Vasoactive intestinal peptide receptors in rat liver after partial hepatectomy

Luis G. GUIJARRO,* Alain COUVINEAU,† M. Sol RODRIGUEZ-PENA,* Maria G. JUARRANZ,* Nieves RODRIGUEZ-HENCHE,* Eduardo ARILLA,* Marc LABURTHE† and Juan C. PRIETO*‡

*Departamento de Bioquímica y Biología Molecular, Universidad de Alcalá, 28871 Alcalá de Henares-Madrid, Spain, and †Institut National de la Santé et de la Recherche Médicale, U-239-Biologie et Physiologie des Cellules Digestives, Faculté de Médecine Xavier Bichat, 16, rue Henri Huchard-75018, Paris, France

We describe the status of vasoactive intestinal peptide (VIP) receptors in regenerating liver. VIP-stimulated adenylate cyclase activity was markedly decreased in proliferating liver 3 days after partial (70 %) hepatectomy. This was associated with a reduced efficacy of VIP (53 % compared with controls), with no change in the potency of the peptide (ED_{50} 0.8 nM). In contrast, forskolin- and guanosine 5'-[β -imido]triphosphate (Gpp[NH]p)-stimulated enzyme activities were not decreased after hepatectomy. The expression of G_s protein subunits (α and β) was studied by cholera toxin-catalysed ADP ribosylation of α_s and by immunoblotting of α_s and β subunits. Both subunits were increased in regenerating liver, further suggesting that the decreased response to VIP was not related to a decreased expression of G_s proteins. In fact, the reduced adenylate cyclase response to VIP in regenerating liver was associated with quantitative and structural changes in VIP receptors. Equilibrium binding data obtained with ^{125} I-VIP indicated the presence of two classes of binding sites, the K_d s of which were not altered after hepatectomy. In contrast, changes in binding capacity (B_{max}) were as follows: 0.11 ± 0.01 and 0.05 ± 0.01 pmol/mg of protein for high-affinity sites in control and hepatectomized rats respectively; and 2.3 ± 0.2 and 0.65 ± 0.03 pmol/mg of protein for low-affinity sites in control and hepatectomized rats respectively. Moreover, affinity labelling experiments showed that the M_r value of ^{125} I-VIP-receptor complexes was higher in regenerating liver than in quiescent hepatocytes, e.g. 58000 and 53000 respectively. It is concluded that VIP receptors are altered in regenerating liver, resulting in a decreased response of adenylate cyclase to the neuropeptide.

INTRODUCTION

Vasoactive intestinal peptide (VIP) is a neuropeptide which is widely distributed in neurons of the central [1] and peripheral [2] nervous systems. Although the highest VIP concentrations have been found in the brain and gastrointestinal tract [3], VIP is also present in many other organs, including the liver, which possesses VIPergic fibres around the hepatic blood vessels [4] and in the hepatic lobule [5], as shown by immunohistochemical techniques. Evidence that VIP may play a significant role in the liver includes the demonstration of its stimulatory effect upon glycogenolysis, by both increasing phosphorylase *a* and decreasing synthase *a* activities [6], and upon gluconeogenesis, through the inhibition of pyruvate kinase activity [7]. Furthermore, VIP causes hyperglycaemia in dogs *in vivo* [8] and promotes glucose output from rabbit liver slices [9] and from isolated hepatocytes [10]. VIP has also been shown to be involved in the control of bile output [11]. On the other hand, the liver acts as a primary site for VIP clearance from portal blood [12,13].

VIP receptors have been purified [14] and characterized as a glycoprotein [15] in liver, where they mediate the stimulation of adenylate cyclase [16,17] through the guanine nucleotide regulatory protein G_s [18,19].

Although pharmacological and biochemical studies have documented the properties of VIP receptors in quiescent adult hepatocytes, very little is known about VIP receptors and their coupling to the cyclic AMP production system during the rapid cell proliferation that follows partial hepatectomy [20]. We have investigated this issue for several reasons. (a) VIP controls several biological functions or enzyme activities which are altered

following hepatectomy. Indeed, glycogenolysis decreases and gluconeogenesis and ureogenesis increase [21] in rat liver 3 days after partial hepatectomy. Moreover, the expression of cyclic AMP-dependent protein kinase subunits is altered during liver regeneration [22]. (b) Cyclic AMP is an inhibitor of the G_1/S transition phase in the cellular cycle so that the cells are apparently in G_0 when the levels of the cyclic nucleotide are increased [23]. (c) There are several lines of evidence that correlate inversely the adenylate cyclase activity stimulated by glucagon and the proliferation produced in fetal liver [24] after acute intoxication with CCl_4 [25], after hepatectomy [26] or in hepatomas [27]. (d) Previous studies have shown a dramatic increase of VIP receptors in human intestinal cells in culture when cells stop dividing and undergo differentiation [28].

We report here on the effect of partial hepatectomy on VIP receptors, G_s and VIP-stimulated adenylate cyclase activity, in order to obtain knowledge on the possible role of VIP in the metabolic alterations and the rapid proliferation in the liver after hepatectomy.

EXPERIMENTAL

Highly purified natural porcine VIP was obtained from Professor V. Mutt (Karolinska Institute, Stockholm, Sweden). Bacitracin, phenylmethanesulphonyl fluoride (PMSF), BSA, guanosine 5'-[β -imido]triphosphate (Gpp[NH]p), 3-isobutyl-1-methylxanthine (IBMX) and cholera toxin were purchased from Sigma. [^{32} P]NAD⁺ was obtained from New England Nuclear-Dupont. ^{125} I-VIP was prepared by the chloramine-T method at a specific radioactivity of 250 Ci/g as described [29].

Abbreviations used: VIP, vasoactive intestinal peptide; PMSF, phenylmethanesulphonyl fluoride; Gpp[NH]p, guanosine 5'-[β -imido]triphosphate; IBMX, 3-isobutyl-1-methylxanthine; DTSP, dithiobis(succinimidylpropionate).

‡ To whom correspondence should be addressed.

It displayed the same activity as native VIP in stimulating cyclic AMP accumulation in the cultured cell line HT-29 [30]. The antisera specific for the α_s (A572) and β (U49) subunits of Gs proteins were generously provided by Dr. Suzanne M. Mumby and Dr. Alfred G. Gilman (University of Texas Health Science Center, Dallas, TX, U.S.A.). Chemicals and unstained protein markers (myosin, M_r 200 000; phosphorylase *b*, M_r 92 500; BSA, M_r 68 000; ovalbumin, M_r 43 000; carbonic anhydrase, M_r 29 000) for SDS gel electrophoresis were purchased from Bethesda Research Laboratories.

Surgical procedures and preparation of liver membranes

Adult male Wistar rats (180–200 g) were fed with a standard rat diet *ad libitum* pre- and post-operatively, and maintained on 12-h-light/12-h-dark cycles. Partial hepatectomy, removing about 70% of liver, was performed under pentobarbitone anaesthesia according to the procedure of Higgins & Anderson [31]. Sham-operated animals, referred to as controls, underwent an abdominal incision, digital manipulation of the liver and closure. Rats were killed by decapitation 3 days after surgery. Crude liver membranes were prepared as described [32]. As an index of plasma membrane enrichment, the specific activity of Na^+/K^+ -ATPase was measured [33] in crude membranes and in the liver homogenates from the control and hepatectomized groups. The same purification factor (about 2-fold) was obtained for both groups. Protein concentrations of membranes were measured by the method of Lowry *et al.* [34], using BSA as a standard.

Binding studies

VIP binding to rat liver membranes was studied as previously described [32]. Briefly, membranes (about 0.05–0.10 mg of protein/ml) were incubated for 30 min at 30 °C with 50 pM- ^{125}I -VIP in the absence or presence of 0.01–100 nM unlabelled VIP in 0.25 ml of assay buffer consisting of 60 mM-Hepes, 2% (w/v) BSA and 0.1% (w/v) bacitracin, pH 7.5. VIP binding to rat intestinal epithelial cells (jejuno-ileal and colonic cells) was studied in a similar manner, as previously described [35]. Briefly, cells (about 0.2 mg of protein/ml) were incubated for 90 min at 15 °C in 0.5 ml of a medium containing 35 mM-Tris/HCl buffer (pH 7.5), 50 mM-NaCl, 1.4% (w/v) BSA, and 1 mg of bacitracin/ml with 50 pM- ^{125}I -VIP in the absence or presence of 0.1–100 nM unlabelled VIP. After the addition of 200 μl of ice-cold 60 mM-Hepes buffer (pH 7.5), membrane- or cell-bound peptide was obtained by centrifugation (13 000 *g*, 2 min) and radioactivity was counted in a LKB Autogamma counter. ^{125}I -VIP specific binding was expressed in fmol of VIP/mg of protein. Non-specific ^{125}I -VIP binding was determined in the presence of 0.3 μM unlabelled VIP and corresponded to about 25% of total binding. Binding data are expressed as specific binding, i.e. total minus non-specific binding. The analysis of binding data was performed according to Scatchard [36] using the EBDA program and by the non-linear curve-fitting program LIGAND [37]. The integrity of ^{125}I -VIP in the incubation mixture was measured by precipitation with 10% trichloroacetic acid as described [38]. Less than 10% of labelled VIP was degraded within the incubation period for both control and hepatectomized rats.

Adenylate cyclase assay

Adenylate cyclase activity was measured as described elsewhere [39], with minor modifications. Briefly, membranes (0.3 mg of protein/ml) were incubated with 1.5 mM-ATP, 5 mM- MgSO_4 , 1 μM -GTP, an ATP-regenerating system (7.4 mg/ml of phosphocreatine and 1 mg of creatine kinase/ml), 1 mM-IBMX, 1 mM-EDTA and tested substances (VIP or forskolin) in 0.1 ml of 25 mM-triethanolamine/HCl buffer (pH 7.4). When

Gpp[NH]p was tested, GTP was omitted from the incubation medium. After 20 min of incubation at 30 °C, the reaction was stopped by heating the mixture for 3 min. After refrigeration, 0.9 ml of an alumina slurry (0.25 g/ml in triethanolamine/HCl buffer, pH 7.6) was added and the suspension was centrifuged (3000 *g*, 5 min). The supernatant was taken for assay of cyclic AMP by the method of Gilman [40].

Cross-linking of ^{125}I -VIP to membranes and SDS/PAGE analysis

Cross-linking experiments were carried out as previously described [32]. Membranes (about 0.5 mg of protein/ml) were incubated for 30 min at 30 °C in 5 ml of the binding assay buffer containing 0.5 nM- ^{125}I -VIP. To determine non-specific binding, parallel samples were incubated in the presence of an excess (0.3 μM) of unlabelled VIP. After incubation, the reaction was stopped by addition of 35 ml of ice-cold 60 mM-Hepes buffer (pH 7.5). The membrane suspension was centrifuged for 15 min at 30 000 *g*, and the pellet was resuspended in 1 ml of 60 mM-Hepes buffer (pH 7.5) containing 1 mM-dithiobis(succinimidyl-propionate) (DTSP). The cross-linking reaction was stopped after 15 min at 4 °C by adding 0.5 ml of ice-cold 60 mM-Hepes buffer (pH 7.5) containing 60 mM-lysine as a reagent quench. The mixture was centrifuged for 15 min at 30 000 *g*, and the pellet was solubilized with 0.1 ml of 60 mM-Tris/HCl buffer (pH 6.8) containing 10% glycerol, 0.001% Bromophenol Blue and 3% SDS. After heating for 30 min at 60 °C, the suspension was centrifuged for 20 min at 30 000 *g* and aliquots of the supernatant were submitted to SDS/PAGE, using procedure of Laemmli [41] as described [32]. The gels were run, fixed and dried as described previously [42]. Dupont films (Cronex-4) were then exposed to the gels for 1–7 days at –70 °C, using an intensifying screen.

Cholera toxin-catalysed ADP-ribosylation

ADP-ribosylation of membranes was carried out as previously reported [18]. After activation of the toxin [18], membranes (0.5 mg of protein/ml) were incubated with the toxin (50 μg /ml) in 0.1 M-phosphate buffer, pH 7.5, containing 5 mM-ATP, 50 μM -GTP, 5 μM - ^{32}P -NAD $^+$, 2 mM-EDTA, 5 mM- MgCl_2 and an ATP-regenerating system. After 30 min at 30 °C, the reaction was stopped by precipitation with 10% (w/v) trichloroacetic acid and submitted to SDS/PAGE as described [42].

Immunodetection of α_s and β subunits of G $_s$ proteins

Membranes were solubilized in SDS sample buffer, and proteins were run on an SDS/10%-polyacrylamide gel as described [42]. The transfer of proteins to nitrocellulose and immunodetection of α_s and β subunits of G $_s$ proteins using antisera for the α_s subunit (A572) and the β subunit (U49) were carried out as described [43]. Briefly, the nitrocellulose sheets were cut into slices, preincubated with 50 mM-Tris/HCl, pH 8.0, 2 mM- CaCl_2 , 80 mM-NaCl, 0.2% (v/v) Nonidet P40 and 5% (w/v) non-fat dry milk. Antisera were diluted in the same buffer and incubated for 1 h at room temperature. After washing, bound immunoreactive proteins were revealed using ^{125}I -labelled goat antibodies against rabbit IgG, and immunoblots were exposed for 1–2 days at –80 °C to a Trimax-type XM film (3M) with a 3M Trimax intensifying screen.

Data processing and statistical analysis

Data are derived from representative experiments, each of which was repeated at least three times in triplicate. Results are expressed as the means $\pm$ S.E.M. Where appropriate, statistical analysis was performed by Student's *t* test, and differences between paired values were considered significant when $P < 0.05$.

RESULTS

As shown in Fig. 1, VIP-stimulated adenylate cyclase activity was markedly decreased in liver membranes from rats hepatectomized 3 days previously as compared with sham-operated rats. This was due to a reduced efficacy of VIP (53 % of control); however, there was no change in the potency of the peptide, which half-maximally stimulated enzyme activity at 0.7

Fig. 1. Effect of increasing concentrations of VIP on adenylate cyclase activity in crude rat hepatic membranes from control (○) or hepatectomized (●) rats

Basal cyclic AMP levels were subtracted from the stimulated data and were 7.3 ± 1.0 and 5.5 ± 0.9 pmol cyclic AMP/min per mg of protein for control and hepatectomized rats respectively. Values are the means $\pm$ S.E.M. of five separate experiments performed in triplicate. The significance of the difference with respect to the corresponding control values is given by * $P < 0.05$.

Fig. 3. Autoradiograph of $[^{32}\text{P}]\text{ADP-ribosylated liver membrane proteins}$

Liver membranes from control rats (lanes A and B) and hepatectomized rats (lanes C and D) were incubated in the presence of $[^{32}\text{P}]\text{NAD}^+$, with (lanes A and C) or without (lanes B and D) cholera toxin. This experiment is representative of three others. For details, see the Materials and methods section.

and 0.8 nM in membranes from control and hepatectomized rats respectively. We also tested the response of adenylate cyclase to forskolin, which is assumed to act directly upon the catalytic subunit of adenylate cyclase [44], and to Gpp[NH]p, which acts via G_s proteins. The ability of both compounds to stimulate enzyme activity was not decreased after hepatectomy (Fig. 2), suggesting that a decreased level of adenylate cyclase or G_s protein was not responsible for the decreased enzyme response to VIP. Further experiments measured the α and β subunit of G_s proteins in regenerating liver. Liver membranes from hepatectomized and control rats were first treated with cholera toxin in the presence of $[^{32}\text{P}]\text{NAD}$ under conditions in which the G_s protein is labelled by ADP-ribosylation of its α subunit [18]. It appeared that cholera toxin induced incorporation of ^{32}P in the M_r 42000 α subunit of G_s in both membrane preparations (Fig. 3). However, the intensity of the labelling was higher in

Fig. 2. Stimulatory effects of forskolin (a) and Gpp[NH]p (b) on adenylate cyclase activity in liver plasma membranes derived from control (○) or hepatectomized (●) rats

Basal levels of cyclic AMP were subtracted from the corresponding stimulated values. Data are expressed as the means $\pm$ S.E.M. of five separate experiments performed in triplicate.

Fig. 4. Immunodetection of the α_s and β subunits of G_s .

Liver membranes from control rats (lanes A and B) and hepatectomized rats (lanes C and D) were resolved on SDS/PAGE as described in the Materials and methods section. Proteins were transferred to nitrocellulose and the immunodetection was achieved using anti- α_s antiserum (A572; lanes A and C) and anti- β antiserum (U49; lanes B and D). This experiment is representative of three others. For details, see the Materials and methods section.

membranes from hepatectomized rats than from controls (Fig. 3), indicating an increase of M_r -42000 cholera toxin substrates during liver regeneration. Immunoblots using anti-(α_s subunit) antibodies also showed that the M_r -42000 α_s subunit was present in higher amount in liver membranes during regeneration (Fig. 4). The same holds true for the β subunits of G proteins, since immunoblots using anti-(β subunit) antibodies showed a greater amount of the M_r -35000 β subunit in membranes from hepatectomized rats than from controls (Fig. 4). Taken together, these experiments support the idea that the G_s protein was overexpressed during liver regeneration and was not responsible for the decreased VIP-stimulated adenylate cyclase activity.

VIP binding to liver membranes was found to be markedly decreased after hepatectomy (Fig. 5). Scatchard analysis [36] of the data gave an upwardly concave curve (results not shown), indicating heterogeneity of binding. The analysis of the binding data by the computer program LIGAND [37] resulted in a best fit for a model of two VIP-binding sites possessing different affinities and capacities. Liver membranes from hepatectomized rats displayed a significant decrease in the maximum VIP-binding capacity without changes in the affinities of the VIP receptor (Table 1). For comparison, we have also investigated

Fig. 5. Specific ^{125}I -VIP binding to liver plasma membranes from control (●) and hepatectomized (○) rats.

Membranes (0.05–0.10 mg of membrane protein/ml) were incubated with 50 pM- ^{125}I -VIP for 30 min at 30 °C in the presence of increasing concentrations of unlabelled VIP. The data are the means $\pm$ S.E.M. of seven separate experiments performed in triplicate. The significance of the difference with respect to the corresponding control values is given by * $P < 0.05$.

VIP receptors in isolated jejuno-ileal and colonic cells from hepatectomized and control rats. It appeared that the two classes of VIP receptors observed in these two cell types displayed an increased B_{max} in hepatectomized rats compared with controls, while the corresponding affinity values were unchanged (Table 1).

Further experiments were conducted to identify VIP receptors at the molecular level in regenerating liver after hepatectomy. For this purpose, the cross-linker DTSP was used to covalently attach ^{125}I -VIP to membranes from hepatectomized and control rats. SDS/PAGE analysis of cross-linked complexes indicated two specifically labelled bands in each membrane preparation, with a much lower labelling in hepatectomized rats than in

Table 1. Characteristics of VIP receptors in control and hepatectomized rats

K_d is the dissociation constant, in nM; B_{max} is the maximum binding capacity, in pmol of VIP/mg of protein. Each value is the mean $\pm$ S.E.M. of 7–8 separate experiments. The significance of the difference with respect to the corresponding control values is given by * $P < 0.05$.

	Control rats		Hepatectomized rats	
	High-affinity receptors	Low-affinity receptors	High-affinity receptors	Low-affinity receptors
Liver membranes				
K_d	0.07 ± 0.01	7.70 ± 0.01	0.05 ± 0.01	6.00 ± 0.60
B_{max}	0.11 ± 0.01	2.30 ± 0.20	$0.05 \pm 0.01^*$	$0.65 \pm 0.03^*$
Jejuno-ileal cells				
K_d	1.90 ± 0.10	94.00 ± 9.90	1.70 ± 0.10	102.00 ± 10.30
B_{max}	0.08 ± 0.01	2.30 ± 0.20	$0.13 \pm 0.01^*$	$4.80 \pm 0.10^*$
Colonic cells				
K_d	2.30 ± 0.40	80.00 ± 5.70	2.10 ± 0.20	77.00 ± 6.60
B_{max}	0.08 ± 0.01	3.30 ± 0.70	$0.12 \pm 0.01^*$	$4.90 \pm 0.20^*$

Fig. 6. Affinity cross-linking of ^{125}I -VIP to liver membranes from control rats (lanes A and B) or hepatectomized rats (lanes C and D)

Membranes were incubated in the presence of ^{125}I -VIP, with (lanes A and D) or without (lanes B and C) $0.3\ \mu\text{M}$ native VIP. Labelled membranes were treated with $1\ \text{mM}$ -DTSP and submitted to SDS/PAGE. This experiment is representative of three others. For details, see the Materials and methods section.

control rats (Fig. 6). Interestingly, the apparent M_r of the cross-linked ^{125}I -VIP-receptor complex represented by the lower band was higher in regenerating liver than in quiescent hepatocytes from control rats, e.g. 58000 versus 53000 respectively. This slight difference was consistently observed in three separate experiments. The minor upper band has been interpreted to be the association of the VIP-receptor complex to a subunit of G_s [19]. As expected, the labelling of the bands was completely abolished by $0.3\ \mu\text{M}$ unlabelled VIP.

DISCUSSION

This study demonstrates that the expression of VIP receptors and the adenylate cyclase response to VIP are reduced during liver regeneration. This extends to a neuropeptide receptor previous observations indicating alterations to receptors for growth factors [45–47] and hormones [26] after hepatectomy. The decreased VIP-stimulated adenylate cyclase activity (Fig. 1) in regenerating liver is most likely related to the observed loss of VIP receptors (Fig. 5). Indeed, the enzyme response to forskolin or Gpp[NH]p is not altered, suggesting that the catalyst of adenylate cyclase and the G_s protein are not involved in the decreased response. Surprisingly, Western blots of α_s and β subunits of G proteins and cholera toxin-catalysed ADP-ribosylation of an M_r -42000 substrate suggest that G_s proteins are overexpressed during liver regeneration. This is apparently inconsistent with the impaired adenylate cyclase response to VIP in liver membranes from partially hepatectomized rats. However, it is known that G proteins are in vast excess over receptors in plasma membranes [48] and that they therefore are not limiting factors in receptor function, even allowing for the fact that many other receptors will use these same coupling proteins.

The reason why VIP receptors are less abundant in regenerating liver than in quiescent adult hepatocytes and the mechanism of this alteration are not known. The mechanism is not known either for any other receptors previously shown to be altered after partial hepatectomy [45–47]. It could be related to a lower level of VIP receptor mRNA due to transcriptional or post-transcriptional control. The recent purification to homogeneity of VIP receptors from porcine liver [14] should provide the means of answering this question. Post-translational alterations in VIP receptors may also result in a decreased number of active receptors after hepatectomy. Such a modification is indicated by

the altered migration of covalently labelled receptors from hepatectomized rats on SDS/PAGE. The apparent M_r of VIP-receptors complexes is higher in membranes from hepatectomized rats (58000) than from control rats (53000). In the ontogenic development of the rat intestine [49] and calf pancreas [50] the marked changes in the stoichiometric parameters of the VIP-receptors complexes, have been also correlated with modifications to the M_r of VIP receptors identified in cross-linking experiments. Previous studies indicated the occurrence of alterations in the glycosylation of proteins during rat liver regeneration [51,52]. It is therefore tempting to speculate that an altered glycosylation of VIP receptors during liver regeneration plays a role in the increase in M_r of VIP receptors. Preliminary results from our laboratory indicate that cross-linked ^{125}I -VIP-receptor complexes from hepatectomized rats have a lower ability to bind to concanavalin A-Sepharose than corresponding complexes from control rats (J. C. Prieto, unpublished work). Further experiments are clearly needed to investigate this issue. Two classes of VIP-binding sites with different affinities were defined when analysing the stoichiometric data, as is also the case in the majority of studies on VIP binding [53], whereas two ^{125}I -VIP-labelled bands were observed in cross-linking studies of liver membranes in both control and hepatectomized rats. The lower band (M_r 53000) has been ascribed to VIP-receptor complexes [14] and the minor upper band to complexes between the VIP receptor and a subunit of G_s [19]. However, further investigations are needed to clarify if the two bands are related to the heterogeneity observed in the VIP binding affinities and capacities.

Whatever the biochemical events underlying the decrease of VIP receptors in regenerating liver, this phenomenon may be related to a ligand-induced down-regulation of receptors. Indeed, VIP induces a down-regulation of its receptors in cultured cells [54,55]. Under normal conditions, low concentrations of VIP are present in portal blood, originating from a washout of VIP released by nerve endings in the gastrointestinal tract [13]. Elevated circulating levels of VIP have been described in liver diseases [56], in accordance with the major role of liver in the inactivation of blood-borne VIP [13]. Whether or not an elevated circulating level of VIP during liver regeneration is responsible for a VIP-induced down-regulation of the hepatic VIP receptors remains to be determined. Such a mechanism may be responsible for the decreased number of liver glucagon receptors after partial hepatectomy [26], which is associated with an elevated circulating glucagon level [57].

Contrary to VIP receptors in the liver, intestinal VIP receptors are slightly increased following hepatectomy. This observation suggests that the decreased expression of VIP receptors in regenerating liver is specific to this organ, and does not favour the idea of a general down-regulation of VIP receptors in the hepatectomized rat. This may be due to the fact that VIP in the intestinal tract is released locally by nerve endings [13], whereas it enters into the liver through the portal blood.

The physiopathological implications of the decrease in the VIP receptor number and VIP stimulation of adenylate cyclase may be related to hepatic proliferation, as suggested by the loss of the stimulatory effect of glucagon adenylate cyclase after hepatectomy [26] or in other proliferative states of the liver [24,25,27]. Since receptors are either decreased [45] or increased [46] after partial hepatectomy, it may be suggested that a fine balance in the expression of various type of neurohormonal receptors, including those for the neuropeptide VIP, is responsible and/or necessary for the proliferation and subsequent differentiation of hepatocytes.

Recent studies by Nair and colleagues [58] have revealed that atrial natriuretic hormone receptors are increased in the

membranes of regenerating livers. Although the physiological role of the atrial natriuretic hormone receptor in the liver is not known, these authors suggested that the regenerating liver system offers a useful model to pursue such studies. The same holds true for VIP receptors, which are decreased during liver regeneration, but whose precise physiological role(s) remains conjectural.

We thank Luis Monje for assistance in the preparation of illustrations. This research was supported by Dirección General de Investigación Científica y Técnica (Grant PM89-96), Actions intégrées Franco-Espagnoles, Accord INSERM/CSIC, Association pour la Recherche sur le Cancer, Fondation pour la Recherche Médicale and Universidad de Alcalá.

REFERENCES

- Magistretti, P. J., Dietl, M. M., Hof, P. R., Martin, J. L., Palacios, J. M., Schaad, N. & Schorderet, M. (1988) *Ann. N. Y. Acad. Sci.* **527**, 110–129
- Sundler, F., Ekblad, E., Grunditz, T., Hakanson, R. & Uddman, R. (1988) *Ann. N. Y. Acad. Sci.* **527**, 143–167
- Said, S. I. (1984) *Peptides* **5**, 143–150
- Sasaki, Y., Kamada, T., Hayashi, N., Sato, N., Kashahara, A., Fusamoto, H., Shiosaka, S., Tohyama, M. & Shiotani, Y. (1984) *Hepatology* **4**, 1184–1189
- Bioulac-Sage, P., Lafon, M. E., Saric, J. & Balandaud, C. (1990) *J. Hepatol.* **10**, 105–112
- Wood, C. L. & Blum, J. J. (1982) *Am. J. Physiol.* **242**, E262–E272
- Feliu, J. E., Mogena, M., Silvestre, R. A., Monge, L. & Marco, J. (1983) *Endocrinology (Baltimore)* **112**, 2120–2127
- Said, S. I. & Mutt, V. (1970) *Science* **169**, 1217–1218
- Kerins, C. & Said, S. I. (1973) *Proc. Soc. Exp. Biol. Med.* **142**, 1014–1017
- Matsumura, M., Akiyoshi, H. & Fujii, S. (1977) *J. Biochem. (Tokyo)* **82**, 1073–1076
- Nyberg, B., Einarsson, K. & Sonnenfeld, T. (1989) *Gastroenterology* **96**, 920–924
- Misbin, R. I., Wolfe, M. N., Morris, P., Buynitzky, S. J. & McGuigan, J. E. (1982) *Am. J. Physiol.* **243**, G103–G111
- Brook, C. W., Sewell, R. B., Shulkes, A. & Smallwood, R. A. (1988) *Regul. Peptides* **20**, 311–322
- Couvineau, A., Voisin, T., Guijarro, L. & Laburthe, M. (1990) *J. Biol. Chem.* **265**, 13386–13390
- Nguyen, T. D., Williams, J. A. & Gray, G. M. (1986) *Biochemistry* **25**, 361–368
- Bataille, D., Freychet, P. & Rosselin, G. (1974) *Endocrinology (Baltimore)* **95**, 713–721
- Desbuquois, B., Laudat, M. H. & Laudat, P. (1973) *Biochem. Biophys. Res. Commun.* **53**, 1187–1194
- Couvineau, A., Amiranoff, B. & Laburthe, M. (1986) *J. Biol. Chem.* **261**, 14482–14489
- Couvineau, A., Rouyer-Fessard, C., Voisin, T. & Laburthe, M. (1990) *Eur. J. Biochem.* **187**, 605–609
- Bucher, N. L. R. & Swaffield, M. N. (1964) *Cancer Res.* **24**, 1611–1625
- Huerta-Bahena, J., Villalobos-Molina, R. & García-Sainz, J. A. (1983) *Biochim. Biophys. Acta* **763**, 112–119
- Ekanger, R., Vintermyr, O. K., Houge, G., Sand, T. E., Scott, J. D., Krebs, E. G., Eikhom, T. S., Christoffersen, T., OGREID, D. & Doskeland, S. O. (1989) *J. Biol. Chem.* **264**, 4374–4382
- Paston, I. H., Johnson, S. & Anderson, W. B. (1975) *Annu. Rev. Biochem.* **44**, 491–522
- Blazquez, E., Rubalcava, B., Montesano, R., Orci, L. & Unger, R. H. (1976) *Endocrinology (Baltimore)* **98**, 1014–1023
- Mourelle, M. & Rubalcava, B. (1981) *J. Biol. Chem.* **256**, 1656–1660
- Leffert, H. L., Alexander, N. M., Faloon, G., Rubalcava, B. & Unger, R. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4033–4036
- Mourelle, M., Morris, H. P. & Rubalcava, B. (1980) *Toxicology* **15**, 173–180
- Laburthe, M., Rousset, M., Rouyer-Fessard, C., Couvineau, A., Chantret, I., Chevalier, G. & Zweibaum, A. (1987) *J. Biol. Chem.* **262**, 10180–10184
- Laburthe, M., Bataille, D. & Rosselin, G. (1977) *Acta Endocrinol.* **84**, 588–599
- Laburthe, M., Rousset, M., Boissard, C., Chevalier, G., Zweibaum, A. & Rosselin, G. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2772–2775
- Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12**, 186–202
- Couvineau, A. & Laburthe, M. (1985) *Biochem. J.* **225**, 473–479
- Scharschmidt, B. F., Keefe, E. B., Blankenship, N. M. & Ockner, R. K. (1979) *J. Lab. Clin. Med.* **93**, 790–799
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Prieto, J. C., Laburthe, M. & Rosselin, G. (1979) *Eur. J. Biochem.* **96**, 229–237
- Scatchard, G. (1949) *Ann. N. Y. Acad. Sci.* **51**, 660–675
- Munson, P. J. & Rodbard, D. (1980) *Anal. Biochem.* **107**, 220–239
- Freychet, P., Roth, J. & Neville, D. M. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1833–1837
- Houslay, M. D., Metcalfe, J. C., Warren, G. B., Hesketh, T. R. & Smith, G. A. (1976) *Biochim. Biophys. Acta* **436**, 489–494
- Gilman, A. G. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **67**, 305–312
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Laburthe, M., Breant, B. & Rouyer-Fessard, C. (1984) *Eur. J. Biochem.* **139**, 181–187
- Mumby, S. M., Kahn, R. A., Manning, D. R. & Gilman, A. G. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 265–269
- Seamon, K. B. & Daly, J. W. (1981) *J. Biol. Chem.* **256**, 9799–9801
- Earp, H. S. & O'Keefe, E. J. (1981) *J. Clin. Invest.* **67**, 1580–1583
- Scott, C. D. & Baxter, R. C. (1990) *Endocrinology (Baltimore)* **126**, 2543–2549
- Gruppiso, P. A., Mead, J. E. & Fausto, N. (1990) *Cancer Res.* **50**, 1464–1469
- Padrell, E., Herberg, J. T., Monsatirsky, B., Floyd, G., Premont, R. T. & Iyengar, R. (1987) *Endocrinology (Baltimore)* **120**, 2316–2325
- Chastre, E., Emami, S., Rosselin, G. & Gespach, C. (1987) *Endocrinology (Baltimore)* **121**, 2211–2221
- Le Meuth, V., Farjandon, N., Bawab, W., Castre, E., Rosselin, G., Guilloteau, P. & Gespach, C. (1991) *Am. J. Physiol.* **260**, G265–G274
- Kato, S. & Akamatsu, E. (1985) *Biochem. J.* **229**, 521–528
- Bartles, J. R., Zhang, L. Q., Verheyen, E. M., Hospodar, K. S., Nehme, C. L. & Fayos, B. E. (1991) *Dev. Biol.* **143**, 258–270
- O'Dorisio, M. S. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, 192–195
- Luis, J., Muller, J. M., Abadie, B., Martin, J. M., Marvaldi, J. & Pichon, J. (1986) *Eur. J. Biochem.* **156**, 631–636
- Boissard, C., Marie, J. C., Hejblum, G., Gespach, C. & Rosselin, G. (1986) *Cancer Res.* **46**, 4406–4413
- Hunt, S., Vaamonde, C. A., Rattassi, T., Berian, M., Said, S. I. & Papper, S. (1979) *Arch. Int. Med.* **139**, 994–996
- Cornell, R. P. (1981) *Am. J. Physiol.* **240**, E112–E118
- Nair, B. G., Steinke, L., Yi-Ming, Y., Rashed, H. M., Seyer, J. M. & Patel, T. B. (1991) *J. Biol. Chem.* **266**, 567–573