INTERRELATIONSHIP OF INSULIN AND GLUCAGON RATIOS ON CARBOHYDRATE METABOLISM IN ISOLATED HEPATOCYTES CONTAINING HIGH GLYCOGEN[+]

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SUMMARY

The effect of physiological concentrations of glucagon and insulin on glycogenolysis was studied in the presence and absence of substrates in isolated hepatocytes containing high glycogen. In the absence of substrates glucagon stimulated glycogenolysis at 10^{-14}M concentration, and addition of 100 μunits of insulin partially inhibited glucagon stimulated glycogenolysis (10^{-14}M to 10^{-11}M). However, in the presence of substrates, insulin completely inhibited glucagon stimulated glycogenolysis (10^{-14}M to 10^{-11}M), indicating that molar glucagon and insulin ratios control carbohydrate metabolism in liver. Additional studies showed incorporation of amino acid into protein was linear for only 3 to 4 hr in cells containing low glycogen, whereas in cells containing high glycogen, incorporation was linear for 8 to 10 hr.

It has been implicated that insulin in combination with glucagon participates in the regulation of glucose and amino acid metabolism in hepatic tissues (1). Relative concentrations of these two hormones determine whether glucose is added or removed from the extracellular compartment, and whether amino acids are utilized for protein biosynthesis or gluconeogenesis. Unger (2) has postulated that molar glucagon to insulin ratios may control carbohydrate metabolism. In this communication we report the effect of glucagon and insulin on glycogenolysis at

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physiological concentrations under various experimental conditions with isolated hepatocytes and provide the first direct evidence to support this hypothesis.

MATERIALS AND METHODS

Male, fed Cox rats (160-200 g) were used for all studies. All rats were maintained on Purina Laboratory Chow and tap water fed ad libitum. Rats received their food either placed on the floor (for high glycogen levels) or suspended in wire baskets (for low glycogen levels) as it was previously observed that glycogen levels were significantly increased in animals that had easy access to food as compared to rats that had to obtain their food from suspended wire baskets (3). Rat liver cells were isolated by collagenase in vivo perfusion technique as described previously (4, 5). Cells from two rats were pooled together as all incubations could be carried out with the same cell preparations. Approximalety 60-75 mg of cells were incubated in 3 ml of Umbreit-Ringer 25 mM bicarbonate buffer with various concentrations of hormones and with and without substrates at 37°C and 90 oscillations per min (6). The vials were gassed with 95% O_2 and 5% CO_2 for 5 min at time zero and after each 2 hr of incubation. At the end of incuba-

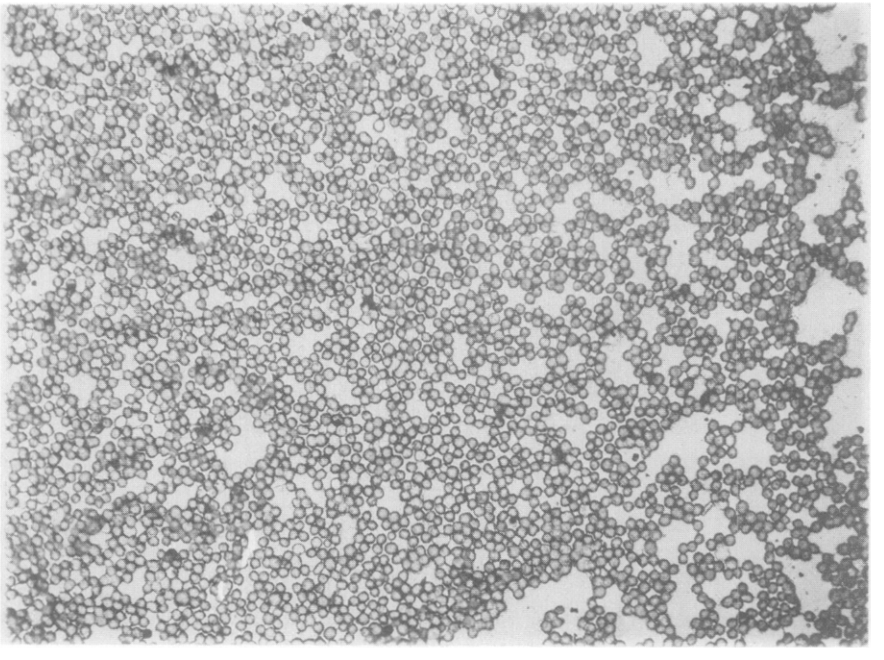

Figure 1. Light micrograph (80 x mag) of normal isolated liver cells just prior to incubation. Liver cells are completely dissociated from each other and demonstrate intact cell membranes and not a single broken cell may be seen. They are also completely free of broken connective tissue and red blood or Kupffer cells.

tion the medium was assayed for glucose by the glucose oxidase method (7). Protein was precipitated by the addition of trichloroacetic acid to the entire vial contents and radioactivity into protein was assayed as described previously (8).

RESULTS AND DISCUSSION

Figure 1 shows typical isolated hepatocytes used in the present study. It can be seen from this figure that cells are completely dissociated, demonstrate distinct cell membranes and not a single broken cell may be seen in the entire field. They are also completely free from broken connective tissue and red blood or Kupffer cells and all cells exclude trypan

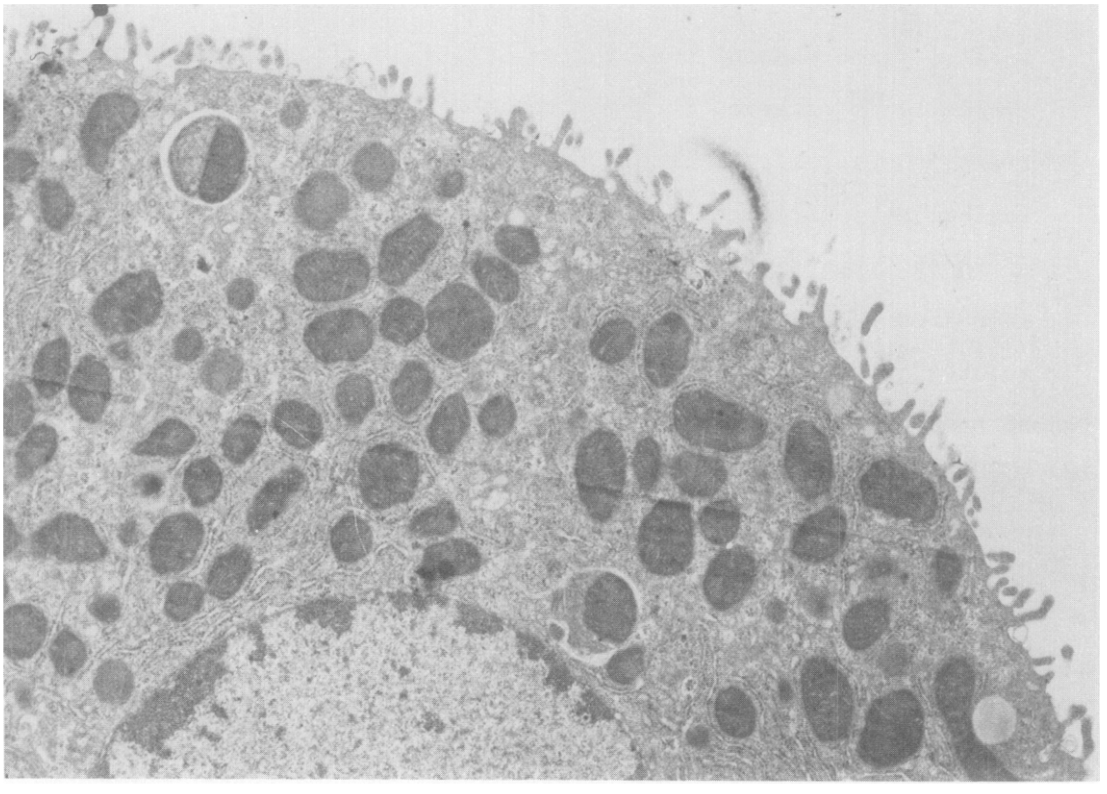

Figure II: Electron micrograph (16000 x mag) of a normal hepatocyte after isolation. Mitochondria and rough endoplasmic reticulum are normal. Parallel strands of polyribosomes are also present and do not show any presence of vacuoles. Large number of microvilli are also present on the surface of the cell membranes.

blue. Figure II is an electron micrograph of hepatocyte immediately after the isolation. It can be seen that cell shows intact cell membrane,normal mitochondria, parallel strands of rough endoplasmic reticulum and abundant glycogen granules and do not show any vacuoles as seen in other cell preparations that have been prepared by using high concentration of both collagenase and hyaluronidase (9-11). In addition large numbers of microvilli are also present on the surface of cell membranes which may be essential to obtain hormonal responses at physiological concentrations. These microvilli are missing in cell preparations that have been prepared by using high concentration of both collagenase and hyaluronidase (9-11). Furthermore no mitochondrial swelling or depletion of cytoplasmic enzymes (5) is observed in these cells. Using such cell preparations we were able to obtain hormonal response at normal physiological concentrations of both insulin and glucagon. The results of these studies are summarized in Table I and II. It can be seen from Table I that the addition of glucagon (10^{-14}M) stimulated glycogenolysis in isolated hepatocytes that contained high amounts of glycogen. Much higher concentrations (10^{-9}M) of glucagon were needed to obtain stimulatory effects on glycogenolysis in isolated hepatocytes that contained low glycogen, as reported previously (12). Less than a two-fold (12) increase in glycogenolysis was observed previously with cells containing low glycogen when glucagon concentrations were increased from 10^{-10}M to 10^{-6}M, whereas more than a three-fold increase is observed in the present studies under similar conditions in hepatocytes that contained high glycogen.

Addition of 100 μunits of insulin (4×10^{-11}M) partially blocked the glycogenolysis when the glucagon concentration was

TABLE I

EFFECTS OF VARIOUS CONCENTRATIONS OF GLUCAGON ON GLYCOGENOLYSIS AND ITS INHIBITION BY INSULIN IN ISOLATED HEPATOCYTES CONTAINING HIGH LEVELS OF GLYCOGEN INCUBATED IN THE ABSENCE OF SUBSTRATE[+]

Hormone Concentration	µmoles glucose released in the medium per gram per hour
None	66.2 ± 7.3
10^{-6}M Glucagon	220.5 ± 22.5
10^{-6}M Glucagon + 100 µUnits Insulin	206.4 ± 20.0
10^{-7}M Glucagon	214.3 ± 21.0
10^{-7}M Glucagon + 100 µUnits Insulin	200.0 ± 19.0
10^{-8}M Glucagon	195.6 ± 18.0
10^{-8}M Glucagon + 100 µUnits Insulin	180.0 ± 12.0
10^{-9}M Glucagon	182.2 ± 16.2
10^{-9}M Glucagon + 100 µUnits Insulin	167.0 ± 12.0
10^{-10}M Glucagon	165.3 ± 15.0
10^{-10}M Glucagon + 100 µUnits Insulin	148.1 ± 12.3
10^{-11}M Glucagon	145.3 ± 13.0
10^{-11}M Glucagon + 100 µUnits Insulin	108.0 ± 13.0
10^{-12}M Glucagon	125.6 ± 9.0
10^{-12}M Glucagon + 100 µUnits Insulin	89.0 ± 9.2
10^{-13}M Glucagon	105.5 ± 8.6
10^{-13}M Glucagon + 100 µUnits Insulin	72.8 ± 8.1
10^{-14}M Glucagon	83.6 ± 9.8
10^{-14}M Glucagon + 100 µUnits Insulin	73.6 ± 9.5
10^{-15}M Glucagon	68.8 ± 9.2
10^{-15}M Glucagon + 100 µUnits Insulin	66.8 ± 8.6

[+]Approximately 60-75 mg of cells were incubated in 3 ml of Umbreit-Ringer 25 mM bicarbonate buffer containing various concentrations of glucagon and insulin for 1 hr. Initial liver glycogen levels were in the range of 340 ± 35 µmoles glucose per gram before perfusing. Isolated liver cells had glycogen in the range of 276 ± 32 µmoles glucose per gram. Values are expressed as µmoles glucose released in the medium per gram per hour.

TABLE II

EFFECT OF VARIOUS CONCENTRATIONS OF GLUCAGON ON GLYCOGENOLYSIS AND ITS INHIBITION BY INSULIN IN THE ISOLATED HEPATOCYTES CONTAINING HIGH LEVELS OF GLYCOGEN INCUBATED IN THE PRESENCE OF SUBSTRATE+

Hormone Concentration	μmoles glucose released in the medium per gram per hour
None	58.8 ± 6.2
10^{-6}M Glucagon	206.0 ± 22.0
10^{-6}M Glucagon + 100 μUnits Insulin	155.0 ± 12.0
10^{-7}M Glucagon	180.0 ± 20.0
10^{-7}M Glucagon + 100 μUnits Insulin	142.0 ± 11.0
10^{-8}M Glucagon	166.0 ± 18.0
10^{-8}M Glucagon + 100 μUnits Insulin	130.0 ± 10.0
10^{-9}M Glucagon	148.0 ± 15.0
10^{-9}M Glucagon + 100 μUnits Insulin	115.0 ± 11.0
10^{-10}M Glucagon	134.0 ± 12.0
10^{-10}M Glucagon + 100 μUnits Insulin	102.0 ± 9.0
10^{-11}M Glucagon	112.7 ± 9.8
10^{-11}M Glucagon + 100 μUnits Insulin	68.0 ± 8.0
10^{-12}M Glucagon	101.0 ± 9.0
10^{-12}M Glucagon + 100 μUnits Insulin	63.2 ± 8.0
10^{-13}M Glucagon	88.9 ± 10.2
10^{-13}M Glucagon + 100 μUnits Insulin	65.0 ± 6.0
10^{-14}M Glucagon	76.2 ± 7.0
10^{-14}M Glucagon + 100 μUnits Insulin	60.5 ± 6.0
10^{-15}M Glucagon	60.3 ± 7.1
10^{-15}M Glucagon + 100 μUnits Insulin	62.2 ± 6.8

+The conditions were as in Table I except that the incubation mixture also contained 5 mM lactate and 5 mM of amino acids mixture (6).

in the range of 10^{-14}M to 10^{-11}M in the absence of added substrates (Table I), whereas at higher concentrations of glucagon it was

much less effective. However, in the presence of substrates (Table II) such as 5 mM lactate and 5 mM amino acids mixture, addition of 100 μunits of insulin completely blocked the glycogenolysis at all but the highest concentrations of glucagon. These results clearly show that a ratio of insulin to glucagon controls the hepatic carbohydrate metabolism, and provide the first direct evidence that molar ratios of glucagon to insulin may be important in the control of carbohydrate metabolism in various disease states, as suggested by Unger (1, 2). We have previously reported (3) that the glycogen content in the liver can be increased in animals that have easy access to food as compared to those rats that have to obtain food from suspended wire

TABLE III

INCORPORATION OF ^{14}C PHENYLALANINE INTO PROTEIN BY ISOLATED HEPATOCYTES* CONTAINING LOW AND HIGH GLYCOGEN

Period of Incubation	^{14}C Phenylalanine Incorporated into Protein dpm/mg	
	Low Glycogen**	High Glycogen***
1	530 ± 65	582 ± 72
2	820 ± 89	880 ± 86
3	1080 ± 120	1205 ± 108
4	1405 ± 130	1830 ± 128
6	1686 ± 160	2663 ± 150
8	2063 ± 180	3580 ± 210
10	2230 ± 210	4752 ± 320

*Approximately 60-75 mg of cells were incubated in 3 ml of Umbreit-Ringer 25 mM bicarbonate buffer containing 5.5 mM glucose, 5 mM lactate, 100 μunits of insulin and 5 mM of amino acids mixture containing 0.5 μCi of ^{14}C phenylalanine (6). Glycogen levels were 130 ± 25** and 220 ± 35*** μmoles glucose as glycogen per gram of cells.

baskets. These isolated hepatocytes containing high glycogen respond to physiological concentrations of glucagon (10^{-14}M) as in the present studies and also to insulin (4×10^{-11}M) as reported previously (13-15). This is in contrast to previously reported studies (12, 16-19) where much higher concentrations (10^{-9}M to 10^{-6}M) of these hormones are needed to obtain similar responses. It may be that high intracellular glycogen content helps to maintain *in vivo* metabolic characteristics of isolated cells.

Studies on the amino acid incorporation into protein in low and high glycogen-containing hepatocytes is summarized in Table III. It can be seen from this table that U-^{14}C phenylalanine into protein was linear for 8 to 10 hr in the presence of glucose, lactate and amino acids mixture. In cells containing low glycogen only a small increase in the incorporation of U-^{14}C phenylalanine was observed under similar conditions. It was also observed that more than 50% of the radioactivity incorporated into protein was present in the supernatant fraction. This suggests that newly synthesized protein was being rapidly released into the medium. The incorporation of amino acids into protein presumably represents active protein synthesis by isolated hepatocytes and is not due to bacterial growth since both penicillin and streptomycin (750 γ/3 ml) were added to the incubation medium. If antibiotics were not added large numbers of bacteria were noted at the end of 6 hr of incubation. No bacterial growth was observed when incubations were carried out in the presence of antibiotics.

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