Guanine Nucleotide Regulation of VIP Binding to Rat Peritoneal Macrophage Membranes

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SEGURA, J. J., J. M. GUERRERO, R. GOBERNA AND J. R. CALVO. *Guanine nucleotide regulation of VIP binding to rat peritoneal macrophage membranes*. PEPTIDES 13(5) 953–955, 1992.—In the present study, we have examined the effect of guanine nucleotides on VIP binding to rat peritoneal macrophage membranes. Both guanosine 5'-triphosphate (GTP) and its nonhydrolyzable analog guanosine 5'- β , γ -imidotriphosphate [Gpp(NH)p] inhibited, in a dose-dependent manner, the VIP binding to its specific binding sites. Half-maximal inhibition (IC_{50}) was observed at $5.4 \pm 0.5 \mu M$ GTP. The inhibitory effect of GTP was due to an increase of the dissociation rate of peptide bound to membranes. The specificity of the binding inhibition was assessed from the lack of action of the other nucleotides tested. These results directly suggest the coupling of VIP binding sites with guanine nucleotide binding proteins in rat peritoneal macrophage membranes.

Vasoactive intestinal peptide (VIP) Rat peritoneal macrophages VIP binding sites G-proteins
Guanine nucleotides

ACCUMULATING experimental evidence supports the concept that VIP plays a role in communication between the nervous and immune systems (12). In this context, specific binding sites for VIP have been demonstrated both in human and rat lymphoid cells (3,4,10), as in monocytes (19). Furthermore, recently we have characterized functional specific binding sites for VIP in rat peritoneal macrophages (16,17). In many systems for hormones and neuropeptides it has been well established that guanine nucleotides are involved in the coupling of receptors to their effectors through guanine nucleotide binding proteins. The receptor–guanine nucleotide binding protein interaction is manifested, in the context of binding experiments, as a decrease in agonist affinity in the presence of guanine nucleotides (9). Thus, it has been demonstrated that guanine nucleotides regulate VIP binding to its receptors in several tissues (1,5,7,14). In this paper, we have investigated the regulatory role of guanine nucleotides on VIP-binding site interaction in isolated plasma membranes of rat peritoneal macrophages.

METHOD

Materials

Synthetic rat VIP was purchased from Peninsula Laboratories Europe (Merseyside, UK); ethylenediaminetetraacetic acid (EDTA), *N*-[2-hydroxyethyl]piperazine-*N'*-[2-hydroxypropane-sulfonic acid] (HEPES), bacitracin, bovine serum albumin (BSA), guanosine 5'-triphosphate (GTP), guanosine 5'- β , γ -imidotri-

phosphate [Gpp(NH)p], and other nucleotides were from Sigma (St. Louis, MO); phenylmethylsulfonylfluoride (PMSF), *N* α -p-tosyl-L-lysine chloro-methyl ketone (TLCK), and leupeptin were from Boehringer Mannheim (FRG); carrier-free Na [¹²⁵I] (IMS 30, 100 mCi/ml) was obtained from Radiochemical Center (Amersham, UK). Synthetic rat VIP was radioiodinated by the chloramine-T method to a specific activity of about 800 Ci/mmol (16). Purification of labeled tracer was performed on a Sephadex G-50 column (1 $\times$ 30 cm) eluted with 0.2 M acetic acid containing 0.5% (w/v) BSA and 0.03% (w/v) bacitracin. All other chemicals were reagent grade.

Macrophage Membranes Preparation

Rat peritoneal macrophages were obtained from Wistar rats as described previously (16). Quickly, macrophages were resuspended in 5 mM HEPES (pH 7.5 at 4°C) containing 0.1 mg/ml bacitracin, 0.01 mg/ml leupeptin, 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 tube 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 (2) using bovine serum albumin as standard.

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TABLE 1
EFFECT OF VARIOUS NUCLEOTIDES ON THE EQUILIBRIUM OF [¹²⁵I] VIP SPECIFIC BINDING
TO RAT PERITONEAL MACROPHAGE MEMBRANES

Nucleotide	Concentration (M)							
	10 ⁻¹⁰	10 ⁻⁹	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³
GTP	102 ± 2	94 ± 2*	84 ± 3*	73 ± 5*	57 ± 4*	51 ± 3*	38 ± 4*	29 ± 4*
Gpp(NH)p	103 ± 5	96 ± 4	95 ± 7	90 ± 5	78 ± 4*	50 ± 5*	45 ± 6*	37 ± 5*
GDP	101 ± 3	98 ± 3	94 ± 9	92 ± 6	81 ± 7*	78 ± 5*	69 ± 5*	52 ± 6*
GMP	103 ± 5	102 ± 4	96 ± 5	91 ± 6	89 ± 5*	89 ± 4*	87 ± 8*	81 ± 8*
ATP	104 ± 8	103 ± 9	103 ± 8	101 ± 13	91 ± 7	91 ± 13	65 ± 15	52 ± 9*
CTP	106 ± 6	105 ± 7	103 ± 7	92 ± 12	89 ± 16	85 ± 9	70 ± 13	57 ± 14
UTP	105 ± 6	102 ± 8	102 ± 9	103 ± 7	100 ± 9	82 ± 11	61 ± 16	58 ± 15

Membranes (20 µg/ml) were incubated with 45 pM [¹²⁵I] VIP at 15°C for 60 min, in the absence (control) or presence of increasing concentrations of various nucleotides (10⁻¹⁰ to 10⁻³ M). Results are expressed as percentage of control values. Each value is the mean ± SEM of 15 (GTP), six (GDP, GMP, and GppNHp), or four (ATP, CTP, and UTP) experiments performed in triplicate. Control value: 100%. Statistical significance was determined by Student's *t*-test. A value of *p* < 0.01 (one-tailed) was considered statistically significant. **p* < 0.01.

Binding Studies

In standard conditions, membranes (20 µg/ml) were incubated at 15°C in 0.5 ml of 50 mM Tris-HCl buffer (pH 7.5 at 15°C) containing 1.6% (w/v) bovine serum albumin and 1.2 mg/ml bacitracin with 45 pM [¹²⁵I] VIP and native rat VIP (1 µM). Membrane-bound peptide was separated after 60-min incubation by centrifugation, then washed, and the radioactivity was determined. Data are reported as specific binding, i.e., total tracer bound minus the amount of tracer that was not displaced by 1 µM VIP. Each individual experiment was performed in triplicate.

RESULTS

At apparent equilibrium, by incubating at 15°C during 60 min, the binding of [¹²⁵I] VIP to rat peritoneal macrophage

FIG. 1. Effect of GTP on dissociation of [¹²⁵I] VIP bound to rat peritoneal macrophage membranes. Membranes (20 µg/ml) were incubated at equilibrium (60 min at 15°C) with 45 pM [¹²⁵I] VIP. Then (zero time), dissociation of [¹²⁵I] VIP-receptor complexes was induced at 15°C by the addition of an excess of 10⁻⁷ M native VIP (○), 10⁻⁵ M GTP (■), or buffer alone (●). Results are expressed as percentage of [¹²⁵I] VIP specifically bound at zero time dissociation. Each point is the mean of three separate experiments performed in triplicate. For clarity, standard errors are not indicated; they are always below 12% of the mean values.

membranes was competitively inhibited by native VIP (data not shown). The binding data suggested the presence of both high- and low-affinity binding sites with *K_D* values of 0.60 ± 0.08 and 275 ± 39 nM, respectively, and *B_{max}* of 580 ± 71 and 72,500 ± 810 fmol VIP bound/mg protein, respectively (data not shown).

The specific binding of [¹²⁵I] VIP was sensitive to guanine nucleotides. Thus, GTP inhibited, in a dose-dependent manner and in a wide range of concentrations (0.1 nM to 1 µM), the [¹²⁵I] VIP specifically bound at equilibrium to rat peritoneal macrophage membranes (Table 1). A GTP concentration as low as 1 nM induced a significant inhibition (*p* < 0.01). Half-maximal inhibition (*IC*₅₀) was observed at 5.4 ± 0.5 µM and maximal inhibition was achieved at 1 mM GTP. The inhibitory effect of GTP on the equilibrium of [¹²⁵I] VIP binding to membranes was a rather specific phenomenon (Table 1). Among various nucleotides tested, only GTP and its nonhydrolyzable analog Gpp(NH)p caused a 50% inhibition of tracer binding when they were present in the incubation medium at 10 µM (*p* < 0.01). Both GTP and Gpp(NH)p at 1 mM caused inhibition, at 71% and 63%, respectively. Other nucleotides assessed were practically ineffective to inhibit [¹²⁵I] VIP specific binding at 10 µM.

When further investigated, the inhibitory effect of 10⁻⁵ M GTP on [¹²⁵I] VIP binding to membranes was shown to be due to a dramatic increase in the peptide-receptor complexes dissociation rate (Fig. 1). In the presence of an excess of buffer alone, a slow dissociation was observed. The presence of an excess of VIP (10⁻⁷ M) induced the dissociation of 55% and 70% of peptide-membrane complexes after 30 min and 120 min, respectively. The time course of dissociation did not follow simple first-order kinetics. The dissociation pattern can be explained by the existence of more than one class of VIP binding sites. The dissociation of [¹²⁵I] VIP can be considered as the sum of two first-order processes with apparent rate constants of about 1.5 × 10⁻³ and 3.2 × 10⁻² min⁻¹ for the high-affinity slow dissociating and the low-affinity fast dissociating site, respectively.

The presence of an excess (10⁻⁵ M) of GTP induced the dissociation of 61% and 92% of peptide-membrane complexes after 30 min and 120 min, respectively. Thus, GTP increased the whole dissociation rate but, especially, increased the rate constant of the high-affinity slow dissociating binding site, that was 3.4 × 10⁻³ min⁻¹ in the presence of 10⁻⁵ M GTP.

DISCUSSION

The present study shows that guanine nucleotides regulate the binding of [¹²⁵I] VIP to rat peritoneal macrophage mem-

branes, and this is the first time that is shown a regulation by guanine nucleotides of VIP binding to cells implicated in the immune response. Inhibition of binding was observed in a wide range of GTP concentrations. Half-maximal inhibition (IC_{50}) was observed at $5.4 \pm 0.5 \mu M$, a value similar to those obtained in other VIP receptor systems (5,7). The inhibitory effect of GTP was specific and only Gpp(NH)p, a nonhydrolyzable analog of GTP, has a similar potency. These results are in good agreement with previous reports (5,14). Other nucleotides tested, such as GDP, GMP, ATP, UTP, and CTP, only inhibited the binding of VIP to membranes at concentrations as high as 1 mM, a finding in good accordance with earlier studies (1,5,7,14).

Our data indicate that GTP exerts an inhibitory effect on VIP binding to membranes by increasing the dissociation rate of the peptide-binding site complexes. This effect of GTP has also been observed in other VIP binding systems (5,7,11,14,15). Guanosine 5'-triphosphate was more potent than VIP in inducing VIP-binding site complexes dissociation. Similar results have been previously described in lung membranes (15) and in fetal calf pancreas (11). However, GTP did not increase equally the dissociation rates of the two classes of binding sites. The dissociation rate of the high-affinity binding site was 2.3-fold higher in the presence of 10^{-5} M GTP ($3.4 \times 10^{-3} \text{ min}^{-1}$) than in the presence of 10^{-7} M VIP ($1.5 \times 10^{-3} \text{ min}^{-1}$), whereas that of the low-affinity binding site only was increased 1.3-fold in the presence of GTP. Taking into account that the increase in the dissociation rate caused by GTP is due to a decrease in the receptor affinity for VIP (13), our results suggest that GTP, in rat peritoneal macrophage membranes, decreases especially the affinity

for VIP of the high-affinity binding site population, in accordance with the results found in guinea pig lung membranes (13), where GTP increases the IC_{50} value for VIP, but only increases the K_D for the high-affinity binding site. This effect of GTP increasing the K_D value of high-affinity binding sites could explain its greater potency than VIP in inducing VIP-binding site complexes dissociation.

In many systems for various hormones and neuropeptides, it has been well established that guanine nucleotides are involved in coupling of receptors to their effectors through guanine nucleotide binding proteins (G-proteins). As shown in Table 1, the [^{125}I] VIP binding was effectively inhibited by guanine nucleotides. The order of molar potency for several guanine nucleotide analogues was consistent with that of other receptor systems in which receptors were coupled to G-proteins (20). These results strongly suggest the coupling of VIP binding sites with a G-protein in rat peritoneal macrophage membranes. Since it has been recently described that VIP increases the cyclic AMP production in rat peritoneal macrophages (17), it could be suggested that this effect is mediated by a G_s -protein, as it has been demonstrated in liver (8) and intestine (6). Furthermore, this suggestion is supported by the fact that recently it has been cloned the human VIP receptor in Nalm 6 leukemia preB lymphoblasts (18) and this receptor exhibits 24% homology with other G-protein-coupled receptors.

In conclusion, the results we have presented demonstrating an inhibitory effect of GTP and Gpp(NH)p on VIP binding to rat peritoneal macrophage membranes provide direct evidence of an interaction between the VIP binding site and a G-protein, presumably G_s .

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