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Regulation of liver cell volume and proteolysis by glucagon and insulin

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The effects of insulin and glucagon on liver cell volume and proteolysis were studied in isolated perfused rat liver. The rate of proteolysis was assessed as [^{3}H]leucine release from single-pass-perfused livers from rats which had been prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine. The intracellular water space was determined from the wash-out profiles of simultaneously added [^{3}H]inulin and [^{14}C]urea. In normo-osmotic (305 mosM) control perfusions the intracellular water space was 548 ± 10 μl/g wet mass ($n = 44$) and was increased by 16.5 ± 2.6 % ($n = 6$), i.e. by 85 ± 14 μl/g, after hypo-osmotic exposure (225 mosM). Glucagon (0.1 μM) decreased the intracellular water space by 17 ± 4 % ($n = 4$), whereas insulin (35 nM) increased the intracellular water space by 9.3 ± 1.4 % ($n = 15$). Also, in isolated rat hepatocyte suspensions insulin (100 nM) caused cell swelling by 10.7 ± 1.8 % ($n = 16$), which was fully reversed by glucagon. In perfused liver, insulin-induced cell swelling was accompanied by a hepatic net K^+ uptake (4.5 ± 0.2 μmol/g) and an inhibition of proteolysis by 21 ± 2 % ($n = 12$); further addition of glucagon led to a net K^+ release of 3.8 ± 0.2 μmol/g ($n = 7$) and fully reversed the insulin effects on both cell volume and proteolysis. Similarly, insulin-induced cell swelling and inhibition of proteolysis were completely antagonized by hyperosmotic (385 mosM) cell shrinkage. Furthermore, cell swelling and inhibition of proteolysis after hypo-osmotic exposure or amino acid addition were reversed by glucagon-induced cell shrinkage. There was a close relationship between the extent of cell swelling and the inhibition of proteolysis, regardless of whether cell volume was modified by insulin, glucagon or aniso-osmotic exposure. The data show that glucagon and insulin are potent modulators of liver cell volume, at least in part by alterations of cellular K^+ balance, and that their opposing effects on hepatic proteolysis can largely be explained by opposing effects on cell volume. It is hypothesized that hormone-induced alterations of cell volume may represent an important, not yet recognized, mechanism mediating hormonal effects on metabolism.

INTRODUCTION

Hepatic proteolysis is under the control of amino acids, hormones ([1–4]; for reviews see [5,6]) and liver cell volume [7,8]. The mechanisms underlying proteolysis control by amino acids and hormones are not understood [6]; however, recent evidence points to an involvement of cell volume alterations [7–9]. The anti-proteolytic effects of glutamine and glycine could be fully mimicked by equipotent hypo-osmotic cell swelling [7,8], and the inhibition of proteolysis by other amino acids could, at least in part, be ascribed to amino acid-induced cell swelling [8]. The anti-proteolytic effect of insulin in liver parallels the extent of hormone-induced intracellular K^+ accumulation, even when the insulin-induced K^+ uptake was modulated by the nutritional state or a variety of inhibitors [9]. Further, the anti-proteolytic effect of insulin could be mimicked by addition of Ba^{2+}, which, like insulin, causes cellular net K^+ uptake owing to a blockade of Ba^{2+}-sensitive K^+ channels [9]. Whereas the anti-proteolytic effect of insulin was associated with a net K^+ uptake, inhibition of proteolysis after hypo-osmotic cell swelling was accompanied by a depletion of intracellular K^+ [7–9]. Accordingly, alterations of the intracellular K^+ concentration under the influence of insulin and hypo-osmotic swelling could not provide the link to the anti-proteolytic effect. Thus it was suggested that cell swelling resulting from the insulin-induced K^+ accumulation might be the common denominator for the inhibition of proteolysis [9], in line with our previous hypothesis that hormone-induced alterations of cell volume could serve as a second messenger [10]. However, hormones have not yet been identified as modulators of cell volume. The data presented here on the effects of glucagon and insulin on liver cell volume and proteolysis suggest that the opposing effects of glucagon and insulin on hepatic proteolysis can largely be explained by their influence on cell volume. In view of the recent evidence that cell volume modulates cell function in liver [7–17], the present demonstration of a hormonal control of liver cell volume provides a new aspect on the mechanisms involved in hormone action.

MATERIALS AND METHODS

Liver perfusion

Livers from male Wistar rats (120–250 g body wt.), fed *ad libitum* on stock diet (Altromin), were perfused as described previously [18] in a non-recirculating manner with bicarbonate-buffered Krebs–Henseleit saline plus L-lactate (2.1 mM), pyruvate (0.3 mM) and leucine (0.1 mM). The influent K^+ concentration was 5.9 mM. The perfusate was gassed with O_2/CO_2 (19:1); the temperature was 37 °C. In normo-osmotic perfusions, the osmolarity was 305 mosM; hypo-osmotic and hyperosmotic exposure was performed by lowering or increasing the NaCl concentration in the perfusion medium, resulting in corresponding osmolarity changes. Amino acid and hormone additions to influent perfusate were either made by use of precision micropumps or by dissolution in the Krebs–Henseleit buffer. The composition of an amino acid mixture roughly resembling the concentrations found in portal-venous blood in the postprandial state is given in [19].

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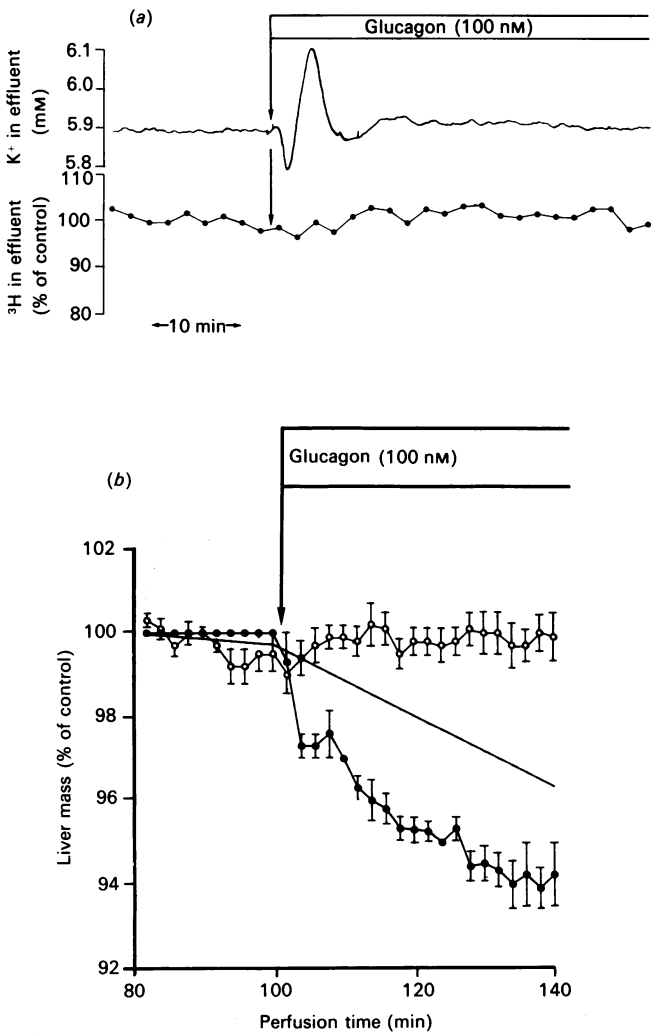

Fig. 1. Effect of glucagon (100 nM) on [K^+] in effluent perfusate and [^{3}H]leucine release (*a*) and liver mass (*b*)

In (*a*) a representative experiment from a series of three different perfusion experiments is shown. Livers from fed rats were prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine, and [^{3}H]leucine release into effluent was monitored as a measure of hepatic proteolysis [7,8]. Symbols in (*b*): ●, liver mass after glucagon addition; ○, liver mass in control experiments, i.e. without hormone addition. The line refers to a theoretical liver mass decrease calculated from hepatic glucose release from the liver in the presence of glucagon, under the assumption that all glucose released from the liver is derived from endogenous glycogen stores. Data are given as means ± S.E.M. ($n = 3$).

Isolated hepatocytes

Isolated hepatocytes were prepared by collagenase treatment as described in [20] and incubated in the above-mentioned Krebs–Henseleit buffer at 37 °C in a shaking water bath with continuous gassing with water-vapour-saturated O_2/CO_2 (19:1). After a 45 min incubation period the packed hepatocyte volume was determined after gentle centrifugation (600 *g* for 60 s) and related to the protein content of the incubation. Effectors (e.g. hormones) on cell volume were added at 30 min of incubation. The control cell volume determined in the absence of effectors was 5.6 ± 0.1 μl/mg of protein ($n = 68$); the control cell volume (after correction for the protein content of the individual incubation) determined in the individual cell preparation was set to 100%, and cell volume changes under the influence of effectors are given as percentages of the control value. We refer to this approach for cell volume determination in isolated hepatocyte suspensions as 'hepatocrit technique'.

Determinations

Liver mass was recorded continuously with a specially constructed balance pan, as described recently [7–9,19]. The liver wet mass at the end of the perfusion experiment after restoration for at least 30 min of normo-osmotic (305 mosM) medium was set to 100%, and the steady-state liver mass changes after aniso-osmotic exposure, amino acid or hormone addition are given on a percentage-change basis.

The rate of proteolysis was assessed by measuring the release of ^{3}H from isolated perfused rat livers after prelabelling of liver proteins *in vivo* by intraperitoneal injection of 200 μCi of L-[4,5-^{3}H]leucine about 16 h before the perfusion experiment as described recently [7–9]. In all experiments, the influent perfusate was supplemented with unlabelled leucine (0.1 mM) in order to prevent reutilization of [^{3}H]leucine for protein synthesis and to accelerate the wash-out of pre-existing free [^{3}H]leucine. Chromatographic analysis of the effluent perfusate revealed that ^{3}H released from the liver was more than 98% associated with leucine [7,8]. As shown recently [7], hypo-osmoticity-induced alterations in [^{3}H]leucine release also paralleled alterations in leucine release from the liver, as determined by amino acid analysis. ^{3}H release into the perfusate during normo-osmotic perfusions amounted to about 500 c.p.m./min per g of liver and was determined by scintillation spectrometry. After an about 80 min pre-perfusion period, ^{3}H release from the liver had reached a steady state, which was maintained for at least 100 min further; in control experiments [^{3}H]leucine release decreased by only 0.06%/min. Radioactivity released under these conditions was seen to be derived from proteolysis.

The effluent K^+ concentration was continuously monitored with a K^+-sensitive electrode (Radiometer, Munich, Germany); volume-regulatory or hormone-induced K^+ fluxes were determined by planimetry of areas under curves [7–9,11].

The portal pressure was routinely monitored with a pressure transducer (Hugo Sachs Electronics, Hugstetten, Germany).

Glucose in effluent perfusate was measured with an enzymic optical assay as described in [21].

Determination of the intracellular water space in perfused liver

The intracellular water space was determined in the intact perfused rat liver by monitoring the wash-out of simultaneously infused [^{3}H]inulin and [^{14}C]urea. In brief, both radioisotopes were added to the influent perfusate for about 5 min, i.e. a time period sufficient to achieve equilibration of [^{14}C]urea and of [^{3}H]inulin in their respective accessible water spaces. Equilibration was achieved when the release of radioactivity into effluent had reached a steady state. Then radioactivity infusion was stopped, and the effluent perfusate collected during the following 3–5 min and assayed for ^{3}H and ^{14}C. By using the effluent $^3H/^{14}C$ ratio found during steady-state infusion of radioactivity and that found during the wash-out period, [^{3}H]inulin and [^{14}C]urea spaces were calculated. The difference between these two spaces reflects a space which is accessible to added [^{14}C]urea but not to [^{3}H]inulin. This space was considered the intracellular water space. It should be noted that measurements of that space were only conducted in livers that were not prelabelled with [^{3}H]leucine. Accordingly, data on proteolysis and cell volume were obtained in different experimental series. This approach for measuring cell volume in the intact perfused rat liver allowed repeated determinations of the intracellular water space within the individual perfusion experiment. Cell volume changes under the influence of effectors were determined as the difference between two consecutive space

Table 1. Effects of glucagon and insulin on hepatic K^+ balance, liver mass and the intracellular water space in perfused rat liver

Net K^+ balance reflects the net effect of the hormone-induced K^+ movements: positive values refer to net K^+ uptake, negative values to net K^+ release. The intracellular water space ('cell volume') was determined from the wash-out of simultaneously added [^{14}C]urea and [^{3}H]inulin (for details see the Materials and methods section) and is expressed as percentage change compared to the space obtained during normo-osmotic perfusion in the absence of hormones: positive values reflect cell swelling, negative values cell shrinkage. Liver mass during normo-osmotic (305 mosM) perfusions and in the absence of further additions was set at 100% in the individual perfusion experiment, and the hormone-induced mass changes are given as percentages of this 100% value; negative values refer to mass decrease. 'Amino acids' refers to experiments in which the influent perfusate contained amino acids in concentrations roughly mimicking those found physiologically in portal-venous blood (for composition see [19]). Data were obtained during steady states and are given as means ± S.E.M. for the numbers of experiments in parentheses; n.d., not determined.

Condition	K^+ balance (μmol/g)	Liver mass change (%)	Cell volume change (%)
Insulin (35 nM)	+4.5±0.2 (15)	< 1%	+9±1 (15)
Glucagon (100 nM)	−0.8±0.2 (12)	−5.7±0.7 (3)	−17±4 (4)
Glucagon (100 nM) in presence of:			
insulin (35 nM)	−3.8±0.2 (7)	−5.2±0.5 (6)	−13±2 (5)
hypo-osmotic perfusion			
(265 mosM)	−0.7±0.2 (4)	−6.9±1.2 (4)	n.d.
(185 mosM)	−1.9±0.6 (4)	−9.8±0.8 (4)	−19±3 (4)
amino acids	0.0±0.2 (3)	−3.4±0.1 (3)	n.d.

measurements (time interval about 30 min) in the individual experiment. Control experiments with repeated determinations of the intracellular water space in 30 min intervals revealed a spontaneous decrease of the water space by 2.4±0.3% ($n = 6$) per 30 min of perfusion. The data on aniso-osmotically and hormone-induced cell volume changes reported in this paper were not corrected for this spontaneous decrease of liver cell volume. The wash-out of added [^{14}C]urea was not significantly delayed when endogenous urea synthesis was stimulated by NH_4Cl: in the presence and absence of added NH_4Cl (1 mM) plus ornithine (2 mM) the effluent urea concentration was 336±32 μM ($n = 4$) and 36±2 μM ($n = 4$) respectively, but [^{14}C]urea radioactivity wash-out was more than 99% complete within 3 min under both conditions. In addition, stimulation of urea synthesis by NH_4Cl (1 mM) plus ornithine (2 mM) had no significant effect on intracellular water space: in four different perfusion experiments the change in intracellular water space after the approx. 10-fold stimulation of urea synthesis by addition of NH_4Cl (1 mM) was 3±3 μl/g, i.e. by 1±1%. However, in this determination the spontaneous decrease of intracellular water space was not taken into account.

Statistics

Data on [^{3}H]leucine release were obtained during steady states. Data from different perfusion experiments are given as means ± S.E.M. (number of experiments).

Materials

L-[4,5-^{3}H]Leucine, [^{3}H]inulin and [^{14}C]urea were from Amersham Buchler (Braunschweig, Germany), and L-lactic acid was from Roth (Karlsruhe, Germany). Insulin and glucagon were from Sigma (Munich, Germany). All other chemicals were from Merck (Darmstadt, Germany).

RESULTS

Effect of hypo-osmotic perfusion on the intracellular water space

In the isolated perfused rat liver, the intracellular ([^{14}C]urea-accessible minus the [^{3}H]inulin-accessible) water space was calculated from the wash-out profiles of simultaneously infused [^{3}H]inulin (which distributes in the extracellular space) and [^{14}C]urea (which distributes in the extra- and intra-cellular spaces). During normo-osmotic (305 mosM) perfusions and in the absence of hormones or amino acids in the influent, the intracellular water space was 548±10 μl/g liver wet wt. ($n = 44$). Exposure of perfused rat liver to hypo-osmotic perfusion fluid (225 mosM) increased the intracellular water space by 16±3% ($n = 6$), i.e. by 85±14 μl/g. By using the 'hepatocrit technique', a similar increase of cell volume, i.e. by 13±2% ($n = 16$) was found on incubation of isolated rat hepatocytes in hypo-osmotic (225 mosM) media. The hypo-osmoticity (225 mosM)-induced increase in the intracellular water space in the intact liver by 85±14 μl/g agrees well with the steady-state increase in perfused liver mass of 9.7±0.2% ($n = 22$) found under these conditions. In line with previous data [11–13], this shows again that the liver mass increase after hypo-osmotic exposure is due to an expansion of the intracellular space. Accordingly, liver mass changes after hypo-osmotic perfusion reflect fairly well the cell volume changes *in situ*.

Effect of glucagon on cell volume, liver mass, K^+ movements and [^{3}H]leucine release

Addition of glucagon (100 nM) to influent perfusate of isolated perfused rat liver led to triphasic K^+ movements, which were completed within 12 min (Fig. 1*a*) and resulted in a small net K^+ release from the liver of 0.8±0.2 μmol/g ($n = 12$) (Table 1). Glucagon (100 nM) decreased the intracellular water space by 17±4% ($n = 4$) and liver mass by 5.7±0.7% ($n = 3$). About 40% of the glucagon-induced liver mass decrease could be ascribed to the stimulation of glycogenolysis by glucagon, which led to a glucose output at a constant rate of 3.66±0.37 μmol/min per g ($n = 3$) during the first 40 min of glucagon addition (Fig. 1*b*).

When liver proteins were prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine about 16 h before the perfusion experiment, addition of glucagon was without effect on [^{3}H]leucine release into effluent perfusate (Fig. 1*a*): when [^{3}H]leucine release during the control period was set to 100%, this value was 101±1% ($n = 3$) in the presence of glucagon. This suggests that glucagon does not affect hepatic proteolysis under these conditions. Similarly to glucagon, cell shrinkage by exposing the liver to hyperosmotic perfusion fluid (385 mosM) led to a decrease of liver mass by 9.3±0.6% ($n = 3$) and of the intracellular water space by 16.3±0.2% ($n = 3$), but was without effect on

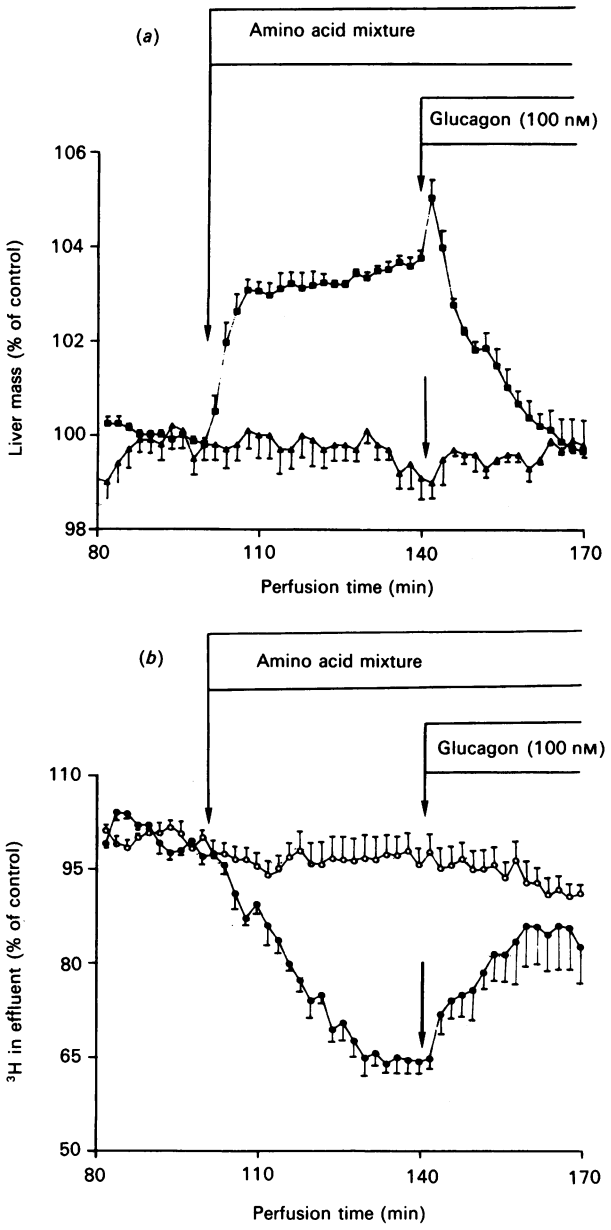

Fig. 2. Effect of an amino acid mixture in physiological concentrations and of glucagon (100 nM) on liver mass (*a*) and [^{3}H]leucine release (*b*) from perfused rat liver

Liver mass in the absence of additions was set to 100% in the individual perfusion experiment. Liver mass increases upon amino acid addition and decreases again after glucagon administration (■). In (*a*) the ▲ symbols refer to control experiments without amino acid or hormone addition. In (*b*) livers from fed rats were prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine, and [^{3}H]leucine release into effluent was monitored as a measure of hepatic proteolysis. Proteolysis is inhibited by amino acids and stimulated by further addition of glucagon (●); ○, control experiments, i.e. without hormone or amino acid addition. Data are given as means ± S.E.M. ($n = 4$).

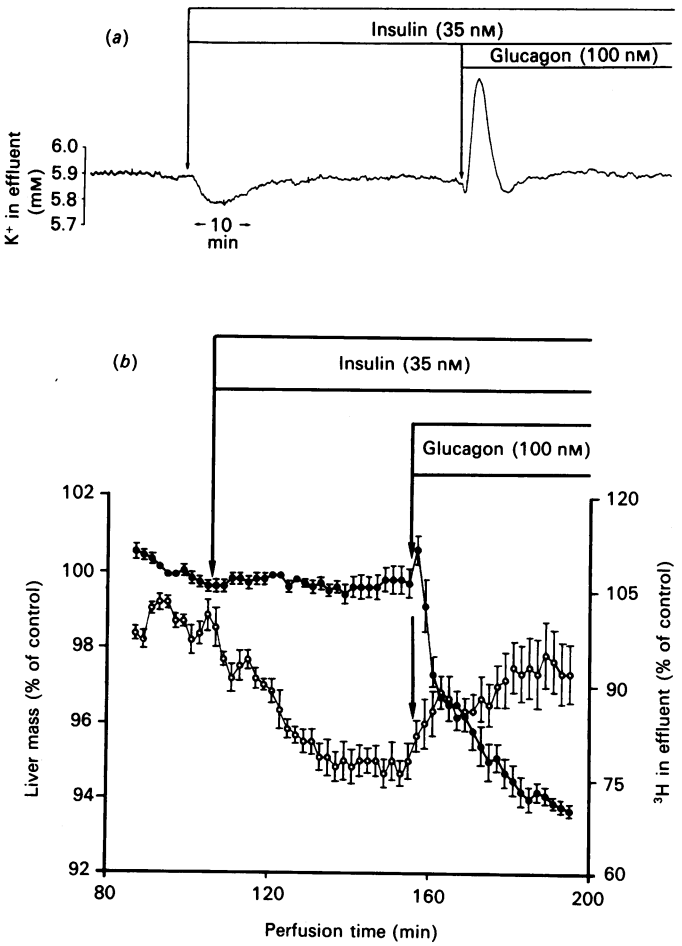

Fig. 3. Effects of insulin (35 nM) and glucagon (100 nM) on [K^+] in effluent (*a*) and [^{3}H]leucine release (○) and liver mass (●) (*b*) in presence of insulin (35 nM)

Insulin induces a net K^+ uptake by the liver via amiloride- and furosemide-sensitive mechanisms [17], whereas glucagon leads to a marked net K^+ efflux from the liver (*a*). Livers from fed rats were prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine, and [^{3}H]leucine release into effluent was monitored as a measure of hepatic proteolysis. Proteolysis is inhibited by insulin and stimulated by glucagon (*b*). Insulin is without effect on liver mass, whereas glucagon decreases liver mass (*b*). Liver mass in the individual perfusion experiment was set to 100% in the absence of added hormones. In (*a*) a representative experiment from a series of six similar perfusion experiments is shown; in (*b*) data are given as means ± S.E.M. ($n = 6$).

[^{3}H]leucine release. [^{3}H]Leucine release was 98.8 ± 2.6% ($n = 3$) during hyperosmotic (385 mosM) perfusion compared with normo-osmotic perfusion. On the other hand, hypo-osmotic (225 mosM) cell swelling inhibited [^{3}H]leucine release by 24 ± 2% ($n = 7$), in line with previous data [7,8].

As shown recently [8], addition of amino acids in concentrations roughly mimicking those found in portal-venous blood increased liver mass by 3.4 ± 0.1% ($n = 3$) and inhibited [^{3}H]leucine release by 35 ± 2% ($n = 3$). Further addition of glucagon (0.1 μM) returned liver mass to the baseline level and counteracted the amino-acid-induced inhibition of [^{3}H]leucine release (Fig. 2).

Antagonism between glucagon and insulin regarding K^+ balance, cell volume and proteolysis

In contrast with glucagon, insulin (35 nM) increased the intracellular water space in perfused liver by 9.3 ± 1.4% ($n = 15$), accompanied by a net K^+ uptake in these experiments of 4.5 ± 0.2 μmol/g ($n = 15$) (Table 1). A similar degree of cell swelling, i.e. by 10.7 ± 1.8% ($n = 16$), was found upon addition of insulin (100 nM) to isolated hepatocyte suspensions, when cell volume was determined with the 'hepatocrit method'. When glucagon (100 nM) was added to perfused rat liver during constant infusion of insulin (35 nM), the intracellular water space decreased

again by 13±2% ($n = 5$), i.e. by 73±9 μl/g ($n = 5$), being accompanied by a marked net K^+ release (Fig. 3*a*, Table 1). Accordingly, glucagon fully counteracted the insulin-induced cell swelling in perfused rat liver. Although insulin led to cell swelling, it had no significant effect on liver mass; however, subsequent cell shrinkage by glucagon decreased liver mass by 5–6% (Fig. 3*b*, Table 1). None of the hormones had detectable effects on the portal perfusion pressure (results not shown).

Whereas cell swelling by insulin inhibited [^{3}H]leucine release by 21±2% ($n = 12$), further addition of glucagon not only abolished insulin-induced swelling, but also largely counteracted the anti-proteolytic effect of insulin (Fig. 3*b*). Whereas insulin alone induced a net K^+ uptake by the liver (Table 1, Fig. 3*a*), further addition of glucagon led to a net K^+ release of 3.8±0.2 μmol/g ($n = 7$). These data show that the antagonistic action of glucagon and insulin on hepatic proteolysis is accompanied by opposing effects on cellular K^+ contents and cell volume.

Interactions between aniso-osmotic cell volume changes and the insulin and glucagon effects on proteolysis and cell volume

When the livers were exposed to hypo-osmolar perfusion fluid (185 mosM), there was a volume-regulatory K^+ efflux of 18.2±1.1 μmol/g ($n = 7$) within 10 min (results not shown). Thereafter liver mass had reached a new, albeit elevated, steady state. In line with recent studies on hypo-osmotic cell swelling and proteolysis [7,8], [^{3}H]leucine release was inhibited by 31.4±0.8% ($n = 4$) (Fig. 4). Further addition of glucagon (100 nM) to the hypo-osmotically (185 mosM) pre-swollen livers decreased liver mass by 9.8±0.8% ($n = 4$) and increased [^{3}H]leucine release by 27.6±2.6% ($n = 4$). Thus about 90% of the anti-proteolytic effect of hypo-osmotic cell swelling was abolished by glucagon-induced cell shrinkage. Determinations of intracellular water spaces in a separate series of perfusion experiments (i.e., similar to those in Fig. 4, but without [^{3}H]leucine prelabelling) revealed that glucagon completely reversed the increase in the intracellular water space resulting from hypo-osmotic (185 mosM) exposure: whereas hypo-osmotic exposure increased the intracellular water space by 18±1% ($n = 4$) (i.e. by 106±8 μl/g), further addition of glucagon lowered it again by 19±3% ($n = 4$) (i.e. by 110±13 μl/g). These data show that both cell swelling and inhibition of proteolysis by hypo-osmotic exposure are almost completely counteracted by glucagon. This suggests that stimulation of proteolysis by glucagon in hypo-osmotically preswollen livers can largely be explained on the basis of glucagon-induced cell shrinkage.

As shown in Fig. 5, the insulin (35 nM)-induced inhibition of [^{3}H]leucine release was fully abolished by subsequent hyperosmotic (385 mosM) cell shrinkage. Under these conditions, hyperosmotic perfusion decreased liver mass by 7.0±0.3% ($n = 3$), and in another series of experiments the intracellular water space was found to decrease by 16±1% ($n = 3$) upon hyperosmotic (385 mosM) exposure. Thus reversal of insulin-induced cell swelling by hyperosmotic cell shrinkage also counteracts the anti-proteolytic effect of the hormone.

Relationship between proteolysis and cell volume under the influence of insulin, glucagon and aniso-osmotic perfusion

As shown in Fig. 6, there was a close relationship between the inhibition of proteolysis and the extent of liver cell volume change under the influence of hypo-osmotic exposure (185–275 mosM). This relationship was also maintained when cell volume was modified by insulin or glucagon, suggesting that the hormones' effects on proteolysis are largely mediated by cell volume changes. It should be noted that corresponding cell volume changes and effects on proteolysis could not be obtained in the same experiment, because ^{3}H radiolabels were employed for determination of both proteolysis and intracellular water space. Accordingly, the data in Fig. 6 compare different series of experiments, which, however, had the same experimental design.

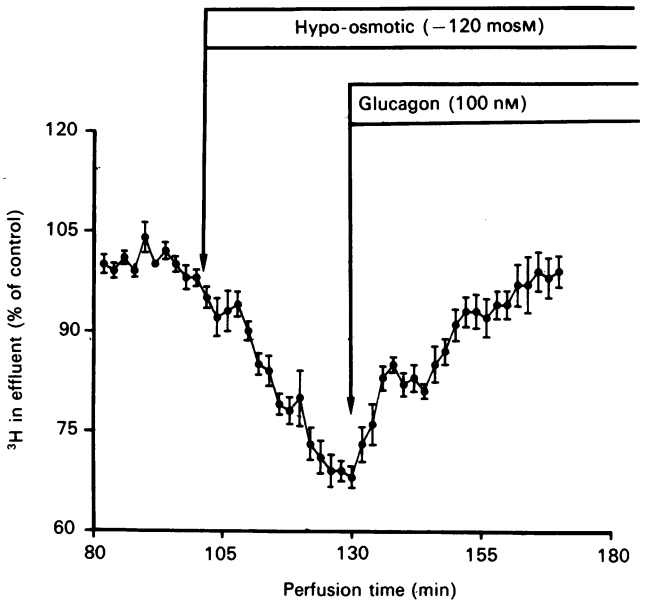

Fig. 4. Effect of glucagon (100 nM) on [^{3}H]leucine release during hypo-osmotic (185 mosM) perfusion

Livers from fed rats were prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine, and [^{3}H]leucine release into effluent was monitored as a measure of hepatic proteolysis. Proteolysis is inhibited during hypo-osmotic cell swelling and stimulated again by further addition of glucagon (100 nM). Data are given as means±S.E.M. from four different experiments.

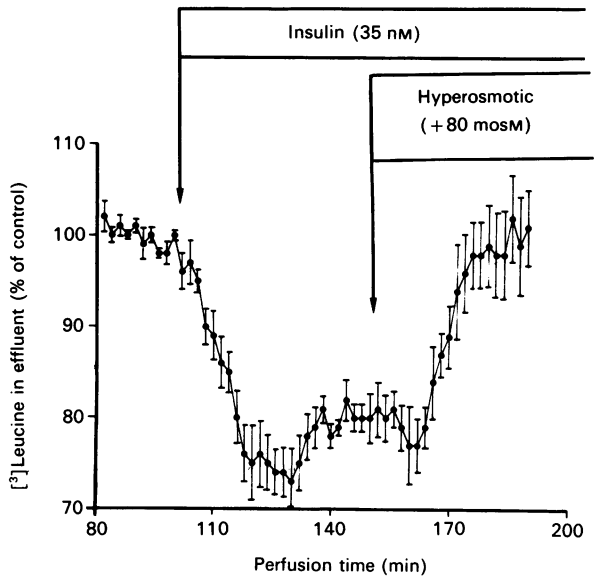

Fig. 5. Effect of hyperosmotic cell shrinkage on proteolysis inhibition by insulin

Livers from fed rats were prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine, and [^{3}H]leucine release into effluent was monitored as a measure of hepatic proteolysis. Inhibition of proteolysis by insulin is counteracted by cell shrinkage caused by hyperosmotic exposure (385 mosM). Data are given as means±S.E.M. from three different experiments.

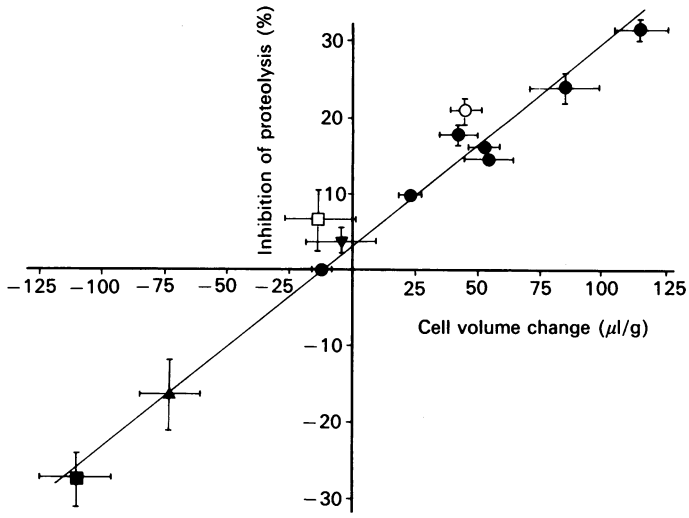

Fig. 6. Relationship between cell volume changes and proteolysis under the influence of insulin, glucagon and hypo-osmotic exposure in perfused rat liver

Aniso-osmotic cell volume changes were achieved by exposing the livers to hypo-osmotic perfusion fluids (185–275 mosM). The concentrations of insulin and glucagon were 35 and 100 nM respectively. Cell volume changes were assessed in the individual perfusion experiment as the difference between the intracellular water spaces found under control conditions (i.e. normo-osmotic perfusion, hormones absent) and those in the presence of the effector. Proteolysis was assessed as [^{3}H]leucine release from perfused livers from fed rats prelabelled *in vivo* by intraperitoneal injection of [^{3}H]leucine. Effects on proteolysis and the intracellular water spaces ('cell volume') were determined in separate experimental series. Control experiments, in which the intracellular water space was determined in 30 min periods without changing the perfusion conditions, revealed that the intracellular water space decreased by 2.4 ± 0.3 % ($n = 6$) per 30 min of perfusion. The data in Fig. 6 were not corrected for the spontaneous perfusion-time-dependent decrease in cell volume under control conditions; this explains why the curve given in Fig. 6 is somewhat moved to the left. For further explanations see text. Data are given as means ± S.E.M. ($n = 3$–7): ●, aniso-osmotic modulation of cell volume (185–305 mosM); ○, insulin (35 nM); ■, glucagon (100 nM) effect during hypo-osmotic perfusion (185 mosM); ▲, glucagon (100 nM) effect in presence of insulin (35 nM); ▼, net effect of glucagon-induced shrinkage plus hypo-osmotic swelling; □, net effect of glucagon-induced shrinkage plus insulin-induced swelling.

Cell volume changes in the individual perfusion experiments were calculated as the difference between two consecutive intracellular-water-space measurements: the first measurement ('control space') was performed about 60 min after the start of the experiment during normo-osmotic perfusion and in the absence of hormones. Thereafter effectors on cell volume were added, and the second water-space measurement was performed about 30 min later. The data in Fig. 6 were not corrected for the 'spontaneous' perfusion-time-dependent decrease in cell volume by 2.4 ± 0.3 % per 30 min ($n = 6$) occurring already in control experiments, i.e. without effector addition (see methods). This explains why the curve shown in Fig. 6 is moved somewhat to the left.

DISCUSSION

Assessment of liver cell volume

Several lines of evidence indicate that the technique employed here for assessment of cell volume changes in the intact liver is a rather sensitive and useful approach. In normo-osmotic perfusion the intracellular water space was 548 ± 10 μl/g ($n = 44$). This value is close to that reported by others in freeze-clamp studies [22] and corresponds well to an extracellular space in freeze-clamped perfused rat livers of about 35 % [12,13]. Changes in the intracellular water-space after hypo-osmotic exposure or insulin were in close agreement with the cell-volume effects determined in isolated hepatocyte suspensions by the 'hepatocrit method'. In addition, the increase in the intracellular water-space after hypo-osmotic exposure could account for about 90 % of the simultaneously observed liver mass increase. In line with previous data [13], the present approach also shows that liver mass changes after aniso-osmotic exposure reliably reflect volume changes of liver cells in the intact organ. Liver mass recordings, however, are not suitable to monitor cell volume changes in response to hormones (Table 1).

Effect of glucagon on proteolysis

Regulation of hepatic proteolysis by glucagon and insulin is long known [1,3,4–6,23]; however, the underlying mechanisms are not understood [6]. In line with earlier studies on proteolysis [23], infusion of glucagon into perfused rat liver in the absence of inhibitors of proteolysis had no effect on [^{3}H]leucine release, probably because under these conditions proteolysis is already maximally activated and cannot be further enhanced by glucagon or hyperosmotic cell shrinkage (the present paper). Stimulation of proteolysis by glucagon, however, becomes apparent when proteolysis was pre-inhibited by either amino acids (Fig. 2) or insulin (Fig. 3). Similarly, hyperosmotic cell shrinkage stimulated proteolysis only when protein breakdown was pre-inhibited in the presence of amino acids [8] or by insulin (Fig. 5), but not in the absence of known inhibitors of proteolysis. Interestingly, glucagon also reversed the inhibition of proteolysis caused by hypo-osmotic liver swelling (Fig. 4). This suggests that the proteolytic action of glucagon is independent of the presence of amino acids or insulin.

Regulation of cell volume by glucagon and insulin

The insulin-induced cell volume increase by 9–10 % is only about 30 % higher than that theoretically expected from the insulin-induced net K^+ (plus an accompanying anion) accumulation inside the cells (Table 1). Accordingly, insulin-induced cell swelling can largely be explained on the basis of cellular net K^+ accumulation. On the other hand, glucagon markedly decreased the intracellular water space, but glucagon-induced cell shrinkage was accompanied by a pronounced net K^+ release only when insulin was present (Table 1). Thus other, not yet identified, mechanisms may be responsible for glucagon-induced cell shrinkage. It should be emphasized that the decrease in urea-accessible water space of 10–20 % induced by glucagon (Table 1) cannot readily be explained by the stimulation of glycogen breakdown. The effect of glucagon on liver mass was significantly lower when amino acids were present (Table 1). This is probably explained by the known effects of glucagon on amino acid transport and metabolism. Whereas a glucagon-induced stimulation of amino acid transport tends to swell the cells, stimulation of intracellular amino acid breakdown tends to dissipate the intra-/extra-cellular amino acid concentration gradient, and accordingly the degree of amino-acid-induced cell swelling.

Role of cell volume in regulating hepatic proteolysis

The picture emerging from this and previous studies [7–9] suggests that cell volume is an important regulator of hepatic proteolysis and a common denominator for proteolysis control by amino acids, aniso-osmotic perfusion and hormones (Fig. 6;

[7–9]). Liver cell swelling induced by hypo-osmotic exposure [7,8], amino acids [7–9], Ba^{2+} or insulin ([9]; the present paper) inhibits proteolysis. Conversely, when proteolysis is not already maximally activated, cell shrinkage induced by either glucagon (the present paper) or hyperosmotic perfusion ([8]; Fig. 5) stimulates proteolysis. In line with a crucial role of cell volume changes in mediating the hormone effects on proteolysis are also the present findings that hyperosmotic cell shrinkage counteracted the anti-proteolytic effect of insulin-induced cell swelling (Fig. 5) and that glucagon-induced cell shrinkage overcame the inhibition of proteolysis induced by hypo-osmotic cell swelling (Fig. 4). Both insulin and hyperosmotic cell shrinkage are known to activate Na^+/H^+ exchange [9,24–26], but exert opposing effects on cell volume and proteolysis ([7–9]; the present paper). Whereas activation of Na^+/H^+ exchange by insulin increases cell volume above the 'resting' level (the present paper), stimulation of Na^+/H^+ exchange after hyperosmotic exposure brings about volume-regulatory increase, but nonetheless the cell remains in a shrunken state [11,24]. This suggests that cell volume changes, rather than the activation of Na^+/H^+ exchange or the associated alterations of intracellular pH are responsible for the effects on proteolysis. The close relationship between proteolysis and cell volume changes, whether induced by glucagon, insulin or aniso-osmotic exposure (Fig. 6), suggests that the hormone effects are mediated by cell volume changes: the anti-proteolytic effect of insulin can largely be mimicked by equipotent hypo-osmotic cell swelling (Fig. 6).

Possible significance

Cell volume changes are important modulators of metabolic liver function [7–17]. The present finding that liver cell volume is also controlled by glucagon and insulin suggests that hormone-induced alterations of cell volume may mediate the known hormonal effects on metabolism, at least in part. According to this hypothesis [10], cell volume changes under the influence of hormones may act like a 'second or third messenger'. In line with this, both glucagon and hyperosmotic exposure lead to cell shrinkage; simultaneously glycogenolysis [11,14] and proteolysis ([8]; Fig. 5) are stimulated, whereas glycogen synthesis is inhibited [15]. Conversely, insulin and hypo-osmotic exposure lead to cell swelling, accompanied by a stimulation of glycogen synthesis [15], an inhibition of proteolysis [7,8], glycogenolysis [11,14] and glycogen phosphorylase *a* activity in isolated hepatocytes (B. Stoll & D. Häussinger, unpublished work). It should be noted that hormone effects on cell volume need not necessarily be accompanied by parallel alterations in mitochondrial volume. Although glucagon leads to liver cell shrinkage, it simultaneously swells the mitochondria (for reviews see [27,28], thereby activating mitochondrial glutaminase [29,30].

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