Regulation of cell volume in the perfused rat liver by hormones

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The effect of hormones on cell volume was studied in isolated perfused rat liver by assessing the intracellular water space as the difference between a [³H]inulin- and a [¹⁴C]urea-accessible space. The intracellular water space (control value $559 \pm 7 \mu\text{l/g}$ of liver; $n = 88$) increased on addition of insulin (35 nM) or phenylephrine (5 μM) by 12 or 8 % respectively, whereas it decreased with cyclic AMP (cAMP; 50 μM), glucagon (100 nM) or adenosine (50 μM) by 9, 13 or 6 % respectively. Both insulin and glucagon exerted half-maximal effects on cell volume and cellular K⁺ balance at hormone concentrations found physiologically in the portal vein. Adenosine-induced cell shrinkage was explained by a net K⁺ release from the liver. Phenylephrine (5 μM) led to cell swelling by about 8 %, which was additive to insulin-induced swelling. Extracellular ATP (20 μM) induced cell shrinkage by about 6 %; this was additive to adenosine-induced shrinkage. Vasopressin (15 nM) did not appreciably change cell volume, but induced marked cell shrinkage when glucagon or cAMP was present. Insulin- and phenylephrine-induced cell swelling was counteracted by cAMP. Hormone-induced changes of intracellular water space could sufficiently explain accompanying liver mass changes induced by glucagon, cAMP, adenosine or vasopressin, but not those by phenylephrine and extracellular ATP. The data show that liver cell volume is subject to hormonal regulation, in part owing to modification of cellular K⁺ balance. Glucagon- and insulin-induced cell volume changes occur already in the presence of physiological hormone concentrations. The effects of Ca²⁺-mobilizing hormones on cell volume are not uniform. In view of the recently established role of cell volume changes in modulating liver cell function, the present findings open a new perspective on the mechanisms of hormone action in liver, underlining our previous hypothesis that cell volume changes may represent a 'second messenger' of hormone action.

INTRODUCTION

Liver cell volume has recently been identified as an important modulator of liver cell function. A close relationship exists between the extent of cell volume changes and the proteolytic activity in liver, regardless of the experimental means of cell volume modulation [1–3]. It recently became clear that hormones such as insulin and glucagon exert marked effects on liver cell volume [3–5] and that the effect of both hormones on proteolysis can almost fully be explained on the basis of hormone-induced cell volume changes [3]. Accordingly, hormone-induced cell volume changes were suggested to act like a 'second messenger' mediating hormone effects on hepatic metabolism, at least in part [3]. However, it remained unclear whether cell volume changes can be expected during physiological variations of insulin and glucagon concentrations in the portal vein. Further, nothing is known about the effects of other hormones on liver cell volume, except for phenylephrine, which was suggested to decrease and to increase the extra- and intra-cellular spaces respectively in perfused rat liver [6]. Accordingly, we have studied the effects of a variety of hormonally active substances and their interplay on the intracellular water space, liver mass and K⁺ fluxes in perfused rat liver.

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 [7] in a non-recirculating manner with bicarbonate-buffered Krebs–Henseleit saline plus L-lactate (2.1 mM) and pyruvate (0.3 mM). The influent K⁺ concentration was 5.9 mM. The perfusate was gassed with O₂/CO₂ (19:1); the temperature

was 37 °C. The perfusate osmolality was 305 mosmol/l. Hormone and radioisotope additions to influent perfusate were made by use of precision micropumps.

Determinations

Liver mass was recorded continuously with a specially constructed balance pan, as described recently [1,2]. The liver wet mass at the end of the perfusion experiment after restoration of normo-osmotic (305 mosmol/l) medium for at least 30 min was set to 100 % in the individual experiment and the steady-state liver mass changes after hormone addition are given on a percentage-change basis.

The effluent K⁺ concentration was continuously monitored with a K⁺-sensitive electrode (Radiometer, Munich, Germany); hormone-induced K⁺ fluxes were determined by planimetry of areas under curves [1,2,5]. When present, a baseline drift was taken into account.

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

Data from different perfusion experiments are given as means $\pm$ S.E.M. (number of experiments).

Determination of intracellular water space

The intracellular water space of perfused livers was determined by means of a [³H]inulin/[¹⁴C]urea wash-out technique as described in detail recently [8]. In brief, both radioisotopes were added to the influent perfusate for about 5 min, i.e. a time period sufficient to achieve equilibration of [¹⁴C]urea and [³H]inulin in their respective accessible water spaces. [³H]inulin served as a marker of the extracellular water space, whereas [¹⁴C]urea, which exhibits distribution space identical with that of [³H]water [8,9], was used as a marker of both the intra- and the extra-cellular water space. Isotope equilibration was achieved when radio-

Abbreviation used: cAMP, cyclic AMP.

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Table 1. Effects of hormones on intracellular water space, liver mass, portal perfusion pressure and K⁺ movements in perfused rat liver

The intracellular water space was determined from the wash-out of simultaneously added [¹⁴C]urea and [³H]inulin (see the Materials and methods section; for further details see [8]). Data on intracellular water space change are given as $\mu\text{l/g}$ of liver and on a percentage-change basis. In the individual perfusion experiment, the intracellular water space in the absence of hormones was set to 100 % and the space change found after a 30 min hormone addition is expressed as a percentage thereof. Under control conditions the intracellular water space was $559 \pm 7 \mu\text{l/g}$ ($n = 88$) and decreased by $2.4 \pm 0.3 \%$ in a second determination 30 min later. Values given in the Table were corrected for this spontaneous decrease of intracellular water space. Positive values reflect cell swelling, and negative values cell shrinkage. Negative values for net K⁺ uptake reflect net K⁺ release. Liver mass changes are given as percentage mass change induced by the hormones after a 30 min hormone addition; liver wet mass in the absence of hormones at the end of the perfusion experiment was set to 100 %. Data were obtained during steady states and are given as means $\pm$ S.E.M. (n = number of experiments). When the effect of a hormone was studied in presence of another hormonally active substance ('Condition'), the latter compound was continuously present in the influent perfusate and was instituted at least 30 min before addition of the hormone under study. Data on perfusion-pressure increase induced by the hormone under study refer to the time point of intracellular space measurement: n.d., not detectable.

Effector	Condition	Change of intracellular water space		Liver mass change (%)	Net K ⁺ uptake ($\mu\text{mol/g}$)	Portal pressure increase (cmH_2O)	<i>n</i>
		($\mu\text{l/g}$)	(%)				
Insulin (2 nM)	Control	$+49 \pm 9$	$+8.2 \pm 1.6$	< 0.5	$+3.2 \pm 0.4$	n.d.	4-5
	Control	$+59 \pm 6$	$+12.2 \pm 1.4$	< 0.5	$+4.5 \pm 0.2$	n.d.	15
Insulin (35 nM)	Phenylephrine (5 μM)	$+60 \pm 7$	$+12.5 \pm 1.6$	$+3.7 \pm 0.7$	$+4.2 \pm 0.5$	n.d.	4
	ATP (20 μM)	$+50 \pm 3$	$+9.0 \pm 0.7$	$+1.0 \pm 0.4$	$+3.7 \pm 0.3$	n.d.	4
Glucagon (100 nM)	Control	-79 ± 17	-13.5 ± 2.7	-5.8 ± 0.4	-0.8 ± 0.2	n.d.	6-12
	Insulin (35 nM)	-60 ± 9	-10.6 ± 1.5	-4.6 ± 0.4	-3.8 ± 0.2	n.d.	5-7
cAMP (50 μM)	Control	-51 ± 11	-8.6 ± 1.4	-5.5 ± 0.3	-0.4 ± 0.7	n.d.	3
	Insulin (100 nM)	-98 ± 9	-15.9 ± 1.1	-8.5 ± 0.3	-3.1 ± 0.2	n.d.	3
	Phenylephrine (5 μM)	-45 ± 17	-8.8 ± 3.3	-2.2 ± 0.7	-3.4 ± 0.2	n.d.	3
	Vasopressin (15 nM)	-91 ± 21	-15.9 ± 1.1	-6.1 ± 1.2	-2.2 ± 0.4	n.d.	3
Adenosine (50 μM)	Control	-35 ± 9	-6.1 ± 1.6	-4.5 ± 1.0	-4.8 ± 0.2	0.2 ± 0.1	8
Vasopressin (15 nM)	Control	-12 ± 10	-2.3 ± 1.9	-3.3 ± 0.8	$+1.2 \pm 0.5$	n.d.	4
	Glucagon (100 nM)	-76 ± 23	-14.9 ± 3.8	-7.3	-2.6 ± 0.6	n.d.	3
	cAMP (50 μM)	-51 ± 6	-10.0 ± 1.0	-4.7 ± 1.6	-3.3 ± 0.2	n.d.	3
Phenylephrine	Control	$+36 \pm 3$	$+7.8 \pm 0.9$	-21.9 ± 2.2	$+0.3 \pm 0.3$	4.6 ± 0.3	7
	Adenosine (50 μM)	$+16 \pm 3$	$+3.6 \pm 0.8$	-16.1 ± 3.3	$+2.3 \pm 1.0$	2.3 ± 0.2	4
ATP (20 μM)	Control	-35 ± 13	-5.8 ± 2.1	$+0.2 \pm 0.8$	-0.8 ± 0.3	1.8 ± 0.2	6
	Adenosine (50 μM)	-37 ± 15	-6.5 ± 2.5	$+4.2 \pm 0.9$	$+0.8 \pm 0.3$	2.4 ± 0.1	4

activity in the effluent perfusate had reached a steady state. Then radioactivity infusion was stopped, and the effluent perfusate collected in 30–60 s samples during the following 3–5 min and assayed for ³H and ¹⁴C. From the effluent ³H/¹⁴C ratio found during steady-state infusion of radioactivity and that found during the wash-out period, [³H]inulin and [¹⁴C]urea spaces were calculated. The difference between both spaces reflects a space which is accessible to added [¹⁴C]urea but not to [³H]inulin, i.e. the intracellular water space. This approach allowed repeated determinations of the intracellular water space during an individual perfusion experiment. From kinetic data on cellular inulin uptake by fluid-phase endocytosis in perfused rat liver as described in [10], the error arising from inulin endocytosis during the 5 min infusion period is negligible, i.e. less than 0.7 % of the intracellular water space. Hormone-induced cell volume changes were determined as the difference between two consecutive space measurements (time interval 30 min) in the individual experiment: after determination of the intracellular water space during control conditions (i.e. in the absence of effectors of cell volume), hormone infusion was started and 30 min later a second determination of the intracellular water space was performed. The difference between these two spaces was ascribed to hormone action. Repeated determinations of the intracellular water space at 30 min intervals in control experiments (i.e. without hormone addition) revealed a spontaneous decrease of the water space by $2.4 \pm 0.3 \%$ ($n = 6$) per 30 min of perfusion. Hormone effects on liver cell volume given in the paper were corrected for this spontaneous decrease of liver cell volume.

Materials

[³H]Inulin and [¹⁴C]urea were from Amersham Buchler (Braunschweig, Germany). L-Lactic acid was from Roth (Karlsruhe, Germany). Insulin, glucagon, phenylephrine and vasopressin were from Sigma (Munich, Germany). Dibutyl cyclic AMP (dibutyl cAMP), ATP and adenosine were from Boehringer (Mannheim, Germany). All other chemicals were from Merck (Darmstadt, Germany).

RESULTS

Insulin, cAMP and glucagon

In line with previous studies [3–5,8], insulin (35 nM) led to cell swelling accompanied by a hepatic net K⁺ uptake, whereas glucagon (100 nM) and dibutyl cAMP (50 μM) led to cell shrinkage (Table 1). None of these hormones affected the portal perfusion pressure. Whereas insulin had no effect on liver mass, liver mass decreased under the influence of cAMP and glucagon roughly to an extent predicted by the decrease in intracellular water space (Table 1). These findings suggest that insulin may decrease the extracellular space in perfused liver, whereas glucagon and dibutyl cAMP exhibit, if at all, only minor effects on the extracellular space in perfused rat liver. Insulin, however, increased liver mass when added as well as phenylephrine (Table 1).

Insulin-induced cell swelling was recently shown to be due to an amiloride- and bumetanide-sensitive [3,5] net K⁺ accumulation

Fig. 1. Dose-response curves for insulin and glucagon effects on cellular K^+ balance in perfused rat liver

When error bars are shown, data represent means $\pm$ S.E.M. ($n = 3-15$). The effect of glucagon on cellular K^+ balance was assessed in experiments in which the influent perfusate contained insulin (2 nM). K^+ balance is positive (negative) when there is a net K^+ uptake (release). In the absence of insulin, glucagon at concentrations up to $0.1 \mu\text{M}$ gave only a slight K^+ release (less than $1 \mu\text{mol/g}$ of liver).

Fig. 2. Concentration-dependence of insulin-induced cell swelling (●) and glucagon-induced cell shrinkage (□, ■)

The effect on cell volume was determined as change in the intracellular water space (see the Materials and methods section and Table 1 legend). In the absence of hormones, the intracellular water space was $559 \pm 7 \mu\text{l/g}$ ($n = 88$). The glucagon effect on cell volume was studied in the absence (□) or presence (■) of a near-physiological insulin concentration (2 nM). Data are means $\pm$ S.E.M. ($n = 3-15$).

inside the liver cells, suggesting stimulation of Na^+/H^+ antiport and of NaCl-KCl co-transport by insulin [5,11,12]. As shown in Fig. 1, insulin-induced net K^+ uptake by the liver was half-maximal at influent insulin concentrations of about 1 nM, i.e. at a concentration found in the portal vein *in vivo* [13]. Similarly, insulin-induced cell swelling was half-maximal at influent insulin concentrations of about 1 nM (Fig. 2).

Glucagon-induced cell shrinkage was maximal at concentrations above $0.1 \mu\text{M}$ and half-maximal at about 0.1 nM (Fig. 2). Glucagon in concentrations up to 100 nM induced a net K^+ release of less than $1 \mu\text{mol/g}$ (Table 1). When, however, insulin was present at a near-physiological concentration of 2 nM, glucagon markedly stimulated K^+ release from the liver (Fig. 1). Under these conditions, net K^+ efflux was effectively modulated by glucagon at concentrations found physiologically in the portal vein, i.e. at 0.1–1 nM [13] (Fig. 1). Also, in the presence of insulin (2 nM), liver cell volume responds very sensitively to glucagon concentrations of 10–1000 pM (Fig. 2). The physiological insulin and glucagon concentrations found in the portal vein of rats were shown to be 1–2 nM and 30–80 pM respectively [13]. Accordingly, our present findings (Figs. 1 and 2) suggest that fluctuations of the portal insulin and glucagon concentrations within the physiological range may effectively modulate liver cell volume and cellular K^+ balance.

Adenosine

Infusion of adenosine ($50 \mu\text{M}$) led to a transient increase of the portal pressure by $8.3 \pm 1.1 \text{ cmH}_2\text{O}$ ($n = 8$) during the first 3 min of administration, largely owing to a stimulation of eicosanoid release by adenosine [14]. In line with previous data [14], the adenosine-induced vasoconstriction and eicosanoid formation was transient and, after a 30 min infusion period, the portal pressure was elevated by only $0.2 \pm 0.1 \text{ cmH}_2\text{O}$ ($n = 8$) above that observed before the onset of adenosine infusion. Determination of the intracellular water space under these conditions revealed a slight, but significant, cell shrinkage by adenosine (Table 1). Adenosine-induced cell shrinkage was accompanied by a net K^+ release from the liver of $4.8 \pm 0.2 \mu\text{mol/g}$ ($n = 8$) during the first 8 min of adenosine addition. Adenosine-induced cell shrinkage was also well reflected by the adenosine-induced decrease in liver mass (Table 1).

Ca^{2+} -mobilizing hormones

Vasopressin (15 nM) did not affect the portal perfusion pressure; it slightly decreased liver mass and the intracellular water space (Table 1). Vasopressin induced triphasic K^+ movements, which resulted together in a small net K^+ uptake of $1.2 \pm 0.5 \mu\text{mol/g}$ ($n = 4$). However, when vasopressin was added during constant infusion of dibutyryl cAMP ($50 \mu\text{M}$) or glucagon (100 nM), vasopressin led to a net K^+ release of $3.3 \pm 0.8 \mu\text{mol/g}$ ($n = 3$) or $2.3 \pm 0.5 \mu\text{mol/g}$ ($n = 4$) respectively, accompanied by marked cell shrinkage (Table 1). The vasopressin-induced decrease in intracellular water space largely accounted for the simultaneously observed liver mass decrease (Table 1), suggesting only a minor effect of vasopressin on the extracellular space.

Throughout a 30 min infusion period, phenylephrine ($5 \mu\text{M}$) increased the portal perfusion pressure by 4.6 ± 0.3 ($n = 7$) cmH_2O , considerably decreased the liver mass, but had no effect on cellular K^+ balance (Table 1). Simultaneously, the intracellular water space increased by about 8% (Table 1). This suggests a decrease in the extracellular space and an increase in the intracellular space by phenylephrine. Cell swelling by insulin (at a maximally effective insulin concentration of 35 nM; see Fig. 2) and by phenylephrine ($5 \mu\text{M}$) were additive (Tables 1 and 2), suggesting the involvement of different mechanisms mediating cell swelling by these compounds. In line with this suggestion,

Table 2. Effect of hormone combinations on intracellular water space

For experimental details see Table 1 legend. The concentrations of insulin, glucagon, phenylephrine, vasopressin, cAMP and ATP were 35 nM, 100 nM, 5 μ M, 35 nM, 50 μ M and 20 μ M respectively, except for *; here the insulin and glucagon concentrations were 2 nM and 100 pM respectively.

Effector	Change of intracellular water space		Liver mass change (%)
	(μ l/g)	(%)	
Insulin + phenylephrine	+91 $\pm$ 6 (4)	+20.2 $\pm$ 1.4 (4)	-14.3 $\pm$ 1.5 (4)
Insulin + glucagon	+12 $\pm$ 14 (4)	+2.8 $\pm$ 2.9 (4)	-4.6 $\pm$ 0.4 (4)
*Insulin + glucagon	-20 $\pm$ 10 (4)	-3.3 $\pm$ 1.6 (4)	-2.5 $\pm$ 0.3 (4)
Insulin + cAMP	-19 $\pm$ 10 (3)	-3.9 $\pm$ 1.7 (3)	-8.5 $\pm$ 0.3 (3)
Phenylephrine + adenosine	+1 $\pm$ 12 (4)	+0.1 $\pm$ 2.5 (4)	-20.4 $\pm$ 3.6 (4)
Phenylephrine + cAMP	-4 $\pm$ 19 (3)	-0.4 $\pm$ 4.4 (3)	-29.3 $\pm$ 1.9 (3)
Adenosine + ATP	-82 $\pm$ 13 (4)	-13.8 $\pm$ 2.3 (4)	-1.1 $\pm$ 2.1 (4)
Vasopressin + glucagon	-132 $\pm$ 24 (4)	-22.7 $\pm$ 3.0 (4)	-13.1 $\pm$ 0.4 (3)
Vasopressin + cAMP	-102 $\pm$ 5 (6)	-17.8 $\pm$ 0.3 (6)	-10.1 $\pm$ 0.7 (4)

insulin-induced net K⁺ accumulation (Table 1) was not affected in the presence of phenylephrine (5 μ M) (Table 1).

Extracellular ATP (20 μ M) is thought to increase cytosolic Ca²⁺ via an inositol 1,4,5-trisphosphate-dependent mechanism following binding to purinergic P₂ receptors [15–17]. Infusion of ATP (20 μ M) into perfused rat liver led to a transient increase in portal pressure (cf. Fig. 3 in ref. [18]). As shown recently [18], the initial pressure overshoot is due to a transient increased formation of eicosanoids from non-parenchymal liver cells, but within about 8 min the perfusion pressure reached a new steady state, which was 1.8 $\pm$ 0.2 cmH₂O (n = 6) above the control level after a 30 min period of ATP infusion. Determination of the intracellular water space under these conditions revealed cell shrinkage by extracellular ATP, whereas liver mass was not significantly altered and was even increased by extracellular ATP when adenosine was present, although ATP-induced cell shrinkage was additive to adenosine-induced shrinkage (Tables 1 and 2). This suggests that extracellular ATP decreases the intracellular space, but increases the extracellular space, in perfused rat liver. The effect of extracellular ATP on hepatic K⁺ balance was close to zero (Table 1).

Additivity of hormone effects on intracellular water space

As shown in Table 1, glucagon-induced cell shrinkage was largely unaffected when liver cells were pre-swollen by insulin. Similarly, both insulin-induced cell swelling and net K⁺ uptake were not significantly affected during phenylephrine-induced pre-swelling or ATP-induced pre-shrinkage. Cell shrinkage induced by ATP was unaffected in the presence of adenosine; the effect of both adenine derivatives on the intracellular water space was additive (Tables 1 and 2). In the presence of dibutyl cAMP or glucagon, vasopressin-induced cell shrinkage was potentiated; addition of vasopressin plus glucagon (or cAMP) proved to be the most effective cell-shrinkage device in perfused liver, whereas highest degrees of cell swelling were observed with the combined addition of insulin plus phenylephrine (Table 2). Liver mass

changes reflected the effects of vasopressin plus glucagon or cAMP on the intracellular water space (Table 2), but not the cell volume alterations induced by the other hormone combinations listed in Table 2.

DISCUSSION

Hormonal regulation of liver cell volume

The data in this paper show that hormones are effective modulators of liver cell volume. Cell volume changes under the influence of the hormonally active substances studied here are remarkably high (Tables 1 and 2). For example, cell swelling by about 20%, as is observed during combined addition of insulin and phenylephrine in normo-osmotic perfusions (Table 2), can only be mimicked by hypo-osmotic liver exposure, when extracellular osmolality is lowered by more than 80 mosmol/l [3,8]. Similarly, an increase of the extracellular osmolality from 305 mosmol/l (normo-osmotic) to 385 mosmol/l by increasing the influent NaCl concentration by 40 mmol/l decreased the intracellular water space by 14 $\pm$ 0% (n = 3), whereas addition of glucagon plus vasopressin lowered the intracellular water space in normo-osmotic perfusions by 23 $\pm$ 3% (n = 4).

The possibility that agonist-induced vasoconstriction, as observed with phenylephrine or extracellular ATP, could interfere with our technique for assessment of the intracellular water space is considered unlikely, because neither insulin-induced cell swelling nor insulin-induced net K⁺ accumulation (Table 1) was affected in the presence of phenylephrine or extracellular ATP, i.e. compounds leading to vasoconstriction. In addition, phenylephrine also led to swelling of isolated hepatocytes (C. Hallbrucker & D. Häussinger, unpublished work).

The mechanisms whereby hormones modify liver cell volume remain in part unclear, and the osmotic effects of net K⁺ movements into or out of the cell (Table 1) will be superimposed on alterations of the distribution of other osmotically active compounds. However, the present data suggest, in line with previous studies on the mechanisms of insulin-induced cell swelling [3,5], that hormonally induced ion movements across the plasma membrane at least contribute to hormonal modulation of cell volume. Theoretically, adenosine-induced cell shrinkage could be explained by cellular K⁺ depletion (Table 1). Assuming an intracellular osmolality of 305 mosmol/l, an intracellular water space of about 550 μ l/g of liver, and that K⁺ release is accompanied by an equimolar amount of osmotically active univalent anions, the amount of K⁺ released under the influence of adenosine is calculated to decrease the intracellular water space by 5.7%, i.e. by about 32 μ l/g. These calculated values would agree well with the actually measured decrease in cell volume by 6.1 $\pm$ 1.6% (n = 8), i.e. by 35 $\pm$ 9 μ l/g (Table 1). The volume effects exerted by glucagon, cAMP and extracellular ATP, however, are not explained by alterations of cellular K⁺ balance (Table 1). Glucagon and cAMP stimulate both Na⁺/K⁺-ATPase [19] and K⁺ release via K⁺ channels [20]. This could theoretically result in a net K⁺ effect close to zero, whereas the cellular loss of Na⁺ (together with an anion) leads to cell shrinkage. Also insulin activates Na⁺/K⁺-ATPase [11,12,19,21], and when glucagon or cAMP is added as well as insulin, their effect on K⁺ channels will be unmasked. Such phenomena could explain why marked net K⁺ efflux from the liver is observed with glucagon and cAMP in the presence, but not in the absence, of insulin (Table 1; see also Fig. 1). Similar considerations could apply for the stimulation of K⁺ efflux by cAMP in the presence of phenylephrine or vasopressin and for stimulation of K⁺ efflux by vasopressin in the presence of glucagon or cAMP (Table 1).

From the simultaneous determination of changes of intracellular water space and liver mass under the influence of hormones, some conclusions can be drawn on the hormones' effect on the extracellular space in perfused rat liver. Liver mass changes were sufficiently explained by the alterations of cell volume elicited by glucagon, cAMP and adenosine, suggesting minor effects on the extracellular space. However, the data in Table 1 would be compatible with the view that the extracellular space decreased under the influence of insulin, vasopressin and phenylephrine, whereas it increased with extracellular ATP.

Interestingly, Ca^{2+} -mobilizing agents, such as vasopressin, phenylephrine and extracellular ATP, exhibit differential effects on the intracellular water space in perfused rat liver, suggesting the involvement of mechanisms distinct from elevation of cytosolic free Ca^{2+} .

The effects of individual hormones on cell volume were largely additive (compare Tables 1 and 2). Phenylephrine- and insulin-induced cell swelling was counteracted by adenosine, cAMP and glucagon. Likewise, cell shrinkage by glucagon, adenosine, ATP and cAMP was increased by further addition of a second hormone known to shrink the cells.

Physiological relevance

The portal concentrations of insulin and glucagon were shown to be 1.2–2.2 nM and 40–80 pM respectively [13]. These concentrations were sufficient to induce marked cell volume alterations in perfused rat liver (Table 1, Figs. 1 and 2). As suggested by the data in Figs. 1 and 2, fluctuations of the portal insulin and glucagon concentrations during a starvation/feeding cycle [13] are expected to modulate cell volume efficiently. Recent studies have shown that liver cell volume changes are potent modulators of metabolic liver function. Cell swelling inhibits proteolysis [1–5] and glycogenolysis [22], and stimulates amino acid uptake, degradation [23,24] and glycogen synthesis [25], whereas cell shrinkage stimulates proteolysis [3–5], glycogenolysis and glycolysis [22], and inhibits glycogen [25] and protein synthesis (B. Stoll & D. Häussinger, unpublished work). Accordingly, cell swelling may be seen as an anabolic signal [3]. In line with this, the known anabolic properties of insulin are accompanied by cell swelling, whereas the 'catabolic hormone' glucagon shrinks the cells. Cell volume changes under the influence of insulin and glucagon have recently been shown to be a major determinant for the effects of both hormones on hepatic proteolysis [3–5], suggesting that hormone-induced alterations of cell volume may act as a 'second messenger' mediating hormone effects [3–5]. In view of the data presented here, one might speculate that cell

volume changes are of physiological relevance for hormone actions and are involved in synergistic and antagonistic hormone interactions.

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