STIMULATION OF GLYCOGENOLYSIS BY EPINEPHRINE AND GLUCAGON AND ITS INHIBITION BY INSULIN IN ISOLATED RAT LIVER HEPATOCYTES*

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SUMMARY: Rat liver hepatocytes were isolated by collagenase in vitro perfusion technique and the effect of epinephrine, glucagon and insulin on glycogenolysis was studied. Both glucagon and epinephrine at the concentration of 10^{-6}M, stimulated gluconeogenesis by 80-100%. Addition of insulin (33 μUnits/ml) completely abolished the epinephrine-stimulated glycogenolysis whereas only 50% inhibition was observed with insulin in glucagon stimulated glycogenolysis. This stimulation was observed within 2-5 min after the addition of the hormones. These results suggest that hepatocytes isolated with low concentrations of collagenase retain glucagon, epinephrine and insulin receptor sites.

It is well known that glucagon, epinephrine and insulin have a number of effects on carbohydrate metabolism in liver. These effects have been previously studied by using perfused liver and liver slices from normal, diabetic and adrenalectomized animals. It was, therefore, of interest to study the effect of these hormones in isolated rat liver hepatocytes. In this paper, we present data on the effects of glucagon, epinephrine and insulin sensitivity on the isolated hepatocytes to these hormones.

MATERIALS AND METHODS

Male, fed, Cox rats (200 g) were used for all these studies. Rat liver parenchymal cells were isolated by collagenase in vitro perfusion technique (1). Approximately 70 mg of weight of cells (140,000 cells/mg) were incubated in 3 ml of Umbreit Ringer 25 mM $NaHCO_3$ buffer (with no albumin) with various concentrations of hormones at 37°C and at 90 oscillations per min as described previously (1,2). At the end of the incubation period, the vial contents were placed in iced conical centrifuge tubes and were

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TABLE I

EFFECT OF VARIOUS CONCENTRATIONS OF GLUCAGON AND EPINEPHRINE ON GLYCOGENOLYSIS IN ISOLATED RAT LIVER HEPATOCYTES FROM FED RATS.*

Glucagon Concentration	μMoles Glucose Released in Medium /g/hr	Delta Change μMoles	Epinephrine Concentration	μMoles Glucose Released in Medium /g/hr	Delta Change μMoles
None	36.6±6.3	-	None	32.5±6.2	-
10^{-6}M	70.0±6.5	33.4	10^{-6}M	59.2±7.1	26.7
10^{-7}M	60.9±6.3	24.3	10^{-7}M	54.0±6.3	21.5
10^{-8}M	53.3±8.7	16.7	10^{-8}M	47.2±8.0	14.7
10^{-9}M	44.3±8.1	7.7	10^{-9}M	38.6±6.0	6.1
10^{-10}M	36.0±7.4	-	10^{-10}M	33.3±5.2	-

*About 70 mg of cells (140,000 cells/mg) were incubated for 1 hr at 37°C in 3 ml of Umbreit Ringer 25 mM $NaHCO_3$ buffer containing various concentrations of glucagon or epinephrine. Each value is the mean of at least 5 values.

then centrifuged at 2000 rpm in an International centrifuge for 10 min. The supernatant was assayed for glucose by the glucose oxidase method (2) and cellular glycogen was assayed in the pellet. All experiments were conducted in duplicate and all values reported are the mean ± standard error of the mean.

RESULTS AND DISCUSSION

The effect of various concentrations of glucagon and epinephrine on glycogenolysis is summarized in Table 1. Both glucagon and epinephrine at concentrations of 10^{-9}M to 10^{-6}M stimulated glycogenolysis. Glucagon was more effective in stimulating glycogenolysis than epinephrine at all concentrations studied. Maximal stimulation was observed at 10^{-6}M with both hormones. In contrast to glucagon, higher concentrations of epinephrine

TABLE II

INHIBITORY EFFECT OF INSULIN ON EPINEPHRINE AND GLUCAGON STIMULATED GLYCOGENOLYSIS IN ISOLATED RAT LIVER HEPATOCYTES FROM FED RATS.*

Hormone Added	μMoles Glucose Released in the medium/g/hr	Delta Change (μMoles)
None	41.3 ± 4.4 (6)	-
Epinephrine (10^{-6}M)	67.3 ± 7.2 (6)	26.0
Epinephrine (10^{-6}M) + Insulin (100 μUnits)	42.2 ± 6.2 (6)	No Change
Glucagon (10^{-6}M)	73.5 ± 5.5 (6)	32.3
Glucagon (10^{-6}M) + Insulin (100 μUnits)	57.0 ± 6.0 (6)	15.7
Insulin (100 μUnits)	39.3 ± 4.5 (6)	No Change
ACTH (0.3 - 0.6 Units)	41.8 ± 6.5 (4)	No Change

*About 70 mg of cells (140,000 cells/mg) were incubated for 1 hr at 37°C in 3 ml of Umbreit Ringer 25 mM $NaHCO_3$ buffer containing various concentrations of hormones as indicated. Each value is the mean of at least 4 values.

(10^{-4}M) inhibited glycogenolysis. The stimulatory effect of these hormones was observed within 2-5 min after their addition to the incubation medium. Albumin (1%) decreased glycogenolysis in the absence or presence of hormone. The stimulatory effect of these hormones was only observed in cell preparations where 95-98% of the cells were intact and retained distinct rounded cell membranes and excluded trypan blue.

The addition of 33 μUnits/ml of insulin alone to the incubation medium had no effect on glycogenolysis but greatly reduced the effect of epinephrine or glucagon (Table II). Insulin completely abolished the stimulatory effect of epinephrine and decreased the effect of glucagon by 50%. ACTH (0.3 - 0.6 units/3 ml) in the presence or absence of insulin had no effect on glycogenolysis. No hormonal effects were observed in those preparations where the cell membranes were grossly damaged. These studies demonstrate that hepatocytes isolated by the method previously described (1) retain glucagon, epinephrine and insulin receptors.

In preliminary studies we have observed that epinephrine or glucagon (10^{-6}M) stimulated hepatic phosphorylase activity by 80-100%. This is in accord with the results on glycogenolysis in that glucagon was more effective in activating phosphorylase than epinephrine at the same concentration. Insulin did not alter the phosphorylase activation observed in the presence of hormone, suggesting that insulin may be acting through glycogen synthetase. This hypothesis has been previously suggested by others (3, 4).

The stimulation of glycogenolysis by epinephrine or glucagon has been shown in perfused liver (5) and in enzymatically isolated liver cells (6, 7). Furthermore, Jefferson et al. (8) have demonstrated with perfused liver that high concentrations of insulin (10 mU/ml) can inhibit the stimulatory effect of epinephrine or glucagon on glycogenolysis. Studies presented here demonstrate that physiological concentrations of insulin (33 μUnits/ml) can reduce the stimulation of glycogenolysis in isolated liver cells. These results suggested that insulin may be involved in the minute-to-minute regulation of carbohydrate metabolism in the liver by modulating the effects of other hormones.

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