SC 2310

Possible role of a placental lipid fraction in the transport of [1-^{14}C]glycine from maternal to fetal blood*

In a recent hypothesis[1], it is assumed that a phosphatido-peptide fraction, which is a constituent of cell membranes, is involved in the active transport of amino acids in liver cells. That such a fraction may actually function as a carrier is supported by the work of LEVINE[2] and BOLIS-MAGISTRETTI AND MUSA[3] on the intestinal absorption of several drugs, such as ammonium quaternary salts and tetracycline.

According to CHRISTENSEN AND STREICHER[4], cell and placental membranes present certain functional similarities. Since an active transport of amino acids from maternal to fetal blood is at present largely accepted[5], it seemed interesting to test whether the proposal of TRIA AND BARNABEI[1] is valid for the placental transport of labelled glycine. The presence of a phosphatido-peptide fraction in rat placenta has been demonstrated by QUINTO and coworkers[6] and by BOTTIGLIONI AND ORLANDI[7].

[1-^{14}C]Glycine (10 μC), dissolved in 1 ml of physiological saline, was injected into the femoral veins of rats in the last stages of pregnancy. At appropriate times, a sample of maternal blood was withdrawn from the heart and the pregnant uterus was removed. Fetal blood was obtained by decapitation of the fetus. Protein-free filtrates were obtained from both maternal and fetal blood by treatment with methanol.

The placentae were homogenized with 5 vol. of cold 10% trichloroacetic acid. After the usual washings, proteins, neutral lipids, phospholipids and the phosphatido-peptide fraction were separated for the determination of radioactivity[9], which was performed in a thin end-window gas-flow Geiger–Müller counter (Tracerlab).

The general analytical methods were those previously reported[9]. [1-^{14}C]Glycine,

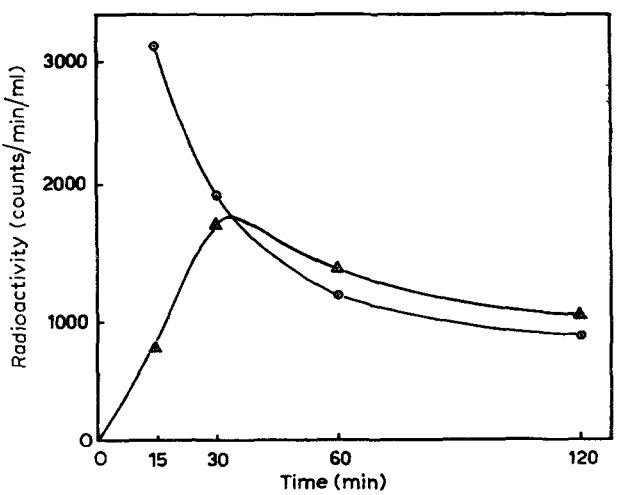

Fig. 1. Radioactivity of protein-free filtrates of blood, after injection of 10 μC of [1-^{14}C]glycine. ⊙—⊙, maternal blood; △—△, fetal blood.

* Some of the results in the present paper were presented at the International Congress of Physiological Sciences of Leiden[8].

having a specific activity of 64 μC/mg, was obtained from the Radiochemical Centre, Amersham (Great Britain).

After injection of 10 μC [1-^{14}C]glycine, the radioactivity of fetal blood rose, and after about 40 min became higher than that of maternal blood (Fig. 1). Although the difference was small, an active transport is indicated by the fact that the concentration of glycine in fetal blood is twice as high as in maternal blood[10]. Autoradiographic experiments, performed on protein-free filtrates from blood, indicated that the radioactivity of both maternal and fetal blood was essentially due to labelled glycine.

During the passage of the labelled compound, placental protein incorporated the radioactivity gradually, while the radioactivity in the phosphatido-peptide fraction reached its maximum rapidly and thereafter diminished (Table 1). The other lipid fractions became negligibly labelled, especially after short experimental periods. Similar results were reported by QUINTO and coworkers[6] and by BOTTIGLIONI AND ORLANDI[7].

TABLE I

INCORPORATION OF RADIOACTIVITY INTO THE PROTEIN AND THE PHOSPHATIDO-PEPTIDE FRACTION, DURING THE TRANSFER OF [1-^{14}C]GLYCINE FROM MATERNAL TO FETAL BLOOD

No. of expts.	*Time after administration of 10 μC [1-^{14}C]glycine (min)*	*Specific activity (counts/min/mg)**	
		Protein	*Phosphatido-peptide fraction***
3	15	7 ± 1	24 ± 5
2	30	20 ± 4	19 ± 4
10	60	60 ± 11	18 ± 4
3	120	79 ± 14	20 ± 3

* ± standard deviation of one observation.

** 6–10 mg of this fraction were obtained from the placentae of each rat. The composition was: total N, 2.3%; total P, 2.8%; amino-nitrogen, 0.1%; amino-nitrogen after 24-h hydrolysis with 6 N HCl, 1.1%. Paper chromatographic experiments on acid hydrolysates showed the presence of most of the common amino acids, with the apparent exception of tyrosine, cysteine and tryptophan.

MACFARLANE[11] recently found that lipid amino acid derivatives are labile to mild alkaline hydrolysis, and suggested that these compounds are hydroxy-amino acid esters of phosphatidylglycerol. Similarly, we found that after treatment with dilute NaOH (1 ml of 0.1 N NaOH added to 5 ml of a chloroform–methanol (2:1, v/v) solution of the labelled phosphatido-peptide fraction) a large part of the radioactivity became water soluble, indicating a labile amino acid linkage. The presence of free glycine in the hydrolysates was shown by paper chromatography and autoradiography. No appreciable effect was observed in the presence of dilute HCl.

The action of some inhibitors of protein synthesis on the placental transfer of labelled glycine is reported in Table II: in the rats given a diet containing 1% of DL-ethionine for 2 days, or injected just before the experiment with puromycin, the transport was strongly reduced. Chloramphenicol, known to be a poor inhibitor of protein synthesis in animal systems, was without effect at least at the doses employed. Ethionine and puromycin inhibited both the incorporation of [1-^{14}C]glycine into the phosphatido-peptide fraction and the placental synthesis of protein.

It is known that in rats fed ethionine, the level of free amino acids in the plasma rises[12]. Similarly, a small increase of free amino acids occurred as an effect of dosing with puromycin. 1 h after the injection of this substance (0.1 g/kg), we had values of 5.1 mg of amino-nitrogen per 100 ml of maternal blood and 8.8 mg of amino-nitrogen per 100 ml of fetal blood, the corresponding values for controls being 4.7 and 8.3. It seems unlikely therefore that the effects observed are a consequence of an alteration in the level of free amino acids in blood.

TABLE II

EFFECTS OF SOME TREATMENTS ON THE PLACENTAL TRANSPORT OF [1-^{14}C]GLYCINE AND ON THE INCORPORATION OF RADIOACTIVITY INTO PLACENTAL FRACTIONS

10 μC of [1-^{14}C]glycine were injected into the femoral vein and the experiment was interrupted 1 h thereafter.

No. of rats	*Treatment*	*Radioactivity of blood (counts/min/ml)*		*Ratio of the radioactivity fetal/maternal*	*Specific activity of placentae (counts/min/mg)**	
		Maternal	*Fetal*		*Protein*	*Phosphatido-peptide fraction*
10	—	1260 ± 153	1430 ± 180	1.12	60 ± 11	16 ± 4
6	DL-Ethionine (1% in the diet for 2 days)	3170 ± 405	1670 ± 240	0.53	18 ± 3	6 ± 2
3	Puromycin (0.05 g/kg)	1900 ± 310	1100 ± 120	0.58	47 ± 14	8 ± 2
3	Puromycin (0.1 g/kg)	2150 ± 60	980 ± 70	0.46	35 ± 3	8 ± 1
5	Chloramphenicol (0.1 g/kg)	1700 ± 130	2000 ± 210	1.18	70 ± 12	15 ± 3

* ± standard deviation of one observation.

The results of the present work indicate that the placental phosphatido-peptide fraction incorporates labelled glycine at a rapid rate and by an alkali-labile linkage. The incorporation into such a fraction parallels the uptake of the same amino acid into the fetal blood, as indicated by the behavior towards puromycin and ethionine. It seems possible therefore that the placental phosphatido-peptide fraction may function as a carrier of amino acids. Further data on chemical composition and kinetic measurements are necessary to confirm this statement.

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