Effect of vasopressin on the regulation of protein synthesis initiation in liver cells

Juan MENAYA, Roberto PARRILLA and Matilde S. AYUSO*

Centro de Investigaciones Biológicas, C.S.I.C., Velazquez 144, 28006 Madrid, Spain

Vasopressin was found to be an effective inhibitor of protein labelling in isolated liver cells. Its effect shows the following distinct characteristics: (1) in contrast with α -adrenergic agonists, its effect is observable under a wide range of cellular Ca^{2+} -loading conditions; (2) it is not influenced by the nutritional state of the animal. The lack of vasopressin effect on valine production, and its ability to decrease protein labelling from near-saturation concentrations of [^3H]valine, indicate that the observed variations in protein labelling reflect actual changes in the rate of protein synthesis. The action of vasopressin is primarily exerted on the initiation step of protein synthesis and this effect is accompanied by a decreased activity of eukaryotic initiation factor 2. Activators of protein kinase C showed similar but not additive effects on protein synthesis, as did vasopressin. It seems plausible to conclude that protein kinase C activation may play an important regulatory role in hepatic protein synthesis as a transducer of hormonal and perhaps other type of signals.

INTRODUCTION

The use of rabbit reticulocyte lysates as an experimental model has allowed the proposal of a mechanism for the regulation of protein synthesis that involves the phosphorylation of the α -subunit of the initiation factor eIF-2 [1–4]. The phosphorylated factor seems to form an unproductive complex with the GDP-exchange factor [5], which catalyses the exchange of a molecule of GDP bound to eIF-2, at the end of the initiation process, for a molecule of GTP [6–9]. The sequestration of the GDP-exchange factor makes it unavailable for the catalysis of the GTP recycling step. There is evidence in favour of a similar mechanism which might operate in non-erythroid cells [10–14]. The eIF-2 as well as the GDP-exchange factor have been isolated from rat liver homogenates [15–18]. Differences in the affinity constant for GDP of eIF-2 and in the subunit composition of GDP-exchange factor isolated from liver and their homologues from reticulocytes [18] have been observed. However, it is not known whether these differences imply different mechanisms of regulation. Neither is it known whether the hepatic eIF-2 factor activity is regulated by a phosphorylation mechanism.

Fluctuations in the rates of hepatic protein synthesis in response to different nutritional states, metabolic or endocrine disorders are well established [19–21]; however, the physiological signals mediating the control as well as their mechanism of action remain to be elucidated. Activation of cyclic-AMP-dependent protein kinases by glucagon does not seem to perturb the hepatic rate of initiation *in vivo* [22,23]. Neither does the activation of Ca^{2+} -dependent protein kinases seem to mediate the action of α -adrenergic agents on hepatic protein synthesis [24].

The activation of protein kinase C by diacylglycerol [25–28] has been involved in the regulation of hepatic metabolism. The activation of a protein kinase, with

similar requirements to those of protein kinase C, has been suggested to play a role in the control of protein synthesis in reticulocytes [29,30]. On these grounds, it was found desirable to determine whether the diacylglycerol–protein kinase C pathway is operative in the regulation of hepatic protein synthesis. For this purpose, we have studied the action of vasopressin on protein synthesis in isolated hepatocytes. Vasopressin is known to display a powerful action in increasing intracellular concentrations of diacylglycerol [31] by promoting the hydrolysis of phosphoinositides [32–37], and also by increasing the flux of carbon from other phospholipids [37]. Our results are compatible with the possibility of the involvement of protein kinase C in the control of the activity of the peptide initiation factor eIF-2.

EXPERIMENTAL

Animals

Male Wistar albino rats weighing 200–220 g were used. Unless indicated otherwise the animals were fed *ad libitum* until used.

Chemicals

[U- ^3H]Valine (10 Ci/ μmol) was purchased from Amersham International (Amersham, Bucks., U.K.). All the reagents were of the highest possible purity, and most were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Collagenase was purchased from Worthington (Freehold, NJ, U.S.A.).

Isolation and incubation of liver cells

The procedure for obtaining liver cells normally loaded or partially depleted of Ca^{2+} has been previously described [24]. Cells were incubated at 36.5 °C in Krebs–Ringer bicarbonate buffer, containing 2% (w/v) Ficoll 70, either in the absence or in the presence of

Abbreviation used: eIF-2, eukaryotic initiation factor 2.

* To whom correspondence and reprint requests should be addressed.

1.3 mM- Ca^{2+} . The pH of the incubation mixture was 7.4 after equilibration with O_2/CO_2 (19:1).

Determination of protein-synthesis activity

L-[^3H]Valine was utilized as the amino acid precursor. This amino acid was chosen because it is poorly oxidized or transaminated by the liver cells [38]. The procedure for determination of the rate of protein labelling from tracer doses or 0.5 mM-L-[^3H]valine (sp. radioactivity 10 Ci/mol) was as previously described [24,39].

Determination of valine production by liver cells

Liver cells suspended in buffer containing Ficoll 70 were incubated at 36.5 °C in a rotary shaker. Portions of the cell suspension were taken at 0, 30 and 60 min of incubation and added to tubes containing cold HClO_4 (final concn. 6%, v/v). After removal of the acid-insoluble material by centrifugation (10000 g for 5 min), the samples were adjusted to pH 7 with 10 M-KOH. The insoluble KClO_4 formed was removed by centrifugation and the supernatant adjusted to pH 2 by addition of 1 M-lithium citrate, pH 1.3. The concentration of valine was determined by automated ion-exchange liquid chromatography.

Determination of the rate of labelling of nascent and total peptides from [^3H]valine

Liver cells (~100–140 mg wet weight/ml) were incubated in Krebs–Ringer bicarbonate buffer containing 2% Ficoll 70 and equilibrated with O_2/CO_2 (19:1). After 15 min of incubation at 36.5 °C, 0.5 mM-[^3H]valine (sp. radioactivity 10 Ci/mol) was added, and 2, 4, 6 and 8 min thereafter 2 ml portions were taken and added to test tubes containing 1 ml of ice-cold buffer, of composition: 150 mM-Tris/HCl, pH 7.6, 3 mM-magnesium acetate, 300 μg of cycloheximide/ml, 1.5% (w/v) sodium deoxycholate and 0.9% (v/v) Triton X-100. After centrifugation at 12500 g for 10 min, the supernatant was separated into two fractions. The first (0.4 ml), used for the determination of the rate of labelling of total peptides, was added to 2 ml of 10% (w/v) trichloroacetic acid and heated at 90 °C for 30 min. After three washes with 10% trichloroacetic acid and one with ethanol, the insoluble material was dissolved in a protein solubilizer and the radioactivity determined by scintillation counting.

The other fraction, used for determination of the rate of labelling of nascent peptides in the polyribosomes, was added to ultracentrifuge tubes containing a layer of 1 M-sucrose/1 mM-magnesium acetate and 10% of liver cell sap as RNAase inhibitor [40,41]. After centrifuging at 150000 g for 3 h at 0 °C, the pellet was washed with ice-cold reagent-quality water, resuspended in 5 ml of ice-cold 10% trichloroacetic acid and washed twice with ice-cold 10% trichloroacetic acid and once with ethanol. After removal of ethanol by evaporation, the pellet was dissolved in a protein solubilizer and the radioactivity determined.

Assay of eIF-2 activity

The activity of the eIF-2 factor was determined by the formation of a ternary complex between eIF-2, GTP and [^{35}S]methionyl-tRNA. The procedure was that described by Gupta *et al.* [42], as modified by Clemens & Pain [43]. Extracts were prepared from livers of rats weighing 120 g. Either 0.2 ml of 0.9% NaCl or 10 nM-vasopressin

dissolved in the same solution was slowly injected into the portal vein; 5 min thereafter, two liver biopsies (approx. 1 g each) were taken and immediately homogenized in 3 vol. of 50 mM-triethanolamine, pH 7.6, containing 5 mM-magnesium acetate, 2.3% (w/v) glycerol, 0.5 mM-dithiothreitol and 120 mM- or 250 mM-KCl. After centrifuging at 10000 g for 10 min at 0 °C, the supernatant was collected and centrifuged again at 150000 g for 3 h at 0 °C. The post-ribosomal supernatant was stored in batches in liquid N_2 . The composition of the incubation mixture for the assay of eIF-2 activity was as described in ref. [43], and contained 5% of post-ribosomal supernatant. This concentration was found to give a linear response in the formation of ternary complexes. The reaction was initiated by the addition of 7×10^6 c.p.m. of [^{35}S]methionyl-tRNA prepared as described by Takeishi *et al.* [44].

RESULTS

Effect of vasopressin on protein labelling from tracer doses of [^3H]valine

Fig. 1 shows the effect of vasopressin on protein labelling in hepatocytes isolated from fed animals and partially depleted of Ca^{2+} . The rate of protein labelling was inhibited whether or not Ca^{2+} was present in the incubation medium. As described previously [24], the addition of extracellular Ca^{2+} caused only a small increase in the rate of protein labelling. A similar inhibitory effect

Fig. 1. Effect of vasopressin and Ca^{2+} on the rate of protein labelling in isolated hepatocytes partially depleted of Ca^{2+}

Hepatocytes partially depleted of Ca^{2+} were obtained from fed rats as described in the experimental section. Rates of protein labelling were determined with tracer doses of [^3H]valine and in the absence (●) and in the presence (○) of vasopressin (5 nM). The points represent mean values of five experiments, run in triplicates, and the vertical bars the S.E.M. The differences between the mean values obtained in the absence and in the presence of vasopressin were statistically significant (by paired *t* test: $P < 0.05$) at any concentration of Ca^{2+} in the extracellular medium.

Fig. 2. Effect of vasopressin and Ca^{2+} on the rate of protein labelling in isolated hepatocytes partially depleted of Ca^{2+} and obtained from starved rats

Hepatocytes were obtained from 48 h-starved rats. Depletion of Ca^{2+} was performed as described in the Experimental section. Rates of protein labelling from tracer doses of [^3H]valine were determined in the absence (●, ○) and in the presence (■, □) of 10 mM-alanine and in the absence (●, ■) and in the presence (○, □) of vasopressin (5 nM). The points are mean values of four experiments, run in triplicate, and the vertical bars the S.E.M. The differences between the mean values obtained in the absence and in the presence of vasopressin were statistically significant at any Ca^{2+} concentration (by paired *t* test: $P < 0.05$).

of vasopressin on protein labelling was observed in hepatocytes obtained from starved animals (Fig. 2), indicating that its action is not influenced by the nutritional status of the animals. Vasopressin was also able to inhibit protein labelling in the presence of alanine, which is known to stimulate the initiation and elongation steps of protein synthesis in starved rats [21]. The data in Fig. 2 indicate that the effect of vasopressin was not dependent on the presence of Ca^{2+} in the extracellular medium. However, Fig. 3(a) shows that 0.1 nM-vasopressin produces maximal inhibition of protein labelling in hepatocytes not depleted of Ca^{2+} , whereas higher concentrations (≈ 1 nM) were needed to attain similar inhibition in hepatocytes partially depleted of Ca^{2+} (Fig. 3b).

Effect of vasopressin on the rates of protein synthesis and proteolysis

The action of vasopressin in inhibiting the rate of protein labelling from tracer amounts of [^3H]valine could be the result of either an inhibition of protein synthesis and/or an increase in the rate of proteolysis. This latter possibility could result in a dilution of the radioactive amino acid precursor, leading to a decreased rate of

Fig. 3. Dose-response of vasopressin on the rate of protein labelling in isolated liver cells

Hepatocytes with normal Ca^{2+} content (a) or partially depleted of Ca^{2+} (b) were obtained from animals starved for 48 h. Rates of protein labelling from tracer doses of [^3H]valine were determined in the presence of the indicated concentrations of vasopressin and either with 1.3 mM- Ca^{2+} (a) or with no Ca^{2+} (b) in the incubation medium. The points are mean values of five experiments and the vertical bars the S.E.M. The differences between the values obtained in the absence and in the presence of any concentration of vasopressin were statistically significant (by paired *t* test: $P < 0.05$).

protein labelling. In order to differentiate between these two possibilities, the effect of vasopressin on the rate of protein labelling from saturating concentrations of [^3H]valine was studied. Under these conditions, changes in the rate of valine production from proteolysis would not have a significant effect on the specific radioactivity of the precursor; therefore the rate of protein labelling should reflect the actual rate of protein synthesis. On the other hand, the effect of vasopressin on the rate of valine production, as an indicator of net protein breakdown [45,46], was also determined. Table 1 shows that vasopressin inhibits the rate of protein labelling without significantly affecting the rate of valine production. These results indicate that the effect of the hormone is exerted primarily on the protein synthesis pathway.

Effect of vasopressin on the initiation and elongation steps of protein synthesis

The kinetics of the incorporation of radioactivity into nascent peptides in polyribosomes was determined in order to elucidate which step of protein synthesis was perturbed by vasopressin. Fig. 4 shows that the incorporation of radioactivity from saturating con-

Table 1. Effect of vasopressin on the rates of protein labelling from saturating concentrations of [3 H]valine and valine production in isolated liver cells

Hepatocytes partially depleted of Ca^{2+} were obtained from 48 h-starved rats. Rates of protein labelling were determined by using 0.5 mM-[3 H]valine (sp. radioactivity 10 Ci/mol) as the precursor amino acid. The values for protein labelling are means $\pm$ S.E.M. for three experiments, each run in duplicate. The values for valine production are means $\pm$ S.E.M. for four experiments. *By paired t test: $P < 0.02$.

	Protein labelling (c.p.m./h per mg wet wt.)	Valine production (nmol/h per g wet wt.)
Control	1068 $\pm$ 60	1.98 $\pm$ 0.2
Vasopressin (5 nM)	812 $\pm$ 54*	2.05 $\pm$ 0.05

Fig. 4. Effect of vasopressin on the kinetics of labelling of nascent polypeptides in polyribosomes in isolated liver cells

Hepatocytes obtained from fed animals were preincubated in the absence (●) and in the presence (○) of 5 nM-vasopressin. The rate of labelling from 0.5 mM-[3 H]valine (sp. radioactivity 10 Ci/mol) was determined as described in the Experimental section. Each point represents the mean of five separate experiments and the vertical bars represent S.E.M. The differences between the means obtained in the absence and in the presence of vasopressin were statistically significant at any time after 2 min (by paired t test: $P < 0.05$).

centrations of [3 H]valine starts to level off after about 6 min of incubation. In the presence of vasopressin the rate of protein labelling did not decrease even after 8 min. If peptide elongation was the only step inhibited, it would be expected that the labelling would reach a maximum before the control did [47,48]. Accordingly, an inhibition of the initiation step should produce kinetics

Fig. 5. Effect of vasopressin on the rate of labelling of total peptides in isolated liver cells

Hepatocytes obtained from fed animals were preincubated in the absence (●) and in the presence (○) of 5 nM-vasopressin. The labelling of total polypeptides from 0.5 mM-[3 H]valine (sp. radioactivity 10 Ci/mol) was determined as described in the Experimental section. The points represent the means of five separate experiments and the vertical bars the S.E.M. The differences between the values obtained at 6 and 8 min in the absence and in the presence of vasopressin were statistically significant (by paired t test: $P < 0.05$).

[47] similar to those shown in Fig. 4. Thus these results strongly indicate that vasopressin inhibits protein synthesis by perturbing the initiation step. To support this conclusion further, the rates of peptide-chain elongation were determined. For this purpose the time of completion of polypeptide chains was obtained by calculating the rate of decay of the ratio between radioactivity incorporated into nascent peptides and the radioactivity incorporated into total peptides (n/t) [48–50]. Fig. 5 shows the kinetics of incorporation of radioactivity from saturating concentrations of [3 H]valine into total peptides. Linear rates of protein labelling were observed during the experimental period. Vasopressin produced a statistically significant inhibition of the rate of protein labelling. The n/t ratios calculated from the data shown in Figs. 4 and 5 allowed us to determine the polyribosomal transit time, which was 4.5 min in both control and vasopressin-treated hepatocytes. *In vivo*, the transit time has consistently been found to be 1.5 min [22]. Thus the rate of polypeptide-chain elongation is 3 times lower in isolated hepatocytes than *in vivo*. The fractional rate of protein synthesis in hepatocytes is 16% of that observed *in vivo* [24]. This implies that, in order to account for the differences in fractional rate, the amount of ribosomes in polyribosomes in isolated hepatocytes should be half that in the intact liver.

Effect of vasopressin on activity of the initiation factor eIF-2

The effect of vasopressin in inhibiting the initiation step of protein synthesis opens the question of whether

Table 2. Effect of vasopressin on the ability of liver extracts to form ternary complexes

The experiment was performed as described in the Experimental section. The values are means $\pm$ S.E.M. for five experiments carried out with liver extracts made with 120 mM-KCl and for three experiments with liver extracts made with 250 mM-KCl. *By *t* test: $P < 0.01$.

[KCl]...	10 ⁻³ $\times$ Radioactivity retained (c.p.m./min per mg of protein)	
	120 mM	250 mM
Control	105 $\pm$ 8	273 $\pm$ 37
Vasopressin	61 $\pm$ 8*	154 $\pm$ 42

its action is mediated by an inhibition of the activity of the initiation factor eIF-2. To answer this question, the ability to form ternary complexes between eIF-2, [³⁵S]methionyl-tRNA and GTP was determined in liver extracts obtained from rats previously treated with vasopressin or with saline. Table 2 shows that pre-treatment with vasopressin significantly decreased the ability of liver extracts to form ternary complexes. The effect was observable in extracts containing either low or high KCl concentration. Since high KCl concentration would release the eIF-2 bound to ribosomal subunits [51,52] and possibly other cellular structures [53,54], it can be concluded that the inhibitory effect of vasopressin is equally exerted on the activity of both free and bound eIF-2.

Effect of activators of protein kinase C on the rate of protein synthesis

The results presented above are compatible with the possibility that vasopressin would inhibit the initiation step of protein synthesis in the intact cell by decreasing the activity of the initiation factor eIF-2. A phosphorylation mechanism is involved in the control of the eIF-2 activity in other mammalian cells [1-4]. Vasopressin is known to display a powerful effect to raise the cellular concentrations of diacylglycerol, which is an activator of protein kinase C [31]. On these grounds, it was decided to study whether activators of protein kinase C would show any effect in perturbing protein synthesis. For this purpose the effects of phorbol 12-myristate 13-acetate, 1,2-dioleoylglycerol and 1-oleoyl-2-acetyl-glycerol, known activators of protein kinase C [55-58], were studied. Table 3 shows that these compounds induced an inhibition of protein labelling from saturating concentrations of [³H]valine similar to that observed with vasopressin. Furthermore, their effects were not additive with that of vasopressin, suggesting that they share the same mechanism of action. Table 3 also shows that 1,3-dioleoylglycerol, which is not effective in activating protein kinase C [59], is unable to alter the rate of protein labelling. This observation seems to rule out any unspecific effect of the diacylglycerol analogues on protein synthesis.

DISCUSSION

The results presented in this paper indicate that vasopressin is able to control the rate of hepatic protein

Table 3. Effects of protein kinase C activators and vasopressin on rates of protein labelling in isolated liver cells

Hepatocytes were obtained from fed rats. The rates of protein labelling from 0.5 mM-[³H]valine (sp. radioactivity 10 Ci/mol) were determined as described in the Experimental section. The values are means $\pm$ S.E.M. for seven experiments, run in triplicate. By paired *t* test: * $P < 0.001$, ** $P < 0.01$, *** $P < 0.02$, **** $P < 0.05$, compared with the value obtained without any addition.

Additions	Vasopressin (10 nM)	Protein labelling (c.p.m./h per mg wet wt.)
None	—	917 $\pm$ 53
	+	719 $\pm$ 25*
4 β -Phorbol 12 β -myristate 13-acetate (10 μ g/ml)	—	751 $\pm$ 62**
	+	690 $\pm$ 51***
None	—	853 $\pm$ 53
	+	693 $\pm$ 20*
1,2-Dioleoylglycerol (25 μ g/ml)	—	734 $\pm$ 51****
	+	629 $\pm$ 26*
None	—	986 $\pm$ 54
	+	786 $\pm$ 55**
1-Oleoyl-2-acetyl-glycerol (25 μ g/ml)	—	818 $\pm$ 60*
	+	772 $\pm$ 86**
None	—	1141 $\pm$ 54
1,3-Dioleoylglycerol (25 μ g/ml)	—	1189 $\pm$ 69

synthesis, acting at the level of the initiation step. This action of vasopressin is consistent with the idea that a stimulation of gluconeogenesis is accompanied by a decreased rate of protein synthesis [60], probably as a result of the increased substrate demand for the synthesis of glucose. From a quantitative point of view the effect of vasopressin is small; however, this does not preclude its physiological significance, since in the intact organ *in vivo* changes in physiological conditions have rarely, if ever, been shown to inhibit protein synthesis by more than 40-50%. Apparently greater inhibitions may render the organ functionally insufficient. Although the rate of protein synthesis in isolated liver cells is only 16% of the rate *in vivo* [24], their responses to nutritional status and to other conditions that affect the rate of protein synthesis tend to be similar to those observed in the intact organ [21,39,61,62].

Our results confirm and extend a previous report [24] which indicates that the activation of Ca²⁺-dependent protein kinases, mediated by a rise in the cytosolic free Ca²⁺ concentrations, does not play a major role in the control of hepatic protein synthesis. Two observations support this view: (1) the addition of Ca²⁺ to the extracellular medium, which should result in an increase in the cytosolic free Ca²⁺ concentration [62], does not significantly affect the rate of protein labelling (Figs. 1 and 2); (2) vasopressin inhibits protein labelling in cells obtained from starved animals (Fig. 2), in the absence of any substrate. Phenylephrine does not perturb the rate of protein labelling in similar conditions [24]. However, both Ca²⁺-mobilizing agents, vasopressin and phenylephrine, seem to have quantitatively similar effects in increasing cytosolic free Ca²⁺ [25], ruling out the

possibility that this ion was the mediator of the action of vasopressin on protein labelling. The total cellular content of Ca^{2+} , rather than transient increases in its cytosolic free concentrations, seems to be the determinant to set the rate of protein labelling. Actually, it can be appreciated that the rate of protein labelling is higher in normal than in Ca^{2+} -depleted cells (Fig. 3).

Higher concentrations of vasopressin are necessary to achieve maximal inhibition of protein labelling under Ca^{2+} -depletion conditions (Fig. 3). This observation suggests that vasopressin may act on protein synthesis through two different mechanisms, one dependent on and another independent of Ca^{2+} , with different affinities for vasopressin. This interpretation is consonant with previous reports indicating the existence of Ca^{2+} -dependent and -independent actions of vasopressin on liver metabolism. The action of vasopressin on the hydrolysis of phosphoinositides seems to be Ca^{2+} -independent [34,37], whereas its effect on the hydrolysis of other phospholipids, including phosphatidylinositol, is Ca^{2+} -dependent [37]. The possibility should also be considered that an effective interaction of vasopressin with its receptor requires extracellular Ca^{2+} . Regardless of the Ca^{2+} -loading conditions, the action of vasopressin leads to increased cellular concentrations of diacylglycerol [37], indicating that this could be the mediator of its effect on protein synthesis, through a stimulation of protein kinase C. This possibility is supported by the following observations. First, phorbol esters and diacylglycerol analogues are as effective as vasopressin in inhibiting protein labelling (Table 2). Second, the effects of phorbol esters and analogues of diacylglycerol are not additive with that of vasopressin (Table 2). These observations seem to support the involvement of the cellular content of diacylglycerol in the response to vasopressin.

The parallelism between the effect of vasopressin inhibiting the initiation step in liver cells and the decreased ability of the eIF-2 factor of liver extracts to form ternary complexes with methionyl-tRNA and GTP could indicate that this factor plays a major role in controlling the rate of initiation. However, the possibility cannot be excluded that the decreased activity of eIF-2 could be the consequence of an interaction with another step, rather than the primary event leading to a decrease in the initiation of peptide synthesis.

How the activation of protein kinase C could bring about an inhibition of the initiation step of protein synthesis requires further elucidation. According to current views on the mechanism of control of eIF-2 activity, based on experiments carried out with other mammalian cells [1-4], protein kinase C might act by phosphorylating the α -subunit of the eIF-2 factor. Failure of protein kinase C to show any direct effect on the activity of eIF-2 from reticulocytes has been recently reported [63]. Therefore, if these results are applicable to the hepatic factor, it would indicate that the action of protein kinase C is probably exerted on other protein(s) that interact with eIF-2. This type of indirect mechanism of action of protein kinase C on the protein synthesis machinery has already been suggested [64].

We express our gratitude to M. J. Arias-Salgado, A. Rodríguez and S. García for their excellent technical assistance. This work was supported in part by grants from

Comisión Asesora de Investigación Científica Y Técnica (431), Secretaría de Estado de Universidades e Investigación (87065) and Fondo de Investigaciones Sanitarias (87/1688 and 87/1689). J. M. is recipient of a fellowship from the Ministerio de Educación y Ciencia.

REFERENCES

- Farrell, P., Balkow, K., Hunt, T., Jackson, R. J. & Trachsel, H. (1977) *Cell* **1**, 187-200
- Farrell, P., Hunt, T. & Jackson, R. J. (1978) *Eur. J. Biochem.* **89**, 517-521
- Ernst, V., Levin, D. H. & London, I. M. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2118-2122
- Leroux, A. & London, I. M. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2147-2151
- Matts, R. L. & London, I. M. (1984) *J. Biol. Chem.* **259**, 6708-6711
- Siekierka, J., Mauser, L. & Ochoa, S. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2537-2540
- Matts, R. L., Levin, D. H. & London, I. M. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2559-2563
- Konieczny, A. & Safer, B. (1983) *J. Biol. Chem.* **258**, 3402-3408
- Salimans, M., Goumans, M., Ames, H., Benne, R. & Voorma, H. O. (1984) *Eur. J. Biochem.* **145**, 91-98
- Pain, V. M., Lewis, J. A., Huvos, P., Henshaw, E. C. & Clemens, M. J. (1980) *J. Biol. Chem.* **255**, 1486-1491
- Mariano, T. M., Siekierka, J. & Ochoa, S. (1986) *Biochem. Biophys. Res. Commun.* **134**, 1160-1166
- Harmon, C. S., Proud, C. G. & Pain, V. M. (1984) *Biochem. J.* **233**, 687-696
- Clemens, M. J., Pain, V. M., Wong, S. T. & Henshaw, E. C. (1982) *Nature (London)* **296**, 93-95
- Panniers, R. & Henshaw, E. C. (1983) *J. Biol. Chem.* **258**, 7928-7934
- Stringer, E. A., Chaudhuri, A. & Maitra, U. (1979) *J. Biol. Chem.* **254**, 6845-6848
- Proud, C. G., Clemens, M. J. & Pain, V. M. (1982) *FEBS Lett.* **148**, 214-220
- Nygard, O., Watermann, P. & Hultin, T. (1980) *Biochim. Biophys. Acta* **608**, 196-200
- Kimball, S. R., Everson, W. V., Myers, L. M. & Jefferson, L. S. (1987) *J. Biol. Chem.* **262**, 2220-2227
- Mathews, R. W., Oronsky, A. & Haschemeyer, A. E. V. (1973) *J. Biol. Chem.* **248**, 1329-1333
- Henshaw, E. C., Hirsch, C. A., Morton, B. E. & Hiatt, H. H. (1971) *J. Biol. Chem.* **246**, 436-446
- Pérez-Sala, D., Parrilla, R. & Ayuso, M. S. (1987) *Biochem. J.* **241**, 491-498
- Martín-Requero, A., Pérez-Díaz, J., Ayuso-Parrilla, M. S. & Parrilla, R. (1979) *Arch. Biochem. Biophys.* **195**, 223-234
- Martín-Requero, A., Martínez-Izquierdo, J. A., Parrilla, R. & Ayuso-Parrilla, M. S. (1981) *Horm. Metab. Res.* **13**, 22-24
- Menaya, J., Parrilla, R. & Ayuso, M. S. (1987) *Biochem. J.* **248**, 903-909
- Charest, R., Blackmore, P. F., Berthon, B. & Exton, J. H. (1983) *J. Biol. Chem.* **258**, 8769-8773
- Charest, R., Prpic, V., Exton, J. H. & Blackmore, P. F. (1985) *Biochem. J.* **227**, 79-90
- Hughes, P., Rye, K. A., Pickford, L. B., Barrit, G. J. & Chalmers, A. H. (1984) *Biochem. J.* **222**, 535-540
- Nishizuka, Y. (1984) *Nature (London)* **308**, 693-698
- de Haro, C., de Herreros, A. G. & Ochoa, S. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6843-6847
- Schatzmann, R. C., Grifo, J. A., Merrick, W. C. & Kuo, J. F. (1983) *FEBS Lett.* **159**, 167-170

31. Bocckino, S. B., Blackmore, P. F. & Exton, J. H. (1985) *J. Biol. Chem.* **260**, 14201–14207
32. Michell, R. H., Kirk, C. J., Jones, L. M., Downes, C. P. & Creba, J. A. (1981) *Philos. Trans. R. Soc. London Ser. B* **296**, 123–137
33. Kirk, C. J. (1982) *Cell Calcium* **3**, 385–387
34. Creba, J. A., Downes, C. P., Hawkins, P. T., Brewster, G., Michell, R. H. & Kirk, C. J. (1983) *Biochem. J.* **212**, 733–747
35. Rhodes, D., Prpic, V., Exton, J. H. & Blackmore, P. F. (1983) *J. Biol. Chem.* **258**, 2770–2773
36. Thomas, A. P., Marks, J. S., Coll, K. E. & Williamson, J. R. (1983) *J. Biol. Chem.* **258**, 5716–5725
37. Pickford, L. B., Polverino, A. J. & Barritt, G. J. (1987) *Biochem. J.* **245**, 211–216
38. Woodside, K. M. & Mortimore, G. E. (1972) *J. Biol. Chem.* **247**, 6474–6481
39. Girbés, T., Susin, A., Ayuso, M. S. & Parrilla, R. (1983) *Arch. Biochem. Biophys.* **226**, 37–49
40. Roth, J. S. (1958) *J. Biol. Chem.* **231**, 1085–1095
41. Shortman, K. (1962) *Biochim. Biophys. Acta* **55**, 88–96
42. Gupta, N. K., Woodley, C. L., Chen, Y. C. & Bose, K. K. (1973) *J. Biol. Chem.* **248**, 4500–4511
43. Clemens, M. J. & Pain, V. M. (1981) *Biochem. J.* **194**, 357–360
44. Takeishi, K., Ukita, T. & Nishimura, S. (1968) *J. Biol. Chem.* **243**, 5761–5769
45. Parrilla, R. & Goodman, M. N. (1974) *Biochem. J.* **138**, 341–348
46. Parrilla, R., Goodman, M. N. & Toews, C. J. (1977) *Pflügers Arch.* **369**, 167–175
47. Li, K. L., Wasan, M. T. & Kisilevsky, R. (1972) *Biochim. Biophys. Acta* **272**, 435–450
48. Ayuso-Parrilla, M. S., Martín-Requero, A., Pérez-Díaz, J. & Parrilla, R. (1976) *J. Biol. Chem.* **251**, 7785–7790
49. Mathews, R. W., Oronsky, A. & Haschemeyer, A. E. V. (1973) *J. Biol. Chem.* **248**, 1329–1333
50. Scornick, O. A. (1974) *J. Biol. Chem.* **249**, 3876–3883
51. Jagus, R. (1983) in *Post-Translational Covalent Modification of Proteins* (Johnson, B. C., ed.), pp. 159–179, Academic Press, New York
52. Thomas, N. S. B., Matts, R. L., Levin, D. H. & London, I. M. (1985) *J. Biol. Chem.* **260**, 9860–9866
53. van Venrooij, W. J., Sillekens, P. T. G., van Eekelen, C. A. G. & Reinders, R. J. (1981) *Exp. Cell Res.* **135**, 79–91
54. Cervera, M., Dreyfuss, G. & Penman, S. (1981) *Cell* **23**, 113–120
55. Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. & Nishizuka, Y. (1982) *J. Biol. Chem.* **257**, 7847–7851
56. Kikkawa, U., Kaibuchi, K., Castagna, M., Yamanishi, J., Sano, K., Tanaka, Y., Miyake, R., Takai, Y. & Nishizuka, Y. (1984) *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **17**, 437–442
57. Kishimoto, A., Takai, Y., Mori, T., Kikkawa, U. & Nishizuka, Y. (1980) *J. Biol. Chem.* **255**, 2273–2276
58. Hannun, Y. A., Loomis, C. R. & Bell, R. H. (1985) *J. Biol. Chem.* **260**, 10039–10043
59. Van de Werve, G., Proietto, J. & Jeanrenaud, B. (1985) *Biochem. J.* **231**, 511–516
60. Ayuso, M. S., Vega, P., González-Manchón, C. & Parrilla, R. (1986) *Biochim. Biophys. Acta* **883**, 33–40
61. Seglen, P. O. & Solheim, A. E. (1978) *Biochim. Biophys. Acta* **520**, 630–641
62. Weigang, K., Wernze, H. & Falge, C. (1977) *Biochem. Biophys. Res. Commun.* **75**, 102–110
63. Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. (1984) *J. Biol. Chem.* **259**, 3077–3081
64. Pelaez, F., de Herreros, A. G. & de Haro, C. (1987) *Arch. Biochem. Biophys.* **257**, 328–338

Received 18 December 1987/22 April 1988; accepted 26 May 1988