding to strychnine-sensitive glycine binding sites, in particular in pons (Danysz et al. 1990, McDonald et al. 1990).

In conclusion, the study demonstrated that exogenously applied serine is taken up by brain structures with different intensity. Its conversion into PC seems to occur at a higher rate than was previously observed in brain slices. The distribution of its uptake in the brain is similar to that of glycine site of the NMDA receptor which may suggest conversion of part of serine into glycine. Both glycine and PC are important in molecular interactions of the mechanisms of memory formation and better understanding of brain metabolism of serine may contribute to understanding its pharmacological effects.

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