Biochem. J. (1992) 287, 217–222 (Printed in Great Britain)

Liver cell volume and protein synthesis

Barbara STOLL,* Wolfgang GEROK,* Florian LANG† and Dieter HÄUSSINGER*‡
*Medizinische Universitätsklinik, Hugstetterstrasse 55, D-7800 Freiburg, Federal Republic of Germany, and †Physiologisches Institut der Universität, Fritz Pregl Strasse 3, A-6010 Innsbruck, Austria

Protein synthesis in isolated rat hepatocytes was determined from the incorporation of [^{3}H]leucine (4 mM) into acid-precipitable material in the presence of amino acids at twice their physiological concentration. Protein synthesis increased linearly with time and incubated cell protein, and was inhibited by cycloheximide by more than 95%. In normo-osmotic incubations containing amino acids at twice the physiological concentration the rate of [^{3}H]leucine incorporation was 5.8 ± 0.2 nmol/h per mg of cell protein ($n = 26$). Hyperosmotic cell shrinkage due to addition of 60 mM-NaCl or 120 mM-raffinose inhibited [^{3}H]leucine incorporation into acid-precipitable material by 60 and 74% respectively, whereas hypo-osmotic cell swelling was ineffective. Inhibition of protein synthesis by adding 120 mM-raffinose was largely counteracted by simultaneous lowering of the NaCl concentration by 60 mM. Glutamine (10 mM) had no effect on protein synthesis in normo-osmotic incubations (320 mosM), but stimulated protein synthesis in hyperosmotically (440 mosM) pre-shrunken cells almost to rates found in normo-osmotic (320 mosM) control incubations. Cyclic AMP and vasopressin inhibited protein synthesis by 23% and 8% respectively, whereas insulin and phenylephrine were ineffective. However, inhibition of protein synthesis by cyclic AMP was about twice as strong in the presence of vasopressin or phenylephrine. When protein synthesis was preinhibited by cyclic AMP, [^{3}H]leucine incorporation was stimulated by glutamine (10 mM), insulin or hypo-osmotic exposure. There was a close relationship between the inhibition of protein synthesis and the extent of hepatocyte shrinkage induced by the above-mentioned effectors, suggesting a role of cell volume in the regulation of hepatic protein synthesis.

INTRODUCTION

Cell volume changes were recently recognized as potent modulators of metabolic liver cell function (for review see Häussinger & Lang, 1991). For example, cell swelling inhibits, whereas cell shrinkage stimulates, hepatic proteolysis (Häussinger *et al.*, 1991; vom Dahl *et al.*, 1991*a*; Hallbrucker *et al.*, 1991*a*,*b*). There was a close relationship between the extent of cell volume changes and the effect on proteolysis regardless of whether cell volume was modified by aniso-osmotic exposure, amino acids or hormones such as insulin or glucagon, suggesting that hormone- and amino acid-induced cell volume changes are a major determinant for proteolysis control in liver (Häussinger *et al.*, 1991). Apparently cell volume changes under the influence of glucagon or insulin are responsible for the hormone effects on proteolysis, and cell volume modulation by these hormones was suggested to act as another cellular messenger of hormone action (Häussinger *et al.*, 1991; vom Dahl *et al.*, 1991*a*,*b*; Hallbrucker *et al.*, 1991*b*). Nothing is known about the interaction between cell volume and hepatic protein synthesis; however, inhibition of protein synthesis after hyperosmotic exposure was observed in cultured mouse myeloma (MPC 11) cells (Nuss & Koch, 1976; Kruppa & Clemens, 1984) or HeLa cells (Saborio *et al.*, 1974). This inhibitory effect was suggested to occur at the level of polypeptide-chain initiation. Amino acids are known to stimulate hepatic protein synthesis (Munro, 1968; Jefferson & Korner, 1969; Pronczuk *et al.*, 1970; Fausto, 1972; Woodside *et al.*, 1974; Seglen, 1978; Seglen & Solheim, 1978), whereas glucagon, vasopressin and ammonia inhibit (Woodside *et al.*, 1974; Seglen, 1978; Kimball & Jefferson, 1990). Insulin led to a slight, but not significant, stimulation of protein synthesis (Mortimore & Mondon, 1970). Both amino acids and hormones have been recognized to alter liver cell volume (Bakker-Grunwald, 1983; Kristensen & Folke, 1984; Baquet *et al.*, 1990; Häussinger *et al.*, 1990*a*, 1991; Wettstein *et al.*, 1990; vom Dahl *et al.*, 1991*a*,*b*; Hallbrucker *et al.*, 1991*a*,*b*,*c*). Accordingly, the present study was undertaken in order to gain insight into a possible role of cell volume in the regulation of hepatic protein synthesis.

MATERIALS AND METHODS

Isolation and incubation of isolated rat hepatocytes

Isolated rat hepatocytes were prepared as described by Hansen *et al.* (1986) from livers of male Wistar rats, fed *ad libitum* on stock diet (Altromin), after collagenase perfusion. After washing, the hepatocytes were incubated at 37 °C in a Krebs–Henseleit buffered saline (final volume 4 ml) supplemented with 6 mM-glucose and an amino acid mixture containing [^{3}H]leucine (4 mM) and other amino acids at concentrations about twice those found *in vivo*, i.e. alanine (0.8 mM), proline (0.2 mM), serine (0.4 mM), glycine (0.6 mM), aspartate (0.06 mM), asparagine (0.14 mM), valine (0.36 mM), isoleucine (0.14 mM), methionine (0.06 mM), tyrosine (0.08 mM), phenylalanine (0.1 mM), histidine (0.1 mM), arginine (0.1 mM), threonine (0.26 mM), lysine (0.5 mM), tryptophan (0.36 mM), cysteine (0.12 mM), glutamate (0.2 mM) and glutamine (1 mM). The incubations were continuously gassed with water-vapour-saturated O_2/CO_2 (19:1). Viability of hepatocytes as assessed by Trypan Blue exclusion at the end of the 2 h incubations was about 90%. The protein content in the incubations was 3–6 mg/ml, except in the experiments given in Table 1, where it was 1.5–9 mg/ml. The incubation volume was 4 ml. Dibutyryl-cAMP, vasopressin and phenylephrine were added at the beginning of the incubations in amounts yielding final concentrations of 50 μM, 100 nM and 5 μM. To maintain maximally active hormone concentrations throughout the 2 h incubation period, these additions were repeated every 20–30 min. Insulin was added at a concentration of 1 μM. In control incubations the osmolarity was 320 mosM; aniso-osmotic conditions were achieved by adding or removing appropriate amounts of NaCl, if not otherwise indicated. Hyperosmotic

Abbreviation used: cAMP, cyclic AMP.
‡ To whom correspondence should be addressed.

exposure (440 mosM) of the hepatocytes for 50 min did not change the cellular ATP content compared with normo-osmotic controls; however, a 15% decrease in ATP was observed when the extracellular osmolarity was increased to 620 mosM.

Determination of [^{3}H]leucine incorporation into protein

[^{3}H]Leucine (125 kBq/ml) at a concentration of 4 mM was present in all incubations. During the 2 h incubation period, samples (0.1 ml) were taken at 20 min intervals (if not indicated otherwise) for immediate protein precipitation by adding 0.15 ml of trichloroacetic acid (1 M) and subsequent centrifugation. The precipitate was washed three times by resuspension and centrifugation, with 250 μl of trichloroacetic acid (0.6 M) in each washing step. The pellet obtained after the last washing step was assayed for ^{3}H radioactivity. Radioactivity recovered here was seen to represent [^{3}H]leucine incorporation into newly synthesized protein. For validation, [^{3}H]leucine incorporation into protein was inhibited by over 95% in the presence of cycloheximide (5 μM). For each time point [^{3}H]leucine incorporation into acid-precipitable material was calculated on the basis of the specific radioactivity of leucine at the beginning of the incubation and was related to the hepatocyte protein content in the incubations. Proteins was determined by the biuret reaction, described in Bergmeyer (1974).

Determination of hepatocyte volume

The intracellular water space of isolated rat hepatocytes was determined as described by Quinlan *et al.* (1983), with some modifications; i.e. [^{3}H]inulin was used as extracellular marker and [^{14}C]urea as marker for intra- plus extra-cellular space, as was previously done and validated in studies on the intact liver (vom Dahl *et al.*, 1991*c*). In brief, hepatocytes were incubated in the above-mentioned medium for 45 min, and then [^{3}H]inulin (20 kBq) and [^{14}C]urea (20 kBq) were added to the incubations. Then 5 min later a 2.5 ml portion of the cell suspension was gently centrifuged (170 *g*) for 30 s. ^{3}H and ^{14}C radioactivity was determined by liquid-scintillation spectrometry in the supernatant and the pellet respectively. The intracellular water space was calculated from the ^{14}C radioactivity found in the pellet after correction for residual extracellular water in the pellet by using the specific radioactivities in the supernatant. Data on intracellular water space ('cell volume') are expressed as μl/mg of cell protein.

Expression of results

If not indicated otherwise, the rate of [^{3}H]leucine incorporation ('protein synthesis') was calculated from the amounts of [^{3}H]leucine incorporated into acid-precipitable material at 20, 40, 60 and 80 min of the individual incubation, by using linear regression analysis. The rates of protein synthesis under the influence of hormones, glutamine or aniso-osmoticity were compared with rates found in normo-osmotic control incubations from the same cell preparation and were expressed as percentages thereof. Accordingly, comparisons were made among different incubations obtained from the same cell preparation. Data obtained from different cell preparations were statistically treated; they are given as means $\pm$ S.E.M. (n = number of cell preparations). When cell volume changes were related to protein synthesis, cell volume was determined after 50 min of incubation and the rate of protein synthesis was measured between 40 and 60 min of incubation.

Materials

[^{3}H]Leucine, [^{3}H]inulin and [^{14}C]urea were from Amersham Buchler (Frankfurt, Germany). Dibutyryl-cAMP, vasopressin, phenylephrine and insulin (bovine pancreas) were from Sigma (Munich, Germany). Collagenase was from Biochrom (Berlin, Germany). All other chemicals were from Merck, Darmstadt, Germany.

RESULTS

Effect of aniso-osmotic exposure and glutamine on protein synthesis in isolated rat liver cells

Protein synthesis in isolated rat hepatocyte suspensions was assessed by measuring the incorporation of added [^{3}H]leucine into acid-precipitable material. Amino acids at twice the physiological concentrations (for composition see the Materials and methods section) were present in order to provide substrate for protein synthesis. [^{3}H]Leucine was present in excess (4 mM), to minimize label dilution by leucine derived from proteolysis during the 2 h incubation period. Under these conditions, ^{3}H incorporation into acid-precipitable material increased linearly over a 140 min incubation period (Figs. 1–3). [^{3}H]Leucine incorporation was inhibited by over 95% in the presence of cycloheximide (5 μM) (Fig. 1), suggesting that ^{3}H incorporation into acid-precipitable material was due to protein synthesis. As shown in Table 1, the rate of [^{3}H]leucine incorporation into acid-precipitable material was independent of the incubated amount of liver cell protein in the range 2–9 mg of cell protein/ml. From this, we assume that the concentration of leucine present in the incubation is sufficiently high to eliminate effects on proteolysis.

As shown in Fig. 1, in hyperosmotic incubations, protein synthesis was inhibited by about 60% when the osmolarity in the incubation medium was raised to 440 mosM by addition of

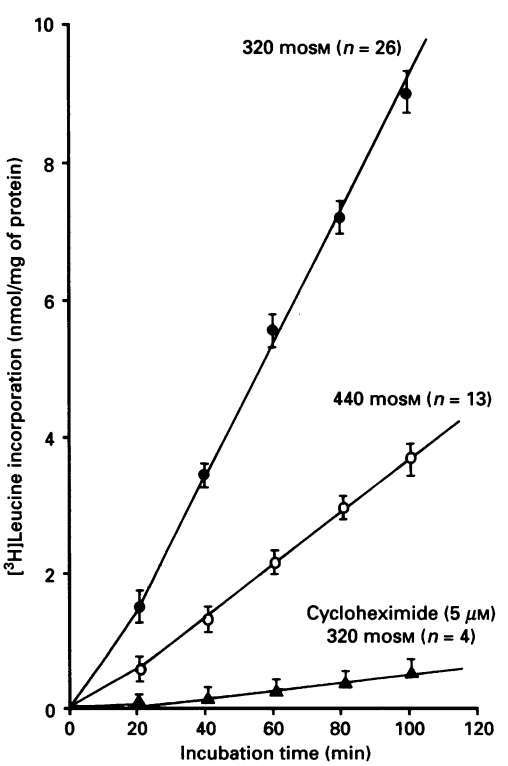

Fig. 1. Effect of hyperosmotic exposure and cycloheximide (5 μM) on [^{3}H]leucine incorporation into acid-precipitable material by isolated rat hepatocytes

For experimental details see the Materials and methods section. Hyperosmotic (440 mosM) exposure was performed by increasing the NaCl concentration in the incubation medium by 60 mM. Results are given as means $\pm$ S.E.M. and are from 4–26 different cell preparations.

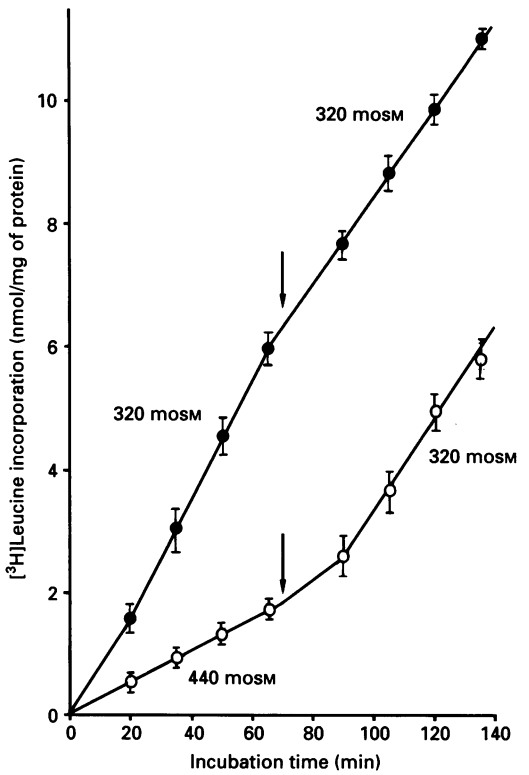

Fig. 2. Reversal of hyperosmoticity-induced inhibition of protein synthesis by re-exposing isolated rat hepatocytes to normo-osmotic media

Isolated rat hepatocytes were incubated for 65 min in normo-osmotic (320 mosM; ●) control or hyperosmotic (440 mosM; ○) medium (4 ml/incubation). Hyperosmoticity was achieved by addition of 60 mM-NaCl. After removal of 0.1 ml samples at 0, 20, 35, 50 and 65 min from the incubations, at 68 min (arrows) 3.5 ml of prewarmed and pre-gassed normo-osmotic (320 mosM) control medium was added to the normo-osmotic incubation, whereas 3.5 ml of a hypo-osmotic (200 mosM owing to removal of 60 mM-NaCl) medium was added to the hyperosmotic incubation (○). Thus normo-osmotic conditions (320 mosM) were created in both types of incubation from 68 to 140 min of incubation. Restoration of normo-osmoticity in hyperosmotic incubations fully abolishes the inhibition of protein synthesis observed during the first 65 min. Data are given as means ± S.E.M. (3 different cell preparations).

NaCl (60 mM). Restoration of normo-osmotic conditions restored the rate of [^{3}H]leucine incorporation into acid-precipitable material to control values (Fig. 2). This reversibility within the individual incubation indicates that inhibition of [^{3}H]leucine incorporation during hyperosmotic (440 mosM) exposure was not due to loss of cell viability. Hyperosmotic cell shrinkage was recently shown to stimulate proteolysis, when the pathway is preinhibited by amino acids (Hallbrucker *et al.*, 1991*a*; vom Dahl *et al.*, 1991*a*). Thus the hyperosmoticity-induced decrease in [^{3}H]leucine incorporation into acid-precipitable material depicted in Figs. 1 and 2 could result from a decrease in the specific radioactivity of added [^{3}H]leucine by unlabelled leucine derived from proteolysis. The extent of such a label dilution of added leucine is expected to increase with the amount of hepatocytes present in the incubation. As shown in Table 1, during hyperosmotic exposure also the rate of [^{3}H]leucine incorporation into acid-precipitable material was independent of the amount of liver cell protein present in the incubations. This indicates that label dilution of added [^{3}H]leucine by unlabelled leucine derived from proteolysis is also negligible during hyperosmotic exposure. Thus inhibition of [^{3}H]incorporation into acid-precipitable material during hyperosmotic cell shrink-

Table 1. Dependence of [^{3}H]leucine incorporation into liver protein on the amount of incubated hepatocyte protein under normo-osmotic (320 mosM) and hyperosmotic (400 mosM) conditions

Hepatocytes were incubated as described in the Materials and methods section with a medium containing amino acids at a concentration twice that found *in vivo*, except for [^{3}H]leucine (4 mM). In normotonic incubations, the osmolarity of the medium was 320 mosM (i.e. 305 mosM in the amino acid- and glucose-free medium); hyperosmotic (440 mosM) incubations were performed by addition of 60 mM-NaCl. Data are given as nmol of [^{3}H]leucine incorporated/60 min per mg of liver protein present in the incubation and represent means ± S.E.M. for the numbers of different cell preparations shown in parentheses. In the individual incubation, the rate of protein synthesis was determined by linear-regression analysis of ^{3}H label recovered in acid-precipitable material at 20, 40, 60 and 80 min of incubation. In all experiments [^{3}H]leucine incorporation was linear with time between 20 and 80 min.

Condition	Hepatocyte protein incubated (mg/ml)	[^{3}H]Leucine incorporation (nmol/60 min per mg of protein)
Normo-osmotic	2.07 ± 0.16 (4)	4.78 ± 0.27 (4)
	3.60 ± 0.22 (4)	5.04 ± 0.36 (4)
	5.20 ± 0.23 (5)	4.93 ± 0.41 (5)
	8.56 ± 0.44 (4)	5.15 ± 0.46 (4)
Hyperosmotic	2.13 ± 0.15 (4)	1.73 ± 0.18 (4)
	3.53 ± 0.22 (4)	2.12 ± 0.30 (4)
	5.12 ± 0.24 (5)	2.04 ± 0.28 (5)
	7.94 ± 0.59 (3)	2.18 ± 0.28 (3)

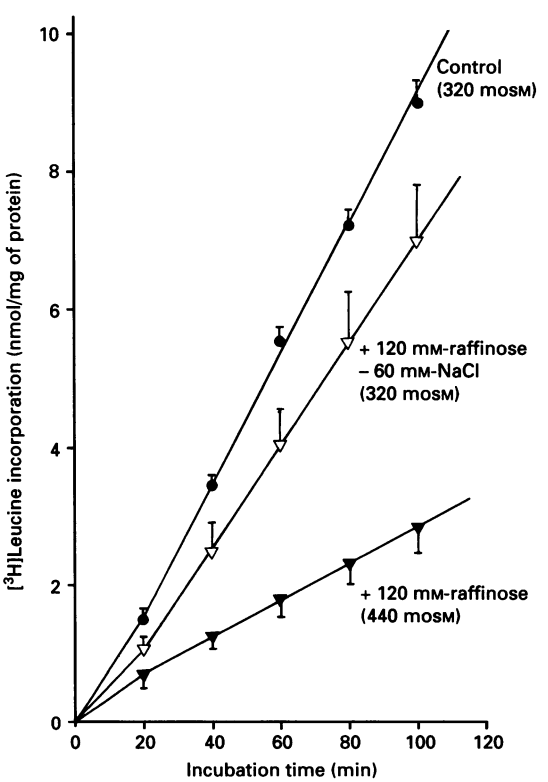

Fig. 3. Effect of raffinose on protein synthesis

Hepatocytes were incubated in normo-osmotic control media (320 mosM) (●). Hyperosmotic (440 mosM) conditions were achieved by adding 120 mM-raffinose (▼). Inhibition of protein synthesis by adding raffinose is largely abolished when simultaneously normo-osmoticity (320 mosM) is maintained by lowering the NaCl concentration by 60 mM (▽). For further details see the Materials and methods section. Data are given as means ± S.E.M. and are from 4–26 different cell preparations.

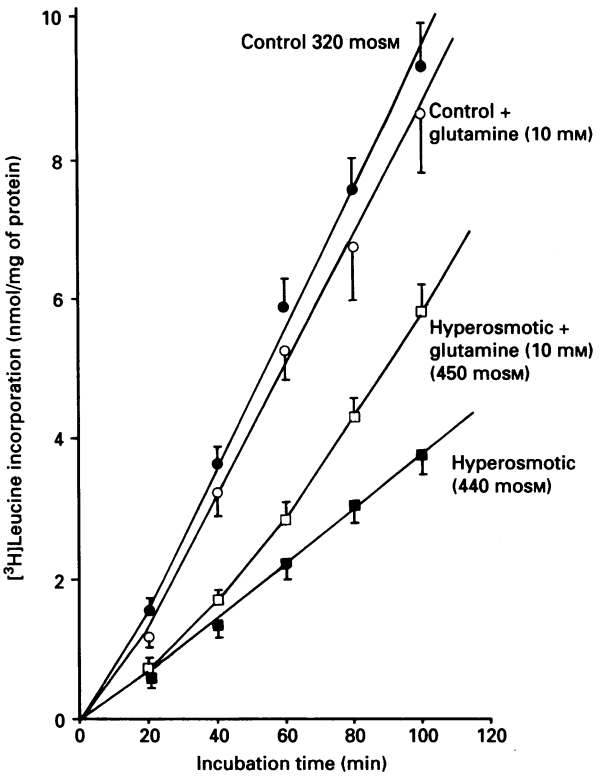

Fig. 4. Effect of glutamine (10 mM) on protein synthesis in isolated rat hepatocytes in normo-osmotic (320 mosM) or hyperosmotic (440 mosM) incubations

Hepatocytes were incubated in normo-osmotic control media (320 mosM) with (○) or without (●) glutamine (10 mM). Hyperosmotic (440 mosM) conditions were achieved by adding 60 mM-NaCl (■). Further addition of glutamine (10 mM) has no significant effect on protein synthesis in normo-osmotic incubations, but stimulates protein synthesis in hyperosmotic incubations (□). Data are given as means±S.E.M. and are from 5–7 different cell preparations.

Table 2. Effect of hormones on [^{3}H]leucine incorporation into liver protein in isolated rat hepatocytes

Hepatocytes were incubated as described in the Materials and methods section with a medium containing amino acids at a concentration twice that found *in vivo* plus [^{3}H]leucine (4 mM). In normo-osmotic incubations, the osmolarity of the medium was 320 mosM; hypo- and hyper-osmotic incubations were performed by addition or withdrawal of 60 mM-NaCl. Glutamine was added at a concentration of 10 mM at the beginning of the incubations. Hormones were added as described in the Materials and methods section. In the individual incubation, the rate of protein synthesis was determined by linear-regression analysis of ^{3}H label recovered in acid-precipitable material at 20, 40, 60 and 80 min of the incubation. For all conditions given, [^{3}H]leucine incorporation was linear between 20 and 80 min of incubation. In normo-osmotic control experiments the rate of [^{3}H]leucine incorporation was 5.77±0.15 nmol/60 min per mg of protein (n = 26). Data obtained in normo-osmotic incubations (control) were set to 100% in the individual cell preparation, and the effects of aniso-osmoticity, hormones and glutamine are presented as percentages thereof. Data are given as means±S.E.M. for the numbers of different cell preparations shown in parentheses. Negative values indicate stimulation of protein synthesis.

Condition	Inhibition of protein synthesis (%)
Vasopressin	8±3 (4)
Insulin	−6±2 (5)
Phenylephrine	−3±1 (5)
Hyperosmotic	59±2 (13)
Dibutyryl-cAMP	23±2 (9)
+glutamine	−2±2 (3)
+hypo-osmotic	12±2 (6)
+insulin	10±2 (7)
+hyperosmotic	74±3 (3)
Dibutyryl-cAMP+vasopressin	43±3 (14)
+glutamine	27±6 (7)
+hypo-osmotic	33±4 (10)
+insulin	37±2 (5)
+hyperosmotic	83±4 (3)
Dibutyryl-cAMP+phenylephrine	38±2 (6)

age is assumed to reflect inhibition of protein synthesis. This view is also augmented by the linearity of [^{3}H]leucine incorporation with time (Figs. 1 and 2), which would not be expected if the specific radioactivity of leucine changed during the incubation period.

Protein synthesis was also inhibited when the extracellular osmolarity was increased by addition of raffinose (120 mM) (Fig. 3), indicating that inhibition of protein synthesis is due to the osmolarity increase, but not to the change of extracellular Na^+ or Cl^- activity. In line with this, inhibition of protein synthesis by adding 120 mM-raffinose was largely prevented when normo-osmoticity was preserved by simultaneous lowering of the NaCl concentration by 60 mM (Fig. 3).

On the other hand, hypo-osmotic exposure by lowering the NaCl concentration in the incubation medium by 60 mM had no effect on protein synthesis [when the normo-osmotic control was set to 100%, the rate of protein synthesis in hypo-osmotic incubations was 99±3% (n = 9)]. Also, addition of glutamine (10 mM), which induces liver cell swelling, was ineffective (Fig. 4). However, glutamine markedly stimulated protein synthesis in hyperosmotically pre-shrunken cells (Fig. 4). As shown in Fig. 4, under these conditions linear rates of protein synthesis were reached after about 50 min, probably owing to progressive cell swelling resulting from a time-dependent intracellular accumulation of metabolites derived from glutamine. From the data for protein synthesis from the 60–100 min of incubation in Fig. 4, rates of [^{3}H]leucine incorporation of 5.16±0.24 (n = 7), 2.34±0.09 (n = 5) and 4.46±0.029 (n = 5) nmol/60 min per mg of protein for the normo-osmotic, hyperosmotic and hyper-osmotic plus glutamine incubations are calculated respectively. Thus inhibition of protein synthesis during hyperosmotic cell shrinkage is largely overcome by glutamine-induced cell swelling.

Effects of dibutyryl-cAMP, vasopressin, phenylephrine and insulin on protein synthesis

Hormones are known to alter liver cell volume (Häussinger *et al.*, 1991; Hallbrucker *et al.*, 1991*b*,*c*; vom Dahl *et al.*, 1991*a*,*b*) and to affect protein synthesis (Mortimore & Mondon, 1970; Woodside *et al.*, 1974; Kimball & Jefferson, 1990). Accordingly, we addressed the question whether cell volume changes are involved in the hormonal regulation of protein synthesis. With all hormones tested, [^{3}H]leucine incorporation into acid-precipitable material rose linearly between 20 and 100 min of incubation. Phenylephrine and insulin had practically no effect on protein synthesis (Table 2); these hormones were recently shown to induce cell swelling (Häussinger *et al.*, 1991; vom Dahl *et al.*, 1991*b*; Hallbrucker *et al.*, 1991*c*). On the other hand, dibutyryl-cAMP and vasopressin, which shrink hepatocytes (Hallbrucker *et al.*, 1991*c*; vom Dahl *et al.*, 1991*b*), inhibited protein synthesis (Table 2). Inhibition of protein synthesis by dibutyryl-cAMP was counteracted by hypo-osmotic exposure, glutamine or insulin (Table 2), i.e. conditions known to swell

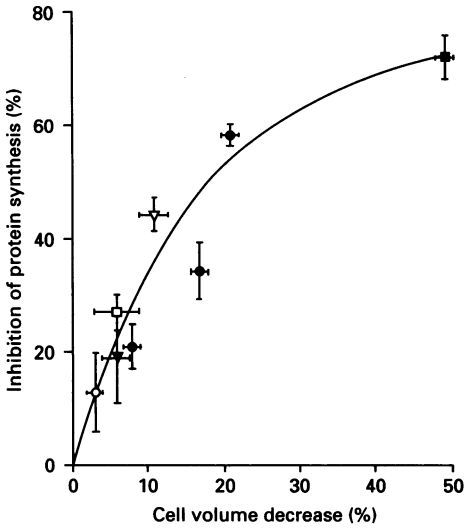

Fig. 5. Relationship between liver cell volume and protein synthesis

Protein synthesis was measured in isolated rat hepatocyte suspensions between 40 and 60 min of incubation; the rate of protein synthesis in normo-osmotic control incubations was set to 100% and the effects of aniso-osmoticity, raffinose, hyperosmoticity plus glutamine, cAMP and vasopressin are expressed as percentage thereof. Cell volume was determined after 50 min of incubation. In the absence of effectors on cell volume (control), cell volume was set to 100% and the effector-induced volume changes are expressed as percentage thereof. Symbols: ●, hyperosmotic (360, 400 and 440 mosM induced by addition of NaCl); ■, hyperosmotic (440 mosM induced by addition of 120 mM-raffinose); ▼, hyperosmotic (440 mosM by addition of NaCl) plus glutamine (10 mM); □, dibutyryl-cAMP; ▽, dibutyryl-cAMP plus vasopressin; ○, vasopressin.

hepatocytes (Häussinger *et al.*, 1991; vom Dahl *et al.*, 1991*b*; Hallbrucker *et al.*, 1991*c*). Inhibition of protein synthesis by vasopressin and dibutyryl-cAMP was higher than expected from simple additivity (Table 2). Similar observations were made recently with respect to cAMP- and vasopressin-induced cell shrinkage in perfused rat liver (vom Dahl *et al.*, 1991*b*). Also, when protein synthesis was pre-inhibited by cyclic AMP plus vasopressin, conditions known to swell the cells, such as addition of glutamine (10 mM), hypo-osmotic exposure or insulin, stimulated protein synthesis, whereas further cell shrinkage by hyperosmotic exposure aggravated the inhibition of protein synthesis (Table 2).

Relation to cell volume

In a separate series of experiments, the intracellular water space of the hepatocytes in suspensions was determined after 50 min of incubation and related to the rate of protein synthesis found between 40 and 60 min of incubation (Fig. 5). The intracellular water space ('cell volume') of the hepatocytes was found to be 2.19 ± 0.04 μl/mg of protein ($n = 24$), when incubated under normo-osmotic conditions with the standard medium containing glucose (5 mM) and amino acids at twice their physiological concentrations. This value compares with a cell volume determined in normo-osmotic incubations without amino acid addition of 2.00 ± 0.08 μl/mg of protein ($n = 5$), indicating that the presence of amino acids already led to significant cell swelling, as was also found for liver cells in the intact organ (Wettstein *et al.*, 1990). Volume changes of isolated rat hepatocytes under different experimental conditions were of similar magnitude to those observed in isolated perfused rat liver (Table 3).

Table 3. Comparison between cell volume effects in isolated rat hepatocytes and isolated perfused rat liver

Cell volume was determined as intracellular water space, which under normo-osmotic control conditions was set to 100%. In normo-osmotic controls the intracellular water space was 559 ± 7 μl/g wet wt. ($n = 88$) in perfused liver and 2.19 ± 0.04 μl/mg of protein in isolated hepatocyte suspensions. Volume changes under the influence of effectors are expressed as percentages thereof. Data on cell volume changes observed in perfused rat liver are taken from previous studies (vom Dahl *et al.*, 1991*b*,*c*), where the influent perfusate contained lactate (2.1 mM) and pyruvate (0.3 mM). On the other hand, cell volume changes in isolated rat hepatocytes were determined with amino acids (twice the physiological concentration). Aniso-osmotic exposure was performed by addition or removal of corresponding amounts of NaCl. Data are given by means ± S.E.M. for the numbers of experiments in parentheses.

Condition	Change in intracellular water space (%)	
	Isolated hepatocytes	Perfused liver
Hypo-osmotic (−120 mosM)	+22±1 (6)	+21±1 (6)
Hyperosmotic (+40 mosM)	−8±1 (3)	−5±1 (3)
(+80 mosM)	−17±1 (9)	−12±1 (3)
Vasopressin	−3±1 (6)	−2±2 (4)
cAMP	−6±3 (6)	−9±1 (3)
cAMP + vasopressin	−11±2 (6)	−16±1 (3)

As shown in Fig. 5, there was a remarkable relationship between the extent of hepatocyte shrinkage and the simultaneously occurring inhibition of protein synthesis. This relationship was preserved regardless of how cell shrinkage was induced, suggesting that cell shrinkage under the influence of cAMP, vasopressin or aniso-osmotic exposure is one mechanism leading to inhibition of protein synthesis.

DISCUSSION

Cell volume and protein synthesis

The rates of protein synthesis reported here for isolated rat hepatocytes are well within the range reported in the literature (Woodside *et al.*, 1974; Seglen, 1978; Everson *et al.*, 1989). The 2-fold higher rates of protein synthesis found by Everson *et al.* (1989) compared with the data given here are probably explained by the 5-fold higher amino acid concentrations employed by those authors and the well-known dependence of protein synthesis in isolated hepatocytes on the amino acid concentration in the incubation (Everson *et al.*, 1989; Seglen, 1978). However, rates of protein synthesis in isolated rat hepatocytes are consistently lower than those found in rat liver *in vivo* (Schreiber *et al.*, 1971; Garlick *et al.*, 1973; McNurlan *et al.*, 1979).

The data in the present paper show that protein synthesis is inhibited after hyperosmotic liver cell shrinkage (Figs. 1, 2, 5, Table 1); this was also described for other cell types (Saborio *et al.*, 1974; Nuss & Koch, 1976; Kruppa & Clemens, 1984). This hyperosmoticity-induced inhibition is counteracted by glutamine, which is known to induce cell swelling. On the other hand, cell swelling by hypo-osmotic exposure or by glutamine (Häussinger *et al.*, 1991; Hallbrucker *et al.*, 1991*a*) did not stimulate protein synthesis under control conditions. Apparently, protein synthesis under control incubations is already maximally active, thereby explaining the lack of effect of hypo-osmotic exposure or glutamine on protein synthesis. Here it should be kept in mind that the presence of amino acids at twice their physiological

concentration already leads to a marked pre-swelling of hepatocytes, owing to cumulative amino acid uptake (Wettstein *et al.*, 1990), and further cell swelling by hypo-osmotic exposure may not stimulate protein synthesis further.

Protein synthesis was also affected by hormones (Table 2), in line with previous reports (Mortimore & Mondon, 1970; Woodside *et al.*, 1974; Kimball & Jefferson, 1990). Again, remarkable parallels were found between hormone effects on protein synthesis and on cell volume. Protein synthesis was inhibited by dibutyryl-cAMP, which shrinks hepatocytes. Both cAMP-induced cell shrinkage (vom Dahl *et al.*, 1991*b*) and inhibition of protein synthesis are potentiated by vasopressin (Table 2). Like hypo-osmotic exposure, cell swelling induced by insulin, phenylephrine (Häussinger *et al.*, 1991; Hallbrucker *et al.*, 1991*b*; vom Dahl *et al.*, 1991*a*,*b*) or glutamine had no effect on protein synthesis under control conditions, but these swelling manoeuvres increased protein synthesis in cells that had been pre-shrunken by cAMP or hyperosmotic exposure (Table 2, Fig. 5). These findings suggest that cell volume changes might be involved in the hormonal regulation of protein synthesis (Fig. 5). It should be noted that [^{3}H]leucine incorporation will pick up overall protein synthesis, whereas cell volume changes may affect the synthesis of individual proteins non-uniformly, as suggested by the recent observation that hypo-osmotic cell swelling increases the tissue mRNA levels for β-actin, but not for tyrosine aminotransferase or albumin (Schulz *et al.*, 1991).

Although our data (Fig. 5) suggest that cell volume may be involved in the regulation of hepatic protein synthesis, it should be emphasized that cell volume may only be one factor among many regulating protein synthesis, and not all findings reported here are consistent with a role of cell volume in regulating overall protein synthesis. For example, phenylephrine counteracts cAMP-induced cell shrinkage (vom Dahl *et al.*, 1991*b*), but further inhibits protein synthesis (Table 2). Likewise, when protein synthesis is pre-inhibited by cAMP plus vasopressin, manoeuvres known to induce cell swelling (such as hypo-osmotic exposure, glutamine or insulin) led to only a small, albeit significant, stimulation of protein synthesis (Table 2). Here, apparently, other mechanisms come into play. Thus the interaction between cell volume changes and other mechanisms involved in the hormonal regulation of protein synthesis remains to be established.

Possible physiological significance

The present data suggest that cell shrinkage inhibits, whereas cell swelling can stimulate, overall protein synthesis (Fig. 5). Just the opposite pattern is found with respect to hepatic proteolysis (Häussinger *et al.*, 1991; vom Dahl *et al.*, 1991*a*; Hallbrucker *et al.*, 1991*a*,*b*). Accordingly, with respect to protein turnover, cell swelling may represent an 'anabolic signal' whereas cell shrinkage is catabolic. These considerations, however, apply only to overall rates of protein synthesis and breakdown, and, when individual proteins are considered, the situation may be quite different. In rat skeletal muscle glutamine was shown to stimulate protein synthesis and to inhibit proteolysis (MacLennan *et al.*, 1987, 1988). The muscle glutamine transporter is known to build up high intra-/extra-cellular glutamine concentration gradients (Rennie *et al.*, 1986) and is expected to modulate cell volume accordingly. One is tempted to speculate that the protein-anabolic effect of glutamine in skeletal muscle (for review see Sugden & Fuller, 1991) also involves cell volume changes, as shown here and in previous studies on liver protein turnover (Häussinger *et al.*, 1990*b*; Hallbrucker *et al.*, 1991*b*).

Received 28 February 1992/16 April 1992; accepted 28 April 1992

We are grateful to Mrs. Petra Ochsenfarth for help in preparing the illustrations. This work was supported by Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 154 and the Gottfried Wilhelm Leibniz-Programm), the Fonds der Chemischen Industrie and the Schilling Stiftung.

REFERENCES

Bakker-Grunwald, T. (1983) Biochim. Biophys. Acta **731**, 239–242
Baquet, A., Hue, L., Meijer, A. J., von Woerkom, G. M. & Plomp, P. J. A. M. (1990) J. Biol. Chem. **265**, 955–959
Bergmeyer, H. U. (ed.) (1974) Methoden der Enzymatischen Analyse, Verlag Chemie, Weinheim
Everson, W. V., Flaim, K. E., Susco, D. M., Kimball, S. R. & Jefferson, L. S. (1989) Am. J. Physiol. **256**, C18–C27
Fausto, N. (1972) Biochim. Biophys. Acta **281**, 543–553
Garlick, P. J., Millward, D. J. & James, W. P. T. (1973) Biochem. J. **136**, 935–945
Häussinger, D. & Lang, F. (1991) Biochim. Biophys. Acta **1071**, 331–350
Häussinger, D., Lang, D., Bauers, K. & Gerok, W. (1990*a*) Eur. J. Biochem. **188**, 689–695
Häussinger, D., Hallbrucker, C., vom Dahl, S., Lang, F. & Gerok, W. (1990*b*) Biochem. J. **272**, 239–242
Häussinger, D., Hallbrucker, C., vom Dahl, S., Decker, S., Schweizer, U., Lang, F. & Gerok, W. (1991) FEBS Lett. **283**, 470–472
Hallbrucker, C., vom Dahl, S., Lang, F. & Häussinger, D. (1991*a*) Eur. J. Biochem. **197**, 717–724
Hallbrucker, C., vom Dahl, S., Lang, F., Gerok, W. & Häussinger, D. (1991*b*) Eur. J. Biochem. **199**, 467–474
Hallbrucker, C., vom Dahl, S., Lang, F., Gerok, W. & Häussinger, D. (1991*c*) Pflügers Arch. **418**, 519–521
Hansen, C. A., Mah, S. & Williamson, J. R. (1986) J. Biol. Chem. **261**, 8100–8103
Jefferson, L. S. & Korner, A. (1969) Biochem. J. **111**, 703–712
Kimball, S. R. & Jefferson, L. S. (1990) J. Biol. Chem. **265**, 16794–16798
Kristensen, L. O. & Folke, M. (1984) Biochem. J. **221**, 265–268
Kruppa, J. & Clemens, M. J. (1984) EMBO J. **3**, 95–100
MacLennan, P. A. Brown, R. W. & Rennie, M. J. (1987) FEBS Lett. **215**, 187–191
MacLennan, P. A., Smith, K., Weryk, B., Watt, P. W., Rennie, M. J. (1988) FEBS Lett. **237**, 133–136
McNurlan, M. A., Tomkins, A. M. & Garlick, P. J. (1979) Biochem. J. **178**, 373–379
Mortimore, G. E. & Mondon, C. E. (1970) J. Biol. Chem. **245**, 2375–2383
Munro, H. N. (1968) Fed. Proc. Fed. Am. Soc. Exp. Biol. **27**, 1231–1237
Nuss, D. L. & Koch, G. (1976) J. Mol. Biol. **102**, 601–612
Pronczuk, A. W., Rogers, Q. R. & Munro, H. N. (1970) J. Nutr. **100**, 1249–1258
Quinlan, P. T., Thomas, A. P., Armston, A. E. & Halestrap, A. P. (1983) Biochem. J. **214**, 395–404
Rennie, M. J., Hundal, H. S., Babij, P., MacLennan, P., Taylor, P. M., Watt, P. W., Jepson, M. M. & Milward, D. J. (1986) Lancet **ii**, 1008–1012
Saborio, J. L., Pong, S. S. & Koch, G. (1974) J. Mol. Biol. **85**, 195–211
Schreiber, G., Urban, J., Zähringer, J., Reutter, W. & Frosch, U. (1971) J. Biol. Chem. **246**, 4531–4538
Schulz, W., Hallbrucker, C., Eickelmann, C., Sies, H. & Häussinger, D. (1991) FEBS Lett. **292**, 264–266
Seglen, P. O. (1978) Biochem. J. **174**, 469–474
Seglen, P. O. & Solheim, A. E. (1978) Eur. J. Biochem. **85**, 15–25
Sugden, M. C. & Fuller, S. J. (1991) Biochem. J. **273**, 21–27
vom Dahl, S., Hallbrucker, C., Lang, F., Gerok, W. & Häussinger, D. (1991*a*) Biochem. J. **278**, 771–777
vom Dahl, S., Hallbrucker, C., Lang, F. & Häussinger, D. (1991*b*) Biochem. J. **280**, 105–109
vom Dahl, S., Hallbrucker, C., Lang, F., Gerok, W. & Häussinger, D. (1991*c*) Biol. Chem. Hoppe-Seyler **372**, 411–418
Wettstein, M., vom Dahl, S., Lang, F., Gerok, W. & Häussinger, D. (1990) Biol. Chem. Hoppe-Seyler **371**, 493–501
Woodside, K. H., Ward, W. F. & Mortimore, G. E. (1974) J. Biol. Chem. **249**, 5458–5463