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HORMONE SENSITIVE CALCIUM UPTAKE BY LIVER MICROSOMES

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SUMMARY: The effects of glucagon and insulin on hepatic microsomal calcium uptake were investigated. Microsomes isolated from perfused rat liver accumulated calcium in the presence of ATP and oxalate. Addition of glucagon to the perfusate significantly increased calcium uptake by microsomes subsequently isolated. In contrast, addition of insulin to the perfusate resulted in a decreased microsomal calcium uptake and inhibition of the glucagon effect. Because the effects of glucagon and insulin on hepatic microsomal calcium uptake are opposite, as are the metabolic effects of these hormones, it is likely that the observed differences are of physiological importance.

INTRODUCTION

Glucose production by the liver is under hormonal control; glucagon and epinephrine increase while insulin decrease glucose production (1,2). The observation that the hyperglycemia evoked by glucagon and epinephrine is associated with hyperkalemia, while the hypoglycemic effect of insulin is accompanied by hypokalemia was reported long ago (3,4,5). More recently an early effect of glucagon and epineph rine on calcium efflux was noted (6). The efflux of calcium is followed by the efflux of K+, which is associated with hyperpolarization of the liver cell membrane (7). The effect of glucagon on calcium movement preceeds not only the potassium efflux, but also the hormonally induced stimulation of hepatic glucose production (8). It has been demonstrated in a variety of experimental conditions that interference with the effects of glucagon on ion redistribution impairs the ability of the hormone to increase glucose release (9). Moreover, under the same experimental conditions, the effects of glucagon on lipid metabolism were also blocked, indicating that the redistribution of ions is probably essential and functionally linked to most, if not all, of the metabolic responses influenced by the hormone (10).

The notion that the ion redistribution is an integral part of the mechanism by which hormones influence liver metabolism is further supported by the observation

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that insulin, which antagonizes the metabolic effects of glucagon, also blocks the effect of glucagon on potassium efflux (11). The exact mechanism by which a redistribution of ions might affect liver metabolism is not known. There are several enzymes in the glycogenolytic and gluconeogenic pathways which are calcium-sensitive (12-15), thus, it is possible that the ion redistribution exerts its influence by altering the activities of key enzymes. Elucidation of the mechanism underlying the hormonally induced ionic movements is essential for the understanding of hepatic regulatory processes. Because it has been reported that the liver microsomal fraction has an energy dependent calcium sequestering system (16), a study of the hormone sensitivity of this system was undertaken. This paper presents the results obtained thus far, which indicate that both glucagon and insulin affect microsomal calcium uptake.

MATERIALS AND METHODS

Liver perfusion. Male, fed Sprague-Dawley rats weighing 200-250 g were used in the experiments. The technique of liver perfusion has been described in detail (8). In essence, livers were perfused in situ with a perfusate composed of Krebs-Ringer bicarbonate buffer pH 7.4 containing 4% bovien albumin (Cohn fraction V) in a recirculating system. The perfusate was oxygened by a water jacketed disk oxygenator which also kept the temperature constant. Hormones, when indicated, were added directly to the perfusate.

Preparation of microsomes. At the end of the perfusion period livers were placed into ice-cold sucrose solution (250 mM) containing Tris (2 mM) and dithiothreitol (1 mM) pH 7.2 and cut into small pieces. Subsequently the liver was homogenized and the microsomal fraction isolated essentially as described by Moore et al (16), with the modification that dithiothreitol was added to the sucrose solution. Homogenization was performed with loosely fitting pestles to maximize the ability of the isolated microsomes to accumulate calcium (personal observation).

Determination of glucose-6-phosphatase. The microsomal marker glucose-6-phosphatase was assayed according to the method of Harper (17). Mitochondria and microsomes were incubated at 37°C with glucose-6-phosphate (0.08 M in citrate buffer, pH 6.5) for 15 min. The inorganic phosphate cleaved off was subsequently measured by the Fiske and Subbarow method (18).

Determination of succinic dehydrogenase. Microsomes were checked for possible contamination with mitochondrial membrane by measuring the mitochondrial enzyme succinic dehydrogenase as described by Pennington (19). The oxidation of succinate was coupled to the reduction of the dye 2-(p-iodophenyl-3-(p-nitrophenyl)-5-phenyl-tetrozolium (INT). Mitochondrial and microsomal preparations, containing 0.11 and 0.12 mg protein per ml, respectively, were incubated at 37°C with 50 mM succinate and 0.1% INT. After 15 min the reaction was stopped with 10% trichloroacetic acid, the formazan produced extracted with ethyl acetate, and its extinction measured at 490 nm.

Determination of 5'nucleotidase. Microsomes and mitochondria were assayed

for 5'-nucleotidase activity in order to determine the extent of possible plasma membrane contamination of the preparations. Microsomes and mitochondria were incubated at 37°C with 10 umoles each of 5'-AMP and $MgCl_2$, essentially as described by Ray (20) except that inorganic phosphate, rather than adenosine, was measured upon termination of the reaction.

Determination of P-450-reductase. Cytochrome P-450 reductase activity was determined according to the method of Williams and Kamin (21). The enzyme was measured in microsomal suspensions (20 mg protein/ml) before and after treatment of such preparations with steapsin (265 ug) to determine whether or not the protease would liberate the reductase activity into the supernatant obtained by high speed centrifugation (106,000x g for 2 hr) of the treated microsomes. The pellet from this high speed centrifugation was itself resuspended and tested for reductase activity before and after solubilization with 1% Renex detergent.

Measurement of calcium uptake. Calcium uptake was measured by using the millipore technique as described by Moore et al. (16) The microsomes (50 ug/ml) were incubated in 30 mM imidazol-histidine buffer pH 6.8, 100 mM KCl, 5 mM $MgCl_2$ 5 mM ammonium oxalate, 5 mM ATP, 25 uM $CaCl_2$ containing ^{45}Ca at 37°C in a Dubnoff water bath. At appropriate times samples were taken, filtered on size 0.45 u millipore filter. The filters were washed with 0.25 M KCl solution (2 ml) followed by water (10 ml). Samples were then filtered with the aid of vacuum and washed with 0.25 M sucrose solution. The radioactivity remaining in the filters was counted in a Delta 300 scintillation counter.

RESULTS AND DISCUSSION

The purity of the prepared microsomal fraction is demonstrated in Table 1. The low values obtained for succinic dehydrogenase activity in the microsomal fraction indicates that the microsomal fraction does not contain particles of mitochondrial origin. The values obtained for glucose-6-phosphatase activity are comparable to previously reported values (17).

In order to determine whether the orientation of the microsomal vesicles is inside out or outside in, cytochrome P-450 reductase activity was measured. Cytochrome P-450 reductase is located outside the endoplasmic reticulum from which the microsomal fraction is derived. If the microsomal vesicles are outside out, then protease treatment would liberate the enzyme activity to the supernatant. That this is the case is demonstrated in Table II. Detergent induced solubilization of the vesicles resulted in a small increase in measureable cyt P-450 activity further showing, that almost all of the enzyme activity is located outside.

The microsomal vesicles prepared from perfused livers accumulate calcium in the presence of ATP and oxalate (Fig. 1). The uptake of calcium is temperature dependent. In the absence of oxalate the calcium taken up does not accumulate.

Table 1. A Comparison of Marker Enzyme Activities in Microsomes and Mitochondria

Preparation	Glucose-6-phosphatase (umoles Pi/g liver/min)	Succinate dehydrogenase umoles formazan/mg prot/15 min.	5'-nucleotidase umoles Pi/mg prot/hr.
Microsomes	11.50±3.70	0.098±0.002	5.54±0.59
Mitochondria	0.11±0.04	1.090±0.070	2.67±0.45

The analytical procedures employed are described in the text. Values reported are the means of determinations on at least three different preparations ± standard deviation.

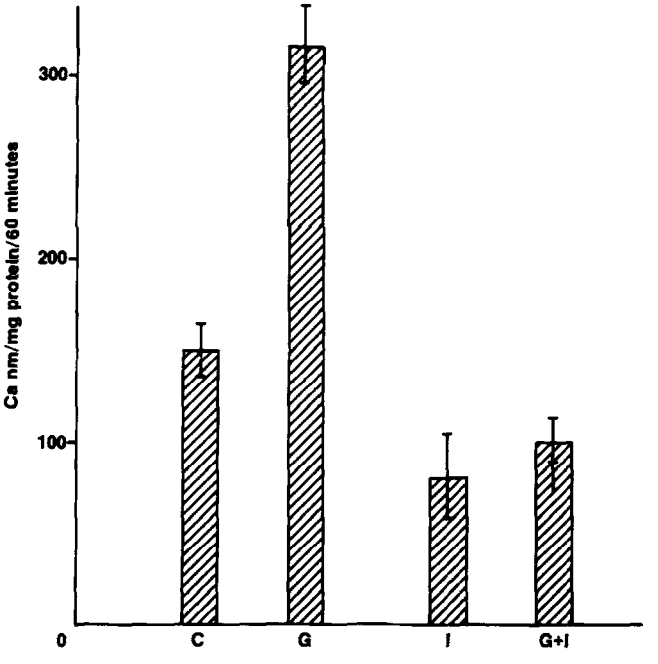

Figure 1. The effects of glucagon and insulin on microsomal calcium uptake livers were perfused in a recirculating system in the presence and absence of glucagon (2.8×10^{-7} M) and insulin (0.04 u/ml). The hormones were added as a single dose directly to the perfusate. After 30 minutes of perfusion the livers were homoginized and microsomes prepared as described in the Methods. C = control. G = glucagon. I = insulin.

Addition of glucagon to the perfusate significantly increased the uptake of calcium (Fig. 1). In contrast, the addition of insulin resulted in a decreased calcium uptake. When insulin was added to the perfusate together with glucagon no stimulation of calcium uptake occurred, indicating that insulin was blocking the effect of glucagon.

The stimulatory effect of glucagon treatment was unexpected in light of previous observations that addition of glucagon to the perfusate resulted in an immediate net release of calcium from the liver (6). In these earlier experiments the liver was perfused with calcium free perfusate, and the possibility that glucagon in the presence of extracellular calcium might increase calcium uptake can not be excluded. It is equally possible that due to the efflux of calcium which follows glucagon administration, the microsomal vesicles isolated from glucagon treated livers are depleted of calcium and therefore take up more calcium than microsomes isolated from untreated liver. This possibility is currently under investigation.

The opposing effect of insulin on microsomal calcium uptake is in line with previous observation about the antagonistic effect of insulin on glucagon induced glucose production and K^+ output. The opposing effects of glucagon and insulin on microsomal calcium uptake could result in changes in calcium concentration in the endoplasmatic reticulum and the cytosol. This change might be functionally related to the metabolic effects of these hormones.

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