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UPTAKE AND METABOLIC RATE OF LIPOSOME-ENTRAPPED [U-^{14}C]GLUTAMATE IN RATS

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Abstract—The metabolic fate of [U-^{14}C]glutamate administered intravenously to rats in aqueous solutions or entrapped in vescicles of phospholipids extracted from rat brain has been studied. When [U-^{14}C]glutamate was administered entrapped in liposomes, higher serum radioactivity levels and higher radioactivity uptake by liver and brain were observed. Rats treated with [U-^{14}C]glutamate entrapped in liposomes exhibited an increased glutamate oxidation shown by increased expired [^{14}C]CO_2 and a modified elimination of the radioactivity in urine.

The importance of phospholipids in the architecture and in the function of cell membranes, as well as in the regulation of the activity of many enzymes and the size of plasma lipoproteins is becoming more and more evident [1].

The results obtained *in vivo* with the administration of phospholipids are attracting increasing interest [2–4]. The recent experiments by Gregoriadis *et al.* [5–13] on the use of liposomes as general carriers, within which drugs or enzymes can be entrapped and homed to target tissues, have opened new therapeutical possibilities.

Moreover, the most recent results on the tissue distribution of enzymes, antibiotics, or chelating agents, administered incorporated within liposomes, indicate a more efficient utilization and a higher level in tissues and extracellular fluids.

The aim of the present work was to investigate the tissue distribution and the catabolic fate of [U-^{14}C]-glutamate entrapped in liposomes formed with brain phospholipids and administered intravenously to rats.

It was found that, when encapsulated in phospholipid vescicles, glutamate distribution and oxidation to CO_2 were significantly modified.

EXPERIMENTAL

Phospholipids were extracted from rat brain according to Kates [14] and suspended in petroleum ether (5 mg/ml). Their composition was determined according to Skipski [15]. [U-^{14}C]glutamate (Radiochemical Centre, Amersham) was diluted with sufficient phosphate buffer (50 mM, pH 6.4) containing 50 mM Na-glutamate, to obtain a radioactivity level of 2.5 μCi/ml.

Liposome preparation. An aliquot of about 20 ml of the phospholipid petroleum ether suspension was slowly evaporated on an equal volume of [U-^{14}C]glutamate-buffered solution. After sonication in nitrogen at 0° for 30 min, the resulting aqueous liposomal suspension was left standing for 24 hr at 4°. The amount of [U-^{14}C]glutamate encapsulated in liposomes was estimated by substracting free glutamate, determined by glutamate dehydrogenase (EC.1.4.1.3), from the total amount of added glutamate. Encapsulated glutamate is, in fact, inaccessible to the enzyme.

Animal experiments. One milliliter of the liposome suspension containing 5 mg/ml phospholipids in the [U-^{14}C]glutamate buffered solution, or 1 ml of the [U-^{14}C]glutamate buffered solution, both containing 2.5 μCi/ml, was injected into the tail vein of male Wistar albino rats weighing 200–250 g. The treated rats were quickly placed in CO_2-trapping or urine collecting cages. After a selected time interval, the animals were killed by decapitation, and blood was collected. Organs were quickly removed and washed with cold 0.9% NaCl solution.

Radioactivity assay. Expired [^{14}C]CO_2 was trapped in 5 ml of an ethanolamine–ethyleneglycol-monomethylether mixture (2:1 v/v) and counted in 10 ml of scintillation liquid containing toluene–ethyleneglycolmonomethylether (2:1 v/v) and diphenyloxazole (5.5 g/l). After carefully removing the blood from the liver and brain, these organs were homogenized in 0.9% NaCl solution. The radioactivity in a 0.2-ml aliquot of the homogenate was measured in 10 ml Instagel-Packard in a Tri-Carb Liquid Scintillation Spectrometer model 3380-Packard. Protein content was determined according to Gornall [16]. Instagel-Packard was used also for serum and urine samples after a brief centrifugation. Counting efficiency of serum, homogenate and urine was the same when labelled glutamate, free or entrapped in liposomes, was added to samples.

RESULTS

Figure 1 shows the time course of [^{14}C]CO_2 expired by rats treated with [U-^{14}C]glutamate entrapped in liposomes in comparison with that eliminated by rats treated with an aqueous solution of the same amount of [U-^{14}C]glutamate (control group). It can be seen that in the first case the amount of expired [^{14}C]CO_2 was higher and its elimination protracted.

Figure 2A shows that serum radioactivity was significantly higher in rats treated with [U-^{14}C]glutamate encapsulated in liposomes than in control rats.

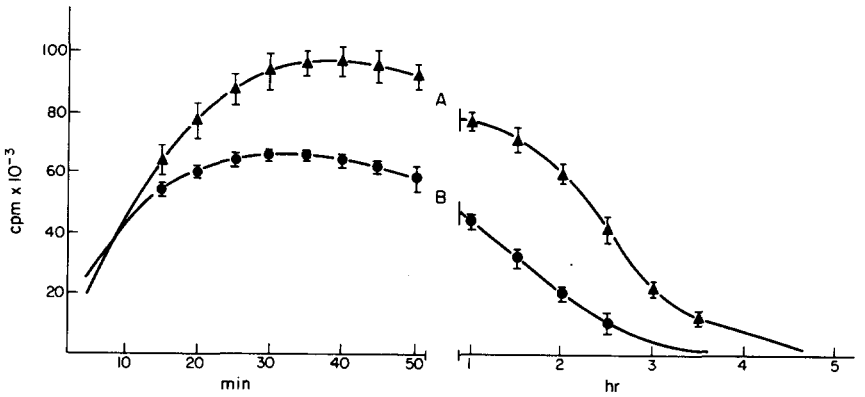

Fig. 1. Time course of $[^{14}C]CO_2$ expired by rats treated with [U-^{14}C]glutamate encapsulated in liposomes (Curve A), or with equimolecular amounts of free [U-^{14}C]glutamate (curve B). Each point on the curve shows the mean ± S.D. of $[^{14}C]CO_2$ expired in a five minutes interval by eight rats.

Moreover, radioactivity in liver and brain was higher in rats treated with [U-^{14}C]glutamate entrapped in liposomes (see Fig. 2B and 2C).

Urinary excretion of radioactivity was prolonged in rats treated with [U-^{14}C]glutamate encapsulated in liposomes in comparison to the controls treated with the same amount of [U-^{14}C]glutamate in aqueous solution (see Fig. 3).

CONCLUSIONS

The results reported in the present paper clearly show the quantitatively different metabolic fate of [U-^{14}C]glutamate when administered entrapped in vescicles made up with phospholipids extracted from rat brain. The delayed clearance of administered [U-^{14}C]glutamate from the blood together with the retarded elimination of radioactivity in urine and of $[^{14}C]CO_2$ by the lungs indicate a modified utilization of glutamate when administered in liposomes.

The greater uptake of liposome-entrapped glutamate by liver probably reflects the higher serum level of liposome-entrapped glutamate. On the other hand, the higher brain uptake may represent an increased crossing of the blood–brain barrier. The latter in fact, is considered more permeable to lipophilic than to hydrophilic molecules. Consequently, it is possible that liposomes entrapping glutamate might cross the blood–brain barrier more easily than glutamate in aqueous solution.

Finally, the higher amounts of expired $[^{14}C]CO_2$ observed in animals treated with glutamate entrapped in liposomes indicate that glutamate preferentially follows a catabolic pathway leading to CO_2 as a consequence of an increased glutamate flux within the tissues. This higher conversion of glutamate into CO_2 might be a consequence of its higher concentration in serum.

From a general point of view, the results indicate the possibility of diverting the *in vivo* distribution and

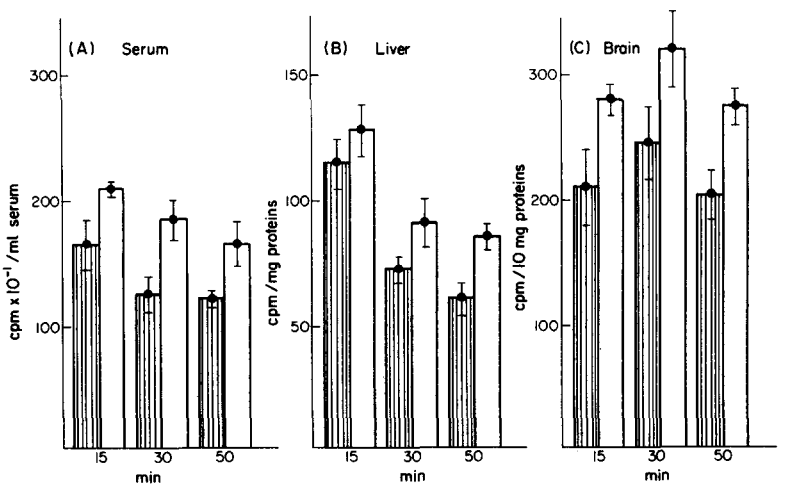

Fig. 2. Time course of radioactivity levels in serum (A), liver (B) and brain (C) homogenates in rats injected with liposome-entrapped [U-^{14}C]glutamate (empty bars) or with free [U-^{14}C]glutamate (filled bars). Each bar shows the mean ± S.D. of radioactivity observed in eight rats.

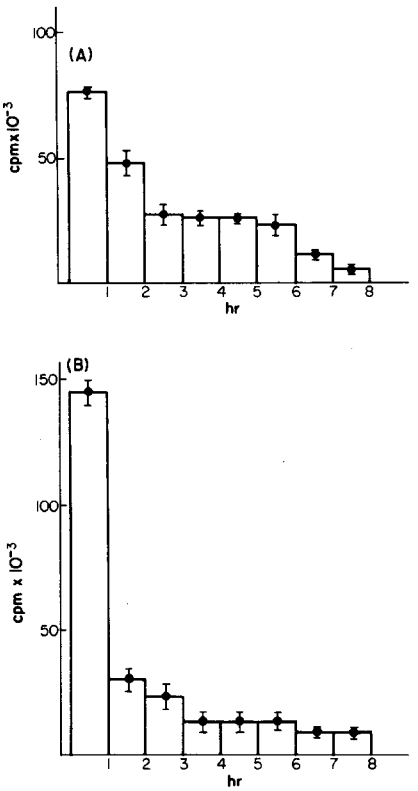

Fig. 3. Urinary excretion of radioactivity after injection of liposome-entrapped [U-^{14}C]glutamate (A) and free [U-^{14}C]glutamate (B). Each bar shows the mean ± S.D. of radioactivity observed in eight rats.

metabolism of utilizable substrates by administrating them encapsulated in liposomes.

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