Volume 222, number 2, 256–260 FEB 05152 October 1987

Characterization of glucacon receptors in Golgi fractions of fetal rat liver

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Received 11 August 1987

The present study was designed to determine if Golgi fractions from fetal rat liver contain glucagon receptors and to characterize the properties of such receptors. Purification patterns of liver plasma membranes and Golgi fractions from fetal and adult rats were similar, as verified by morphological and biochemical approaches. Glucagon binding was greater in plasma membranes of adult than fetal rats, while in Golgi fractions glucagon binding was similar in both groups. The modifications in glucagon binding reflect changes in glucagon receptors. Glucagon association and glucagon receptor inactivation by liver membranes were similar in the two groups of animals, while glucagon degradation was lower in fetal than in adult rats.

Glucagon receptor; Golgi fraction; Fetus; (Wistar rat)

1. INTRODUCTION

Along with binding sites on the plasma membrane, specific receptors on intracellular organelles have been characterized for several hormones and neurotransmitters [1,2], and have been related to receptor internalization, recycling and to inactivation of the ligands [3]. In the case of glucagon receptors, these have been extensively investigated in isolated hepatocytes and purified liver plasma membranes of adult rats [4,5], whereas intracellular glucagon binding sites have not been characterized until very recently [6]. In contrast with adult individuals, in the fetus glucagon receptors have not been characterized in intracellular organelles despite the delayed appearance of glucagon binding sites in the cell surface of hepatocytes as compared with the rapid development of insulin receptors [7] which may be of significance in the metabolic and growth processes of the fetus.

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Accordingly, the present studies were designed to determine whether Golgi fractions from fetal rat liver contain specific receptors for glucagon and to characterize the properties of such receptors.

2. MATERIALS AND METHODS

Male rats weighing 150–200 g with free access to food and water and 21-day-old fetuses of the Wistar strain were used.

Liver plasma membranes or Golgi fractions were obtained from 15 livers from 21-day-old fetuses and 1 liver from an adult rat. Livers were removed from the abdominal cavity through a median incision and immediately homogenized. All operations were performed at 4°C. Highly purified liver plasma membranes were prepared according to Neville [8] as modified by Phol et al. [9], up to step 18. Golgi fractions were prepared as described by Ehrenreich et al. [10], omitting the ethanol treatment of the animals prior to sacrifice. Subcellular fractions were stored in liquid nitrogen until used for biochemical analysis. To provide an index of membrane purification, the binding of ^{125}I-glucagon to its receptors, 5′-nucleotidase [11] and

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00145793/87/$3.50

galactosyltransferase [12] activities were determined at various stages of membrane purification. Membrane protein was determined by the method of Lowry et al. [13].

Mono-^{125}I-glucagon was obtained according to the procedure of Nottey and Rosselin [14] with specific activities of 450–500 μCi/μg. For binding studies, highly purified liver plasma membranes were incubated with Krebs Ringer phosphate buffer and Golgi fractions with 50 mM Tris-HCl buffer, pH 7.4. Incubation mixtures for the measurement of glucagon contained a final volume of 0.25 ml of the corresponding assay buffer, 1% bovine serum albumin, ^{125}I-glucagon (5×10^{-11} M), bacitracin (0.5 mg/ml) and liver membranes (40–60 μg protein). Incubations were carried out at 10°C for 0–8 h, unless otherwise indicated. At the end of the incubation periods, the samples were filtered on Oxoid filters (0.45 μm). The differences between bound radioactivity in the presence or absence of an excess of unlabelled hormone (1 μM) was considered to be specific binding. Non-specific glucagon binding was always lower than 5% of the total. Hormone degradation was studied after incubation of ^{125}I-glucagon with the subcellular fractions at 10°C for 4 h. Tubes incubated under identical conditions without subcellular fractions served as controls. After the incubation periods, aliquots of the supernatants were precipitated with 10% (w/v; final concentration) trichloroacetic acid. The percentage of hormone degraded was calculated as a function of the percentage of ^{125}I-glucagon counts soluble in trichloroacetic acid. Glucagon receptor degradation was studied with the subcellular fractions preincubated in tubes containing the corresponding assay buffer at 10°C for 4 h. The tubes were then equilibrated in a 20°C water bath and ^{125}I-glucagon (5×10^{-11} M) was added. In a second set unlabelled glucagon (1 μM) was also added to measure non-specific binding. Incubations were carried out at 20°C for 90 min. Specific ^{125}I-glucagon binding was expressed as a percentage of binding observed with no preincubation. Results are expressed as mean ± SE. For statistical comparisons Student's t-test was used.

3. RESULTS AND DISCUSSION

The distribution of glucagon binding and marker enzyme activities between plasma membranes and Golgi fractions are shown in fig.1 and tables 1 and 2. After purification of liver plasma membranes the protein yield was similar in the two experimental groups. Thus, protein content (mg/10 g wet liver; n = 4) in liver homogenates was: for 21-day-old fetuses, 1405 ± 78; for adult rats, 1242 ± 344; in highly purified liver membranes of 21-day-old fetuses 1.4 ± 0.7 and adult rats 1.3 ± 0.1. However, in the Golgi fractions of

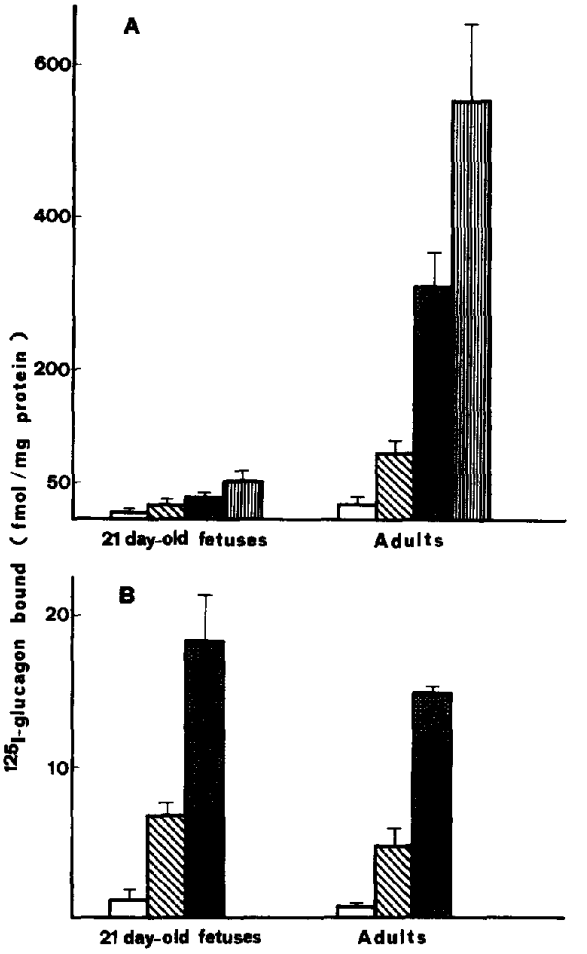

Fig.1. Glucagon binding during the purification of highly purified liver plasma membranes (A) and Golgi fractions (B) obtained from 21-day-old fetuses and adult rats. Means ± SE, n = 4. Data presented correspond to specific binding. (A) Homogenate, ▭; first sediment (1500 g), ▨; partially purified plasma membranes, ■; highly purified plasma membranes, ▥. (B) Nuclear-mitochondrial, ▭; microsomal (initial), ▨; Golgi fractions, ■.

Table 1

5'-Nucleotidase activity during purification of liver plasma membranes obtained from fetal and adult rats

Fraction	5'-Nucleotidase (μmol 5'-AMP hydrol/ mg protein per h)		Purification index	
	21 F	A	21 F	A
Homogenate	0.3 ± 0.1	0.7 ± 0.1	1	1
First sediment (1500 g)	0.7 ± 0.2	1.6 ± 0.2	2.2	2.3
Partially purified liver membranes	1.6 ± 0.3	4.8 ± 0.7	5.2	6.9
Highly purified liver membranes	3.6 ± 0.1	8.1 ± 0.3	11.9	11.8

Means ± SE, $n = 4$. 21 F, 21-day-old fetuses; A, adults

Table 2

Galactosyltransferase activity during purification of Golgi fractions obtained from fetal and adult rats

Fraction	Galactosyltransferase (pmol galactose transferred/mg protein per min)		Purification index	
	21 F	A	21 F	A
Nuclear-mitochondrial	2 ± 0.1	4 ± 1	1	1
Microsomal (initial)	11 ± 2	11 ± 4	5.5	5.7
Golgi fractions	19 ± 4	38 ± 0.2	9.8	9.4

Means ± SE, $n = 4$. 21 F, 21-day-old fetuses; A, adults

fetal rat livers a greater ($P < 0.01$) protein yield was obtained as compared with adult animals. In fact, the protein content (mg/10 g wet liver; $n = 4$) in the nuclear-mitochondrial fraction was: in 21-day-old fetuses, 485 ± 26; in adult rats, 743 ± 29; in Golgi fractions of 21-day-old fetuses 15 ± 4 and adult rats 7 ± 0.5. These findings further support the observations that the cells of the younger animals have a well developed and prominent Golgi apparatus. The purified Golgi fractions obtained from the livers of fetal and adult rats as observed with the electron microscope were also homogeneous, showing the typical patterns of spherical or ellipsoidal vesicles and vacuoles. 5'-Nucleotidase and galactosyl transferase activities (tables 1 and 2) were significantly lower ($P < 0.01$) in fetal than in adult rats, but at every step in the purification of liver membranes there was a similar increase in the activities of the enzymes; thus close purification indices were obtained in both experimental groups.

Glucagon binding (fig.1) increased progressively at every step of the purification of liver plasma membranes and Golgi fractions. In addition, glucagon binding in highly purified liver plasma membranes was greater ($P < 0.001$) in adult rats than in 21-day-old fetuses, while hormone binding to the Golgi fractions of fetal rats was not statistically different ($P > 0.05$) from that observed in the adult animals. Glucagon binding to highly

purified liver plasma membranes of adult rats was significantly greater ($P < 0.001$) than in Golgi fractions of the corresponding group; however in 21-day-old fetuses this difference was much smaller. Our results show that Golgi fractions of fetal and adult rats contain specific binding sites for glucagon. Although in adult rats, glucagon binding to Golgi membranes was low compared to plasma membranes, the difference seems to be smaller because the Golgi apparatus comprises about 3% of the liver cell mass [15] as compared with the 0.4% of the plasma membrane [16]. These facts are more significant in 21-day-old fetuses, because they have a smaller hepatocyte surface [17] and a greater development of the Golgi apparatus. This suggests that fetal liver cells may have more glucagon receptors located intracellularly than on the cell surface.

To determine whether the changes in ^{125}I-glucagon binding could be related to changes in the capacity of glucagon receptors, the bound/free ratio of the labelled hormone was plotted as a function of the hormone bound to the different groups of liver membranes according to a Scatchard plot (fig.2, panels A and B). The results obtained with this procedure could not be fitted to a single straight line but rather gave a curvilinear graph, compatible with negative cooperativity and at least two orders of binding sites: high affinity/low capacity and low affinity/high capacity. Similar affinity constants ($P > 0.05$) of glucagon binding to liver plasma membranes of both experimental groups were obtained, although the total number of binding sites was greater in adult rats (6.2 ng/0.2 mg protein) than in 21-day-old fetuses (2 ng/0.2 mg protein). In contrast, the total number of glucagon binding sites was similar in Golgi fractions for both groups of animals (3.3 ng/0.2 mg protein). Studies of the kinetic properties of the glucagon receptors of 21-day-old fetuses and adult rats revealed that association rates (fig.3) in liver plasma membranes and Golgi fractions were indistinguishable. Furthermore, the degradation of glucagon receptors (per cent/0.2 mg protein per 4 h) was similar in 21-day-old fetuses (in liver plasma membranes, 28 ± 9; and in Golgi fractions, 39 ± 7) to what was observed in adult rats (in liver plasma membranes, 36 ± 4; and in Golgi fractions, 35 ± 6), suggesting that the changes found in glucagon binding are not

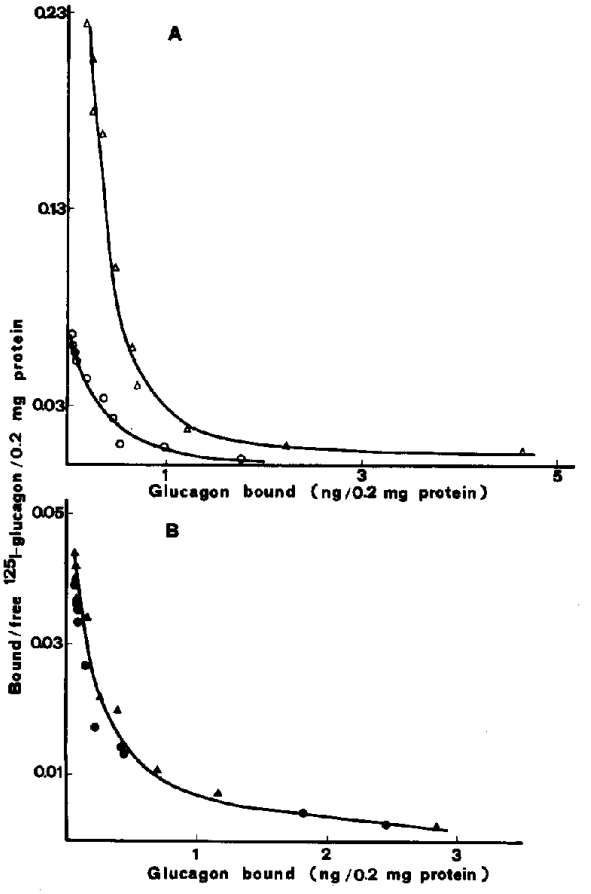

Fig.2. Scatchard analysis of glucagon binding to highly purified liver plasma membranes (A) and Golgi fractions (B) of 21-day-old fetuses and adult rats. Liver membranes were incubated with ^{125}I-glucagon in the absence or presence of unlabelled glucagon (from 0.3 to 1000 ng/ml) at 10°C for 4 h. Each point represents the mean of data obtained with liver membranes of four different rats. Highly purified liver plasma membranes: 21-day-old fetuses (○—○); adult rats (△—△). Golgi fractions: 21-day-old fetuses (●—●); adult rats (▲—▲).

related to alterations by a different stability of the receptor during the incubation periods. Glucagon inactivation (per cent/0.2 mg protein per 4 h) by liver plasma membranes in the presence of bacitracin, was 0.6 ± 0.3 in fetal and 10 ± 0.3 in adult rats, whereas in Golgi fractions the values were 2.3 ± 1.6 and 2.5 ± 1.3 in fetal and adult rats, respectively. However, in the absence of bacitracin hormone inactivation by liver membranes ranges around 19 and 36 per cent in 21-day-old fetuses and adult rats, respectively.

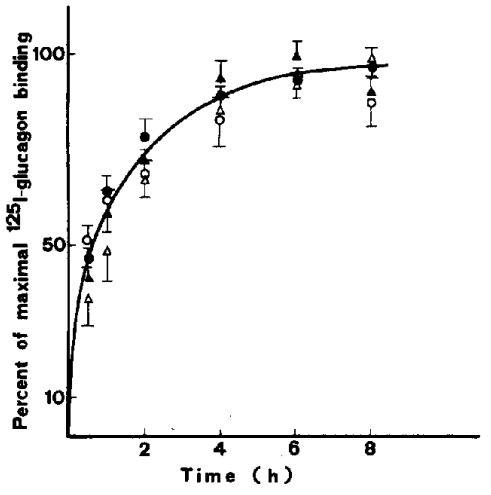

Fig.3. Time course of association of glucagon to highly purified liver plasma membranes and Golgi fractions of 21-day-old fetuses and adult rats. Means ± SE, n = 4. ^{125}I-glucagon was incubated with liver membranes at 10°C. The specific ^{125}I-glucagon binding was determined and plotted as the percentage of maximal ^{125}I-glucagon binding. Highly purified plasma liver membranes: 21-day-old fetuses (○—○); adult rats (△—△). Golgi fractions: 21-day-old fetuses (●—●); adult rats (▲—▲).

The elevation of circulating glucagon levels induces a down-regulation of glucagon receptors [18] and their translocation from the cell surface to the Golgi elements of adult hepatocytes [19], suggesting that the intracellular translocation of glucagon receptors may facilitate the regulation by extracellular glucagon of both the cell surface and intracellular glucagon binding sites. The existence of glucagon receptors in the Golgi fractions of fetal hepatocytes opens the possibility for the study of their relationship with glucagon receptors on the cell surface. Another explanation for glucagon receptors in Golgi fractions is that they are glycosylated in these organelles prior to transport to the cell surface. In this sense it could be suggested that the similar glucagon receptor concentrations in plasma membranes and Golgi fractions of fetal liver cells might be related to a block in the translocation of these receptors to the cell surface.

ACKNOWLEDGEMENTS

This work was supported by grants from the Comisión Asesora de Investigación Científica y Técnica and by the Junta de Castilla y León, Spain.

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