ELSEVIER

Biochimica et Biophysica Acta 1312 (1996) 249–254

BBA Biochimica et Biophysica Acta

Characterization of adenylyl cyclase stimulated by VIP in rat and mouse peritoneal macrophage membranes

David Pozo [a], J. José Segura [a], Isabel Carrero [b], Luis G. Guijarro [b], Juan C. Prieto [b], Juan R. Calvo [a,*]

[a] *Departamento de Bioquímica Médica y Biología Molecular, Facultad de Medicina, Universidad de Sevilla, Avda. Sánchez Pizjuán, 4, 41009-Sevilla, Spain*

[b] *Departamento de Bioquímica y Biología Molecular, Universidad de Alcalá, 28871-Alcalá de Henares, Spain*

Received 2 January 1996; revised 22 March 1996; accepted 2 April 1996

Abstract

Vasoactive intestinal peptide (VIP) stimulated adenylyl cyclase activity in rat and mouse peritoneal macrophage membranes. GTP potentiated the stimulatory effect of VIP so that it was routinely included at 10 μM GTP. Other agents like GTP, Gpp(NH)p, GTP-γ-S, sodium fluoride, and forskolin, at a concentration of 0.1 mM, increased the basal activity of enzyme by 3.1, 5.7, 4.7, 3.6, and 7.8-fold, respectively. The stimulation of adenylyl cyclase by VIP was time, temperature, and membrane concentration dependent. Half-maximal enzyme activation (ED_{50}) was very similar in rat and mouse peritoneal macrophage membranes (1.5 ± 0.1 nM and 1.0 ± 0.1 nM, respectively). However, VIP showed more efficacy in mouse macrophages membranes (about 3.1-fold basal values) than that in rat macrophage membranes (about 2.5-fold basal values). The relative potency of several peptides upon stimulation of adenylyl cyclase activity showed the following potency in both species: VIP = $PACAP_{38}$ = $PACAP_{27}$ > helodermin > PHI > secretin. On the other hand, a M_r-45 kDa α_s subunit of G_s protein was demonstrated by both ADP-ribosylation and immunoblot in mouse and rat peritoneal macrophage membranes. The present results, together other previous, strongly suggest that VIP play an important role in the regulation of macrophage function.

Keywords: Vasoactive intestinal peptide; Adenylyl cyclase; G protein; Peritoneal macrophage; (Rat); (Mouse)

1. Introduction

Vasoactive intestinal peptide (VIP) is a 28 amino acid peptide that is produced by the central and peripheral nervous system [1], where it is considered to behave as a neurotransmitter or a neuromodulator [1], and by immune system [2], where it is considered to behave as an important immunoregulatory peptide [3]. The biological effects of VIP on its target cells are triggers by the interaction of peptide with specific plasma membrane receptors coupled to the enzyme adenylyl cyclase and the subsequent cyclic AMP (cAMP) production. In this context, specific receptors for VIP have been demonstrated in human lymphocytes [4,5], human monocytes [6], both mouse and rat lymphocytes [7,8], and more recently, in mouse and rat peritoneal macrophages [9,10], where it has been shown that VIP receptor is a membrane protein of 52 kDa [9,10] and the VIP binding to its receptor is regulated by guanine nucleotides [11]. On the other hand, VIP has been shown to activate adenylyl cyclase in human lymphocyte membranes [12] and to stimulate cyclic AMP production in human lymphocytes [4,5] and in mouse and rat peritoneal macrophages [9,13]. Finally, VIP has been reported to activate cyclic AMP-dependent protein kinase (PKA) in human lymphocytes [14].

At a molecular level, it is well established in the VIP signal transduction pathway that VIP receptors are coupled to the adenylyl cyclase through a stimulatory GTP binding protein G_s [15,16]. In macrophages, though the cAMP production has been well reported, a complete functional and molecular characterization of the adenylyl cyclase activated for VIP never has been realized. The aim of the present report is to study at functional and molecular level the adenylyl cyclase activated for VIP in rat and mouse peritoneal macrophages.

* Corresponding author. Fax: +34 5 4907048.

PII S0167-4889(96)00044-4

2. Materials and methods

Synthetic rat VIP, pituitary adenylyl cyclase activating peptide (PACAP-27 and PACAP-38), porcine peptide histidine isoleucine (PHI), helodermin, secretin, and insulin were purchased from Peninsula Laboratories Europe (Merseyside, UK). Bacitracin, bovine serum albumin (BSA), 3-isobutyl-1-methyl-xanthine (IBMX), ethylenediaminetetraacetic acid (EDTA), *N*-[2-hydroxyethyl]piperazine-*N'*-[2-hydroxypropanesulfonic acid] (Hepes), forskolin, cholera toxin, GTP, guanosine 5'-β,γ-imidotriphosphate [Gpp(NH)p], guanosine 5'-*O*-(3-triphosphate) (GTP-γ-S), ATP, CTP, creatine phosphokinase, and creatine phosphate were from Sigma (Alcobendas, Spain). Phenylmethylsulfonylfluoride (PMSF), *N*-α-tosyl-L-lysine chloromethyl ketone (TLCK) and leupeptine were from Boehringer Mannheim GmbH (Germany). Chemicals and protein markers for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were from Bio-Rad Laboratories (Richmond, CA, USA). Cyclic AMP assay kits were obtained from Radiochemical Center (Amersham, UK). [^{32}P]NAD was from New England Nuclear-Dupont. Specific antisera against C-terminal (385–394) of α_s subunit of G_s protein was from Calbiochem.

2.1. Preparation of macrophage membranes

Rat and mouse peritoneal macrophages were obtained from Wistar rats and Swiss mouse respectively as described previously [9,10]. Quickly, macrophages were resuspended in 5 mM HEPES (pH 7.5 at 4°C) containing 0.1 mg/ml bacitracin, 0.01 mg/ml leupeptine, 0.01 mg/ml TLCK, 0.05 mg/ml PMSF, and 1 mM EDTA. After 15 min incubation at 4°C, cells were disrupted by sonication for two 10-s bursts at maximal power and tune meter separated by 10-s intervals. The homogenate was centrifugated at $600 \times g$ for 10 min at 4°C. The $600 \times g$ supernatant was centrifugated at $30\,000 \times g$ for 30 min at 4°C. The $30\,000 \times g$ pellet was resuspended in 20 mM Hepes (pH 7.5 at 4°C) containing 0.05 mg/ml PMSF and was immediately frozen at −80°C until used. Proteins were measured by the method of Bradford [17] using bovine serum albumin as standard.

2.2. Adenylyl cyclase assay

Adenylyl cyclase activity was measured as described previously [18]. In a standard assay, membranes (50 μg/ml) were incubated in 0.1 ml of 25 mM triethanolamine-HCl buffer (pH 7.5) containing 1 mM 3-isobutyl-1-methyl-xanthine (IBMX), 5 mM $MgSO_4$, 1 mM EDTA, 1 mg/ml bacitracin, 1.5 mM ATP, 10 μM GTP, and a ATP-regenerating system (7.4 mg/ml creatine phosphate and 1 mg/ml creatine kinase) in the absence or presence of different concentrations of VIP. After 20 min incubation at 30°C, the reaction was stopped by the addition of 2.5 ml methanol. The precipitate was removed by centrifugation, aliquots of the supernatant were evaporated and cyclic AMP was measured by a kit cyclic AMP assay system.

2.3. Cholera toxin-catalyzed ADP-ribosylation

ADP-ribosylation of membranes was carried out as previously reported [19] with minor modifications. Thiol preactivated cholera toxin was incubated at 80 μg/ml with membranes (0.8 mg protein/ml) in 0.25 M phosphate buffer (pH 7.0) containing 5 mM ATP, 50 μM GTP, 1 μM [^{32}P]NAD^+, 2 mM EDTA, 5 mM $MgCl_2$, and ATP-regenerating system. After 45 min at 30°C, the reaction was stopped by ice-cold 10% (w/v) trichloroacetic acid. Protein pellets were washed with acetone and solubilized on SDS-gel loading buffer for SDS-PAGE as described [9].

2.4. Immunodetection of α_s subunit of G_s protein

Membranes were solubilized in SDS sample buffer and proteins were run on a 10% SDS-polyacrylamide gel as described [19]. The transfer of proteins to nitrocellulose and the immunodetection of the α_s of G_s protein using a specific antisera against α_s subunit of G_s protein, were carried out as described [19]. Briefly, the transferred 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 was diluted in the same buffer and incubated for 1 h at room temperature. After washing, immunoreactive protein bound was revealed using [^{125}I]-labelled goat antibodies against rabbit IgG and immunoblots were exposed for 3 days at −80°C to a DuPont Cronex-4 film with an intensifying screen (DuPont Cronex Lightning Plus).

3. Results

The general characteristics of rat peritoneal macrophage membrane adenylyl cyclase are showed in Fig. 1. The basal adenylyl cyclase activity was 9.3 ± 0.7 pmol cAMP/min/mg protein. The GTP and its nonhydrolizable analogs Gpp(NH)p and GTP-γ-S, sodium fluoride, and forskolin stimulated the adenylyl cyclase activity in a dose-dependent manner. At a concentration of 0.1 mM, GTP, Gpp(NH)p, GTP-γ-S, sodium fluoride, and forskolin increased the basal activity of enzyme by 3.1, 5.7, 4.7, 3.6, and 7.8-fold, respectively. The corresponding concentrations required for half-maximal stimulation (ED_{50}) were 0.7 μM, 0.5 μM, 0.6 2 μM, 1.5 μM, and 0.6 μM, respectively. On the other hand, other nucleotide such as CTP, was completely ineffective in modifying basal adenylyl cyclase activity when assessed at concentrations as high as 0.1–1 mM.

VIP-stimulated adenylyl cyclase activity was a time-

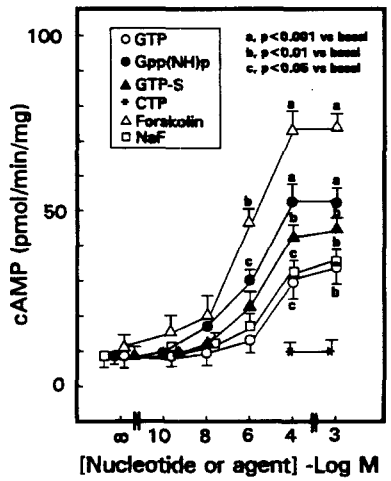

Fig. 1. Effect of increasing concentrations of guanine nucleotides, sodium fluoride and forskolin on the adenylyl cyclase activity of rat peritoneal macrophage membranes. Enzymatic activity is expressed as pmol cAMP formed per min^{-1} mg $protein^{-1}$. Results are the mean of four duplicate experiments. Statistical significance was determined by one-way analysis of variance (ANOVA test) and Bonferroni test.

and temperature-dependent process (Fig. 2) The response was rapid and linear ($r = 0.90$) with time up to 15 min at 30°C and decreased thereafter. At 15°C, the cAMP response decreased in rate and extent, with an apparent steady state reached after 30 min.

Fig. 3 shows the effect of increasing membrane protein concentration on VIP-stimulated adenylyl cyclase. Under standard conditions, the stimulation of adenylyl cyclase activity was linear ($r = 0.98$) with protein concentration up to 50 μg protein/ml. Thus, membrane concentration of 50 μg protein/ml was used in subsequent experiments.

VIP-stimulated adenylyl cyclase activity in a dose-dependent manner both in rat and mouse peritoneal macrophage membranes (Fig. 4). The response occurred in the 0.1–1000 nM range of VIP concentrations. Maximal stimulation was obtained between 0.1–1 μM VIP in both species. However, VIP showed more efficacy in mouse

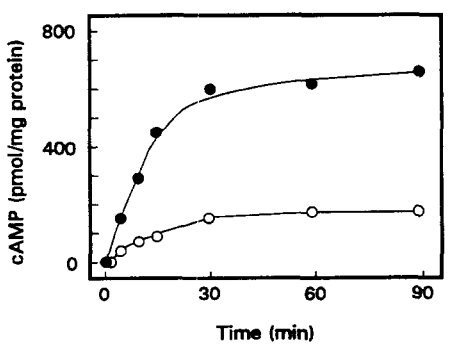

Fig. 2. VIP-induced adenylyl cyclase enzyme activity in rat peritoneal macrophage membranes as a function of incubation time and temperature. Membranes were incubated in the presence of 0.1 μM VIP plus 10 μM GTP at 30°C (●) or 15°C (○). The data correspond to a representative experiment of three performed in duplicated.

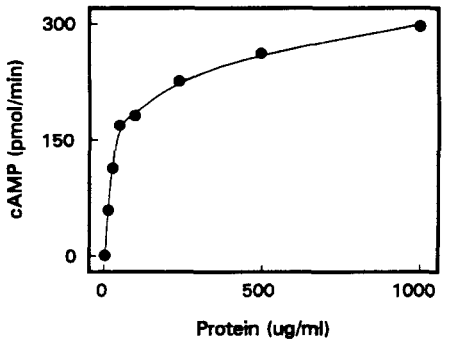

Fig. 3. Effect of protein concentration on adenylyl cyclase activity stimulated by 0.1 μM VIP plus 10 μM GTP. Rat peritoneal macrophage membranes were incubated at 30°C for 15 min. This experiment is representative of two others.

macrophages membranes (about 3.1-fold basal values) than that in rat macrophage membranes (about 2.5-fold basal values). On the other hand, the potency of VIP was very similar in rat and mouse peritoneal macrophage membranes, as indicated by the corresponding ED_{50} values (1.5 ± 0.1 nM and 1.0 ± 0.1 nM, respectively). The effect of VIP on adenylyl cyclase activity in rat and mouse peritoneal macrophage membranes was potentiated by GTP (Fig. 4), since the increase in enzyme activity caused by the nucleotide plus VIP was significantly greater than the sum of the increase caused by each agent acting alone.

The specificity of VIP on adenylyl cyclase activity in rat and mouse peritoneal macrophage membranes was studied using several peptides either structurally related or not to VIP (Fig. 5 and Table 1). The dose-effect curves for peptide stimulation of adenylyl cyclase showed the following potency in both species: VIP = $PACAP_{38}$ = $PACAP_{27}$ > helodermin > PHI > secretin. Under experimental con-

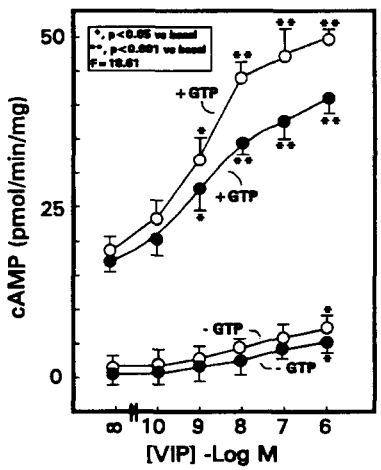

Fig. 4. Dose–effect curves of adenylyl cyclase activity stimulation in rat (●) and mouse (○) peritoneal macrophage membranes by VIP in the absence or presence of GTP 10 μM. Values are the mean of five separate experiments, each performed in duplicate. Statistical significance was determined by one-way analysis of variance (ANOVA test) and Bonferroni test.

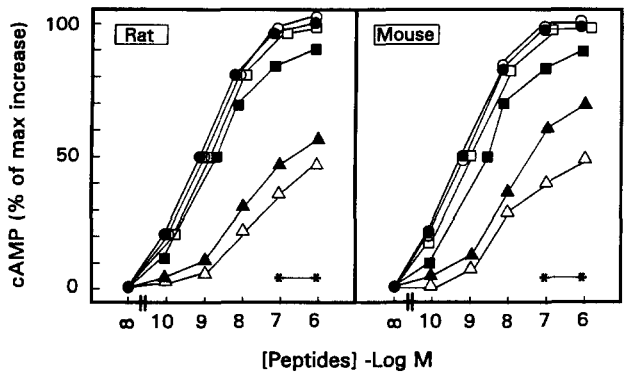

Fig. 5. Effect of increasing concentrations of VIP and other related peptides on adenylyl cyclase activity in rat (left) and mouse (right) peritoneal macrophage membranes. Membranes (50 μg/ml) were incubated for 15 min at 30°C with different concentrations of VIP (●), PACAP-38 (○), PACAP-27 (□), helodermin (■), PHI (▲), secretin (△), and insulin (*) in the presence of 10 μM GTP. Results are expressed as percentage of maximum adenylyl cyclase activity increment above basal. Each point is the mean of three separate experiments performed in duplicate. For clarity, standard errors are not indicated; they are always below 8% of the mean values.

Table 1
Half-maximal stimulation (ED_{50}) of VIP and VIP-family peptides

Peptide	ED_{50} (nM)	
	Rat marophages	Mouse macrophages
VIP	1.5±0.08	1.0±0.11
PACAP-38	1.3±0.1	1.1±0.18
PACAP-27	1.5±0.2	1.3±0.12
Helodermin	2.7±0.1	3.3±0.23
PHI	230±15	171±23
Secretin	1580±251	1420±180

The dose-effect experiments with VIP and VIP-related peptides represented in Fig. 5 served to calculate the corresponding ED_{50} values. The data are the mean±S.E.M. of three separate experiments.

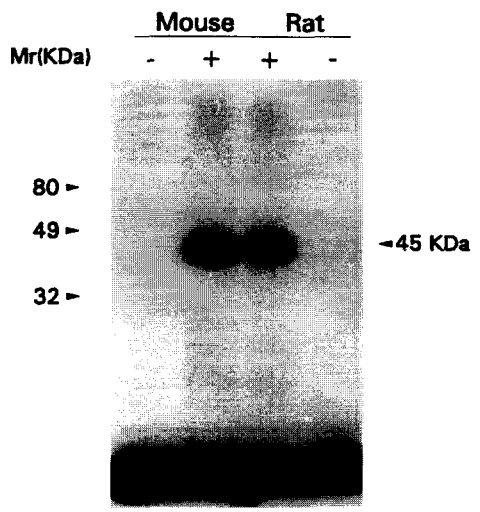

Fig. 6. Autoradiograph of [^{32}P]ADP-rybosylated membrane proteins of mouse and rat peritoneal macrophage membranes. Mouse and rat peritoneal macrophage membranes were incubated with [^{32}P]NAD in the presence (+) or absence (−) of cholera toxin as described in Section 2. This experiment is representative of three others.

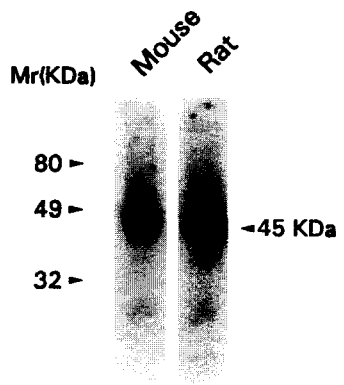

Fig. 7. Immunodetection of α_s subunit of G_s protein of mouse and rat peritoneal macrophage membranes. Plasma membranes from mouse and rat peritoneal macrophages were separated by SDS-PAGE. Proteins were transferred to nitrocellulose and immunoblotted with a specific antisera against C-terminal (385–394) of $G_s\alpha$. This experiment is representative of three others. For details, see Section 2.

ditions used, $PACAP_{38}$, $PACAP_{27}$ or helodermin did not result in a stimulation higher than that obtained with the most efficient peptide (VIP), suggesting that VIP-related peptide acted through a common membrane receptor. Other peptide not structurally related to VIP such as insulin, was ineffective on the stimulation of enzyme activity when assessed at concentrations as high as 0.1–1 μM.

It is well established that adenylyl cyclase activity is stimulated by α_s subunit of stimulatory G protein (G_s). On the other hand, as it is shown in Fig. 4, the stimulatory effect of VIP on adenylyl cyclase activity in rat and mouse peritoneal macrophage membranes was strongly potentiated by GTP. This feature indicates that the functional coupling of VIP receptors to adenylyl cyclase occurs through a G_s protein. Further experiments explored the presence of α_s subunit of G_s protein in rat and mouse peritoneal macrophage membranes by ADP-ribosylation with cholera toxin and by immunoblot with a specific antisera against α_s. Thus, both rat and mouse peritoneal macrophage membranes were treated with cholera toxin in the presence of [^{32}P]NAD under conditions in which the G_s protein is specifically labelled on its α_s subunit by ADP-ribosylation. As it is shown in Fig. 6, cholera toxin induced the labelling by ^{32}P in the M_r-45 kDa α_s subunit in both membrane preparations. As control, no incorporation of ^{32}P radioactivity was detected when the cholera toxin was omitted (Fig. 6). Immunoblot using a anti-α_s subunit antibody also revealed the M_r-45 kDa α_s subunit in both rat and mouse peritoneal macrophage membranes (Fig. 7).

4. Discussion

The present study shows that VIP stimulates in a dose-dependent manner the adenylyl cyclase activity in rat and mouse peritoneal macrophage membranes. Furthermore, in

this report we show experimental evidence that strongly suggests that this stimulation is mediated by a G_s protein. The general characteristics of adenylyl cyclase present in rat peritoneal macrophage membranes, such as its stimulation by GTP, Gpp(NH)p, GTP-γ-S, sodium fluoride, and forskolin are in good agreement with that described in guinea pigs [20]. Maximal stimulation for VIP of the adenylyl cyclase activity was obtained between 0.1–1 μM VIP in both species. However, VIP showed more efficacy in to activate the enzyme in mouse macrophages membranes (about 3.1-fold basal values) than that in rat macrophage membranes (about 2.5-fold basal values). On the other hand, the potency of VIP, as it is indicated by the corresponding half-maximal stimulation (ED_{50}) values, was very similar in rat and mouse peritoneal macrophage membranes (1.5 ± 0.1 nM and 1.0 ± 0.1 nM, respectively). These results are in good agreement with the K_d value of the high-affinity receptors (1.1 ± 0.1 nM in rat and 1.0 ± 0.2 nM in mouse) and with the ED_{50} values for cyclic AMP accumulation (1.2 ± 0.5 nM in rat and 1.0 ± 0.2 nM in mouse) described previously in intact macrophages [9,13,21]. These data suggest that only the binding of VIP with its high-affinity receptors is coupled to the adenylyl cyclase and the subsequent cyclic AMP production. Similar results have been shown for VIP in human lymphocytes [4,5], rat alveolar macrophages [22], and in other cells [23,24].

The effect of VIP upon adenylyl cyclase activity was strongly potentiated by GTP in rat and mouse peritoneal macrophage membranes. This result is in concordance with that observed in other VIP systems such as human lymphocytes [12], rat intestinal membranes [25], and rat seminal vesicle membranes [24]. This feature is other experimental result that suggests that the functional VIP receptors are coupled to adenylyl cyclase through a G_s protein. In fact, we have evidenced the presence of a α_s subunit of 45 kDa by ADP-ribosylation with cholera toxin and by immunoblot with a specific antisera against α_s of G_s protein in both rat and mouse peritoneal macrophage membranes. This G_s protein has been showed by other authors in human lymphocytes [26,27], but it has never been described in rat and mouse peritoneal macrophages. On the other hand, in other tissues such as liver [25], brain [28], pancreas [29], and parotid acini [30], the GTP displayed only a slight potentiating effect on the action of VIP.

Various peptides structurally related to VIP also caused a significant stimulation of adenylyl cyclase activity in rat and mouse peritoneal macrophage membranes. The dose-effect curves for peptide stimulation of adenylyl cyclase showed the following potency in both species: VIP = $PACAP_{38}$ = $PACAP_{27}$ > helodermin > PHI > secretin. Other peptide not structurally related to VIP such as insulin, was ineffective on the stimulation of adenylyl cyclase activity when assessed at concentrations as high as 0.1–1 μM. This pattern of specificity correlates well with the specificity observed in binding and cyclic AMP accumulation studies previously reported in rat and mouse intact macrophages [9,13,21]. Under experimental conditions used, neither $PACAP_{38}$, $PACAP_{27}$, helodermin nor peptide structurally related to VIP, resulted in a stimulation higher than that obtained with the most efficient VIP concentration. An important and interesting feature of these results is that VIP, $PACAP_{38}$, and $PACAP_{27}$ caused a stimulation of adenylyl cyclase in rat and mouse peritoneal macrophage membranes with very similar potency. This datum supports the view that VIP and PACAP acted through a common membrane receptor in both species. The fact that the VIP, $PACAP_{38}$, and $PACAP_{27}$ may share an identical binding site has been previously reported in rat and human liver [31,32], human intestine [33], rat lung [34], and mouse splenocytes [35]. Actually, this common receptor that shows very similar affinities for $PACAP_{27}$, $PACAP_{38}$, and for VIP is denominated VIP type I receptor/PACAP type II receptor and it is clearly different from the so-called PACAP type I receptor observed in rat hypothalamus or rat astrocytes [36,37] that has a high affinity for $PACAP_{27}$ and $PACAP_{38}$ but a very low affinity for VIP.

At present, two types of VIP receptors have been cloned and sequenced. The type 1 receptor (VIP1-R) is the classical VIP receptor and was cloned by screening rat lung [38,39] and the type 2 receptor (VIP2-R), that was cloned from a rat olfactory bulb cDNA library [40], and it seems to be present in regions with neuroendocrine functions where VIP1-R is absent. On the other hand, recently has been cloned a VIP receptor from SUP-T1 lymphoblasts that has 87% sequence homology with the VIP2-R [41]. We consider that the type of VIP receptor involved in the effects showed in this paper is the VIP1-R. In fact, it has been recently shown the gene expression of VIP1-R type in both rat T and B lymphocytes [42]. Furthermore, we have shown for the first time the expression of VIP1-R type in rat peritoneal macrophages [43].

The previous demonstration of the presence of high affinity and specificity VIP receptors in rat and mouse peritoneal macrophages [9,10,21], the sensitivity of VIP binding activity to guanine nucleotides [11], together the ability of VIP to stimulate the adenylyl cyclase activity, argue strongly for a physiological role of VIP in the regulation of macrophage function.

Macrophages play a principal role in many immune functions, but the molecular mechanisms involved are poorly defined. Many hormonally responsive systems are regulated through changes in intracellular cyclic AMP levels. It is well documented that numerous macrophage functions, including lysosomal enzyme secretion [44], phagocytosis [45], cytotoxicity [46], chemotaxis [47], and adhesion [48] are influenced by agents that modulate cyclic AMP levels. With respect to VIP and macrophages, it has been reported that VIP inhibits the respiratory burst in monocytes, the precursor cell of macrophages, and that VIP inhibits substrate adherence capacity of rat peritoneal

macrophage membranes by mechanisms that involve cyclic AMP [49,50]. In this context, our results strongly suggest that VIP could play an important role in regulation of immune function not only acting on lymphocyte functions [51], but also modulating macrophage functions.

Acknowledgements

This work was supported by grant from the DGICYT (PB94-1434).

References

[1] Rostene, W.H. (1984) Prog. Neurobiol. (Oxford) 22, 103–129.
[2] Gomariz R.P., Leceta, J., Garrido, E., Garrido, T. and Delgado M. (1994) Regul. Pept. 50, 177–184.
[3] Calvo, J.R. and Guerrero, J.M. (1993) Trends in Comparat. Biochem. Physiol. 1, 985–998.
[4] Calvo, J.R, Guerrero, J.M., Molinero, P., Blasco, R. and Goberna, R. (1986) Gen. Pharmacol. 17, 185–189.
[5] Guerrero, J.M., Prieto, J.C., Elorza, F.L., Ramírez, R. and Goberna, R. (1981) Mol. Cell. Endocrinol. 21, 151–160.
[6] Wiik, P., Opstad, P.K. and Boyum, A. (1985) Regul. Pept. 12, 145–153.
[7] Ottaway, C.A. and Greenberg, G.R. (1984) J. Immunol. 132, 417–423.
[8] Calvo, J.R., Molinero, P., Jimenez, J., Goberna, R. and Guerrero, J.M. (1986) Peptides 7, 177–181.
[9] Calvo, J.R., Montilla, M.L., Guerrero, J.M. and Segura, J.J. (1994) J. Neuroimmunol. 50, 85–93.
[10] Calvo, J.R., Guerrero, J.M., López-Gonzalez, M.A., Osuna, C. and Segura, J.J. (1994) Peptides 15, 309–315.
[11] Segura, J.J., Guerrero, J.M., Goberna, R. and Calvo, J.R. (1992) Peptides 13, 953–955.
[12] O'Dorisio, M.S., Hermina, N.S., O'Dorisio, T.M. and Balcerzak, P. (1981) J. Immunol. 127, 2551–2556.
[13] Segura, J.J., Guerrero, J.M., Goberna, R. and Calvo, J.R. (1992) Regul. Pept. 37, 195–203.
[14] Guerrero, J.M., Prieto, J.C., Calvo, J.R. and Goberna, R. (1984) Peptides 5, 371–373.
[15] Calvo, J.R., Couvineau, A., Guijarro, L. and Laburthe, M. (1989) Biochemistry 28, 1667–1672.
[16] Couvineau, A., Rouyer-Fessard, C., Voisin, T. and Laburthe, M. (1990) Biochem. J. 187, 605–609.
[17] Bradford, M.A. (1976) Anal. Biochem. 72, 248–252.
[18] Calvo, J.R., Caraballo, M.I., Pozo, D., Segura, J.J., Osuna, C. and Guerrero, J.M. (1995) Peptides 16, 551–557.
[19] Guijarro, L.G., Couvineau, A., Rodríguez-Pena, M.S., Juarranz, M.G., Rodríguez-Henche, N., Arilla, E., Laburthe, M. and Prieto, J.C. (1992) Biochem. J. 285, 515–520.
[20] Verghese, M.W. and Snyderman, R. (1983) J. Immunol. 130, 869–873.
[21] Segura, J.J., Guerrero, J.M., Goberna, R. and Calvo, J.R. (1991) Regul. Pept. 33, 133–143.
[22] Sakakibara, H., Shima, K. and Said, S.I. (1994) Am. J. Physiol. 267, L256–L262.
[23] Carmena, M.J. and Prieto, J.C. (1983) Biochim. Biophys. Acta 763, 414–418.
[24] Rodríguez-Pena, M.S., Guijarro, L.G. and Prieto, J.C. (1991) Peptides 12, 821–824.
[25] Amiranoff, B., Laburthe M. and Rosselin, G. (1980) Life Sci. 26, 1905–1911.
[26] Pessa-Morikawa, T., Mustelin, T. and Anderson, L.C. (1990). J. Immunol. 144:2690–2695.
[27] Fulop Jr, T., Barabas, G., Varga, Z., József, C., Csabina, S., Szucs, S., Seres, I., Szikszay, E., Jeney, Z. and Penyige, A. (1993). Cell. Signal. 5:593–603.
[28] Borghi, C., Nicosia, S., Giachetti, A. and Said, S.I. (1979) Life Sci. 24, 65–70.
[29] Olinger, E.J. and Gardner, J.D. (1979) Gastroenterology 77, 704–713.
[30] Van Bogaert, P., Soukias, Y., Dehaye, J.P., Lambert, M., Poloczek, P., Winaud, J., Mayer, R. and Christophe, J. Regul. Pept. 17, 339–348.
[31] Robberecht, P., Gourlet, P., Cauvin, A., Buscail, L., De Neef, P., Arimura, A. and Christophe, J. (1991) Am. J. Physiol. 260, G97–G102.
[32] Rodríguez-Henche, N., Rodríguez-Pena, M.S., Guijarro, L.G. and Prieto, J.C. (1993) Biochim. Biophys. Acta 1221, 193–198.
[33] Salomon, R., Couvineau, C., Rouyer-Fessard, C., Voisin, T., Lavallée, D., Blais, A., Darmoul, D. and Laburthe, M. (1993). Am. J. Physiol. 264, E294–E300.
[34] Gottschall, P.E., Tatsuno, I., Miyata, A. and Arimura, A. (1990) Endocrinology 127, 272–277.
[35] Tatsuno, I., Gottschall, P.E. and Arimura, A. (1991) Endocrinology 128, 728–734.
[36] Gottschall, P.E., Tatsuno, I. and Arimura, A. (1990) FASEB J. 5, 194–199.
[37] Tatsuno, I., Gottschall, P.E., Koves, K. and Arimura, A. (1990) Biochem. Biophys. Res. Commun. 168, 1027–1033.
[38] Ishiara, T., Shigemoto, R., Mori, K., Takahashi, K. and Nagata, S. (1992) Neuron 8, 811–819.
[39] Sreedharan, S.P., Patel, D.R., Huang, J-X. and Goetzl, E.J. (1993) Biochem. Biophys. Res. Commun. 193, 546–553.
[40] Lutz, E.M., Sheward, W.J., West, K.M., Morrow, J.A., Fink, G. and Harmar, A.J. (1993) FEBS Lett. 334, 3–8.
[41] Svoboda, M., Tastenoy, M., Van Rampelbergh, J., Goossens, J-F., De Neef, P., Waelbroeck, M. and Robberecht, P. (1994) Biochem. Biophys. Res. Commun. 205, 1617–1624.
[42] Gomariz, R.P., Garrido, E., Leceta, J., Martinez, C., Raquel, A. and Delgado, M. (1994) Biochem. Biophys. Res. Commun. 203, 1599–1604.
[43] Delgado, M., Pozo, D., Martinez, C., Garrido, E., Leceta, J., Calvo, J.R. and Gomariz, R.P. (1996) Regul. Pept. in press
[44] Lowrie, D.B., Jackett, B.S. and Ratcliffe, N.A. (1975) Nature 254, 600–602.
[45] Vogel, S.E., Weedon, L.L., Oppenheim, J.J. and Rosenstreich, D.L. (1981) J.Immunol. 126, 441–445.
[46] Schultz, R.M., Pavlidis, M., Stylos, W.A. and Chirigos, M.A. (1978) Sciences 202, 320–321.
[47] Gallin, J.L., Sandler, J.S., Clyman, R.I., Manganiello, V.C. and Vaughan, M. (1978) J. Immunol. 120, 492–496.
[48] Cantarow, W.D., Cheung, H.T. and Sundharadas, G. (1978) Prostaglandins 39, 16–21.
[49] Wiik, P. (1989) Regul. Pept. 25, 187–197.
[50] Segura, J.J., Guerrero, J.M., López-Gonzalez, M.A. and Calvo, J.R. (1993) Cell Adh. Commun. 1, 213–221.
[51] Tseng, J. and O'Dorisio, M.S. (1989) In Neuroimmune networks: Physiology and Diseases (Goetzl, E.S. and Spector, N.H., eds), 105–111, Alan, R.L., New York.