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Cell volume is a major determinant of proteolysis control in liver

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Hepatic proteolysis is inhibited by insulin, amino acids and hypoosmotic cell swelling and is stimulated by glucagon. These effectors simultaneously modulate cell volume in the intact liver, as shown by measurements of the intracellular water space. A close relationship exists between the effect on proteolysis and the accompanying cell volume change, regardless of whether hepatic proteolysis was modified by insulin, glucagon, cyclic AMP, glutamine, glycine, barium of hypoosmotic exposure. It is suggested that cell volume changes exerted by hormones and amino acids play a crucial role in the regulation of hepatic proteolysis.

Glucagon; Insulin; Proteolysis; Perfused liver; Cell volume; Hormone action; Intracellular water space; Glutamine; Glycine; Cyclic AMP; Bumetanide

1. INTRODUCTION

Hepatic proteolysis is under the control of amino acids and hormones, but the underlying mechanisms are not understood ([1–6], for reviews see [7,8]). Recent evidence points to an involvement of cell volume alterations [9–11]. When liver mass was used as an estimate of cell swelling in the intact organ, the antiproteolytic effects of glutamine and glycine could be fully mimicked by equipotent hypoosmotic cell swelling [9,10]. Further the antiproteolytic effect of insulin in liver parallels the extent of hormone-induced intracellular K^+ accumulation, even when the insulin-induced K^+ uptake is modulated by glucagon, the nutritional state or inhibitors of NaCl-KCl cotransport [11]. Indeed, the insulin-induced cellular K^+ accumulation has recently been shown to result in cell swelling, whereas glucagon leads to cell shrinkage [12]. Liver mass recordings and determination of hormone-induced K^+ accumulation, however, are only indirect estimates for cell volume changes in the intact perfused rat liver. Here we report on the relationship between proteolysis and alterations of the intracellular water space in liver under a variety of experimental conditions. The data show that proteolysis control by glutamine, glycine, insulin, glucagon, cyclic AMP and Ba^{2+} is strongly dependent on the extent of the simultaneously occurring changes of the intracellular water space in perfused rat liver.

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2. MATERIALS AND METHODS

L-[4,5-^{3}H]leucine, [^{3}H]inulin and [^{14}C]urea were from Amersham Buchler (Braunschweig, FRG). Insulin, glucagon and bumetanide were from Sigma (Munich, FRG). Dibutyryl cyclic AMP was from Boehringer (Mannheim, FRG). All other chemicals were from Merck (Darmstadt, FRG).

Livers from fed male Wistar rats (120–150 g body weight) were perfused as described previously [9,11,13] in a non-recirculating manner with bicarbonate-buffered Krebs-Henseleit saline plus L-lactate (2.1 mM) and pyruvate (0.3 mM). The perfusate was gassed with O_2/CO_2 (95/5; v/v); the temperature was 37°C. In experiments with Ba^{2+} (1 mM), sulfate was omitted from the perfusion fluid. In normotonic perfusions, the osmolarity was 305 mOsmol/l; hypoosmotic and hyperosmotic exposure was performed by lowering or increasing the NaCl concentration in the perfusion medium, resulting in corresponding osmolarity changes.

The rate of proteolysis was assessed as described and validated recently [9–11] by measuring the release of [^{3}H]leucine from isolated perfused rat livers after prelabelling of liver proteins in vivo by intraperitoneal injection of 200 μCi L-[4,5-^{3}H]leucine about 16 h prior to the perfusion experiment. In these experiments, influent perfusate was supplemented with unlabelled leucine (0.1 mM). In the individual perfusion experiment, the steady state [^{3}H]leucine release from the liver in the absence of effectors on proteolysis was set to 100%; changes of [^{3}H]leucine release under the influence of effectors were expressed as the percentage hereof.

The intracellular water space was determined in the intact perfused rat liver as the difference between a [^{3}H]inulin and a [^{14}C]urea accessible space [12]. Both isotopes were infused together at a constant rate (about 10 kBq/min, each) and equilibrated with their accessible respective compartments. After stopping the isotope infusion, determination of total [^{3}H] and [^{14}C] radioactivity in effluent during a 5 min washout-period allowed the calculation of [^{3}H]inulin accessible and [^{14}C]urea accessible spaces, respectively. The difference between these spaces reflects the intracellular ([^{14}C]urea-accessible) water space. In control experiments, the intracellular water space was found to be 546 ± 9 μl/g ($n = 51$), a value similar to that reported by others [14]. Cell volume changes under the influence of effectors were determined as the difference between 2 consecutive space measurements

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(time interval about 30 min) in the individual experiment. In control experiments the intracellular water space slowly decreased with perfusion time (i.e. by 2.3 ± 0.3% per 30 min); values given in Fig. 1 were corrected for this spontaneous decrease. Effects on intracellular water spaces and on proteolysis were assessed in different series of experiments with the same design.

3. RESULTS AND DISCUSSION

In isolated perfused rat liver, the intracellular water space increased not only upon exposure to hypoosmotic (185–275 mOsmol/l) perfusion fluid, but also when in normotonic (305 mOsmol/l) perfusions the K^+ channel blocker Ba^{2+} (1 mM), insulin (35 nM), glutamine (2 mM) or glycine (2 mM) was added (Fig. 1). In the presence of bumetanide (5 μM), an inhibitor of NaCl-KCl cotransport [15], the insulin-induced increase of the intracellular water space was diminished by about 60% (Fig. 1), i.e. to the same extent as the recently reported inhibition of insulin-induced net K^+ uptake in presence of bumetanide [11,12]. Cell swelling, as reflected by the increase of intracellular water space (Fig. 1), following addition of glutamine and glycine is explained by the Na^+ dependent, concentrative uptake of the amino acids, which creates intra/extracellular amino acid concentration gradients up to 20 [16,17]. Ba^{2+}-induced cell swelling is due to cellular net K^+ accumulation resulting from the blockade of Ba^{2+}-sensitive K^+ channels [11]. Simultaneously, proteolysis was inhibited, as evidenced by a decrease of [^{3}H]leucine release from perfused livers of rats, which were [^{3}H]labelled in vivo by intraperitoneal injection of [^{3}H]leucine about 16 h prior to the perfusion experiment (Fig. 1). On the other hand, dibutyryl-cAMP (50 μM) and glucagon (100 nM) decreased the intracellular water space of perfused rat liver when added on top of insulin or to livers exposed to hypoosmotic perfusion fluid, indicating cell shrinkage (Fig. 1). Under these conditions, the effects of cyclic AMP and glucagon on cell volume were accompanied by a stimulation of proteolysis (Fig. 1). There was a close relationship between the increase of intracellular water space and the extent of proteolysis inhibition, regardless of whether cell volume was modified by glutamine, glycine, insulin, insulin in presence of bumetanide, Ba^{2+}, glucagon, cAMP or hypoosmotic exposure. Accordingly, the antiproteolytic effects of insulin could fully be mimicked by equipotent hypoosmotic or glutamine/glycine-induced cell swelling (Fig. 1). These findings suggest that hormone- and amino acid-induced increases of the intracellular water space are a crucial event in mediating the known antiproteolytic effect of these compounds. Similarly, the known antagonism between insulin and glucagon regarding proteolysis can largely be explained by the opposing effects of these hormones on cell volume. The findings suggest that cell volume changes under the influence of hormones act like a 'second messenger', which mediates some of the hormones' effects on metabolism. In analogy, concentrating amino acid transporters in the plasma membrane may be seen not only as amino acid translocating systems, but also as transmembrane signalling systems inducing via cell volume changes alterations of metabolism. The intracellular mechanisms, which couple cell volume changes to proteolysis control, however, remain elusive.

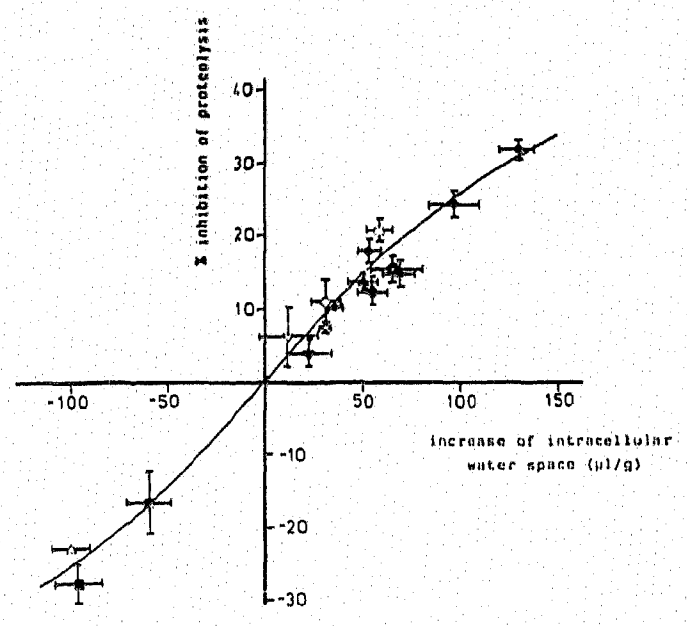

Fig. 1. Relationship between cell volume and proteolysis under the influence of glutamine, glycine, Ba^{2+}, insulin, glucagon, cAMP and hypoosmotic exposure in perfused rat liver.

Anisoosmotic cell volume changes were achieved by exposing the livers to hypoosmotic perfusion fluids (185–275 mOsmol/l). The concentrations of insulin, glucagon and dibutyryl-cAMP were 35 nM, 100 nM and 50 μM, respectively. Glutamine and glycine were added at a concentration of 2 mM, each. Ba^{2+} was added as $BaCl_2$ (1 mM); the concentration of bumetanide was 5 μM. Effects on the intracellular water space and on proteolysis were determined in separate experimental series as described in the methods section. Data on proteolysis inhibition and intracellular water space are in part taken from [9–11] and [12], respectively. Data are given as means ± SEM (n = 3–15). • anisoosmotic exposure (185–275 mOsmol/l); ▲ glutamine; ○ glycine; ⊖ glycine plus glutamine; ◆ Ba^{2+}; □ insulin; ◇ insulin in presence of bumetanide; ■ glucagon effect during hypoosmotic (185 mOsmol/l) swelling; ◩ glucagon effect in presence of insulin; △ cAMP effect in presence of insulin; ▼ net effect of glucagon-induced shrinkage plus hypoosmotic swelling; ▽ net effect of glucagon-induced shrinkage plus insulin-induced swelling.

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