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Guanyl Nucleotide Regulation of Vasoactive Intestinal Peptide Interaction with Rat Liver Membranes

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This study shows the regulatory role of guanyl nucleotides on vasoactive intestinal peptide (VIP) interaction with rat liver plasma membranes. The binding of ^{125}I-VIP to the membranes was partially inhibited by GTP and the GTP-analog Gpp(NH)p in a dose-dependent manner. This effect was mainly due to a dramatic increase of the dissociation rate of the complex tracer-membranes in the presence of guanyl nucleotides. The specificity of the binding inhibition was assessed from the lack of action of the other purine nucleotides tested.

VIP stimulated cyclic AMP production in liver plasma membranes in the range $10^{-10} - 3 \times 10^{-7}$ M. Half-maximal stimulation was observed at 3×10^{-10} M and maximal stimulation (4-fold basal value) at 10^{-9} M VIP. Both GTP and Gpp(NH)p potentiated VIP-stimulated cyclic AMP production since the effect of nucleotide plus VIP was greater than the sum of the effects produced by the two agents separately. Therefore, guanyl nucleotides simultaneously inhibit the binding of VIP to its receptors and potentiate the stimulatory effect of VIP on adenylate cyclase activity in rat liver plasma membranes.

Hormone-responsive adenylate cyclase is today considered (1, 5, 19) as a system consisting at least of three physically distinct units: *a*) the receptor component located at the outer membrane surface and containing a specific site for binding of hormones (glucagon, ACTH, etc.) and neurotransmitters (opiates, dopamine, etc.); *b*) the guanyl nucleotide regulatory component which binds GTP and mediates the activity of the catalytic unit; and *c*) the catalytic unit at the inner face of the membrane bearing the site responsible for cyclic AMP production. The

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formation of the complex hormone-receptor allows the GTP-regulatory unit to react with GTP, thus resulting in a modification of adenylate cyclase activity (activation or inhibition).

The characteristics of vasoactive intestinal peptide (VIP) receptors in rat liver plasma membranes have been described in a previous report from this laboratory (11). A simultaneous effect of guanyl nucleotides on VIP binding to receptors and activation of adenylate cyclase have not yet been described in the literature. In fact, it has been demonstrated that guanyl nucleotides regulate VIP binding to liver (8), brain (16) and intestinal epithelial (3) receptors as well as VIP activation of pituitary (17) and exocrine pancreas (15) adenylate cyclase.

The present article shows the importance of guanyl nucleotides in regulating the interaction of VIP with rat liver plasma membranes. The action mechanisms of guanyl nucleotides have been studied at both VIP receptors and adenylate cyclase steps.

Materials and Methods

Isolation of rat liver plasma membranes, determination of 5′-mononucleotidase and proteins, preparation of ^{125}I-labelled VIP (^{125}I-VIP) and studies on VIP binding to receptors were performed as described in the preceding paper (11).

VIP-induced cyclic AMP production by liver membranes was studied in a standard incubation solution (250 μl final volume) consisting of 50 mM Tris-HCl (pH 7.5), 2 mg/ml bovine serum albumin (BSA), 1 mg/ml bacitracin, 5 mM $MgCl_2$, 0.2 mM 3-isobutyl-1-methylxanthine (IBMX), 1 mM EDTA, 0.1 mM ATP, an ATP-regenerating system (0.1 mg/ml phosphocreatine kinase, 2 mM creatine phosphate) and increasing concentrations of VIP (up to 0.3 μM). The reaction was started with the addition of liver membranes (100 μg protein/ml). Incubations were performed at 15° C for 45 min (maximal cyclic AMP production was observed between 30 and 90 min; data not shown). The reaction was stopped by the addition of 1.25 ml methanol; after removing the precipitate by centrifugation, aliquots of the supernatant were evaporated and cyclic AMP measured by a protein binding assay (10) using a protein kinase isolated from rabbit skeletal muscle (21).

In addition to the substances listed in the preceding paper (11), phosphocreatine kinase and creatine phosphate were purchased from Boehringer, IBMX from Aldrich and the nucleotides guanosine 5′-(β,γ-imido)-triphosphate or Gpp(NH)p, GTP, GDP, GMP, cyclic GMP, ATP, ADP, AMP and cyclic AMP from Sigma. All ather chemicals were reagent grade.

Results

Association studies. Figure 1 (top) shows the time-course of specific binding of ^{125}I-VIP to isolated liver plasma membranes both in the absence and in the presence of guanyl nucleotides. The percentage of nonspecific binding was unaffected by guanyl nucleotides and remained constant throughout the incubation period (about 3-4 % of the total radioactivity added). Both GTP and Gpp(NH)p at 10^{-5} M decreased markedly the equilibrium binding of ^{125}I-VIP. The inhibition of the interaction was already observed after 5 min incubation and was higher with GTP than with Gpp(NH)p. When tracer binding was expressed as the percentage of the maximum value in each of the three conditions studied, the patterns of association were very similar (not shown). This suggests that the initial rate of binding is probably not affected by guanyl nucleotides. Then, the differences in magnitude among the three binding patterns could be due to a guanyl nucleo-

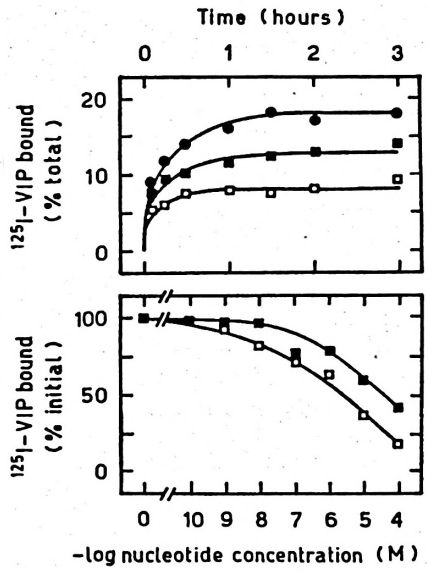

Fig. 1. *Effects of guanyl nucleotides on binding of ^{125}I-VIP to liver membranes.*
Top: membranes were incubated with ^{125}I-VIP at 15° C in the absence (●) and in the presence of 10^{-5} M GTP (□) or Gpp(NH)p (■); the specific binding of the tracer was determined at various periods of time. Bottom: dose-response of GTP and Gpp(NH)p on ^{125}I-VIP binding after 90 min incubation; results are expressed as the percentaje of the radioactivity specifically bound in the absence of nucleotide. Each point is the mean of triplicates.

Table I. *Effect of purine nucleotides on equilibrium of ^{125}I-VIP binding to liver plasma membranes.*
Liver membranes and ^{125}I-VIP were incubated for 90 min at 15° C as previously described (11). The effect of various nucleotides on specific tracer binding is expressed as the percentage of radioactivity bound in the presence of tracer only. Each value is the mean of triplicates. Control value: 100 %.

Nucleotides	Concentration of nucleotides, M		
	10^{-9}	10^{-7}	10^{-5}
Gpp(NH)p	100	74	43
GTP	96	72	23
GDP	102	—	54
GMP	92	88	—
Cyclic GMP	106	98	100
ATP	90	86	76
ADP	102	106	99
AMP	107	99	86
Cyclic AMP	103	109	93

tide-induced increase of the dissociation rate of the bound peptide.

The inhibitory effect of guanyl nucleotides upon ^{125}I-VIP binding to liver membranes was dose-dependent (fig. 1, bottom). The amount of tracer bound at equilibrium was studied in the presence of increasing concentrations of guanyl nucleotides from 10^{-10} to 10^{-4} M. Concentrations of GTP and Gpp(NH)p as low as 10^{-8}-10^{-7} M induced a significant inhibition; maximal effects were observed at 10^{-4} M.

The inhibition of ^{125}I-VIP binding was a rather specific phenomenom (table I). Among some purine mono-, di- and tri-nucleotides as well as cyclic nucleotides tested, only GTP, Gpp(NH)p and, to a lesser extent, GDP were effective in this respect. A small action of ATP at 10^{-5} M can be probably attributed to a contamination of the ATP preparation by GTP, as it has been previously suggested in other systems (20).

Dissociation studies. When further investigated, the inhibitory effects of 10^{-5} M GTP and Gpp(NH)p on binding of ^{125}I-VIP to liver plasma membranes were shown to be due to a dramatic increase in the dissociation rate of the peptide-membrane complex (fig. 2, top). In control conditions, a lapse of up to 2 hours was necessary for dissociation of 50 % of the ^{125}I-VIP specifically bound to membranes at equilibrium. In the presence of guanyl nucleotides, the time for half-dissociation was only about 5 min for GTP and 15 min for Gpp(NH)p, respectively.

The increase of the dissociation rate was also a dose-dependent process (fig. 2, bottom). The effect was noticeable at

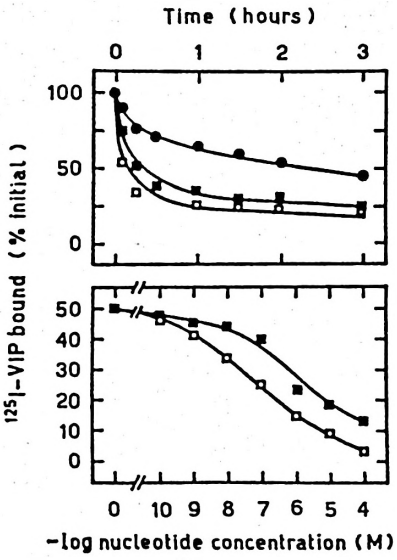

Fig. 2. *Effects of guanyl nucleotides on dissociation of ^{125}I-VIP bound to liver membranes.* Top: membranes were incubated with ^{125}I-VIP for 90 min at 15° C; dissociation of tracer from membranes was induced by the addition of an excess (1 μM) of native VIP, as previously described (11). Experiments were performed in the absence (●) and in the presence of 10^{-4} M GTP (□) or Gpp(NH)p (■); results are expressed as the percentage of radioactivity specifically bound at zero time dissociation. Bottom: dose-response of GTP and Gpp(NH)p on dissociation of ^{125}I-VIP; results are expressed as the ratio of the amount of ^{125}I-VIP bound after 60 min dissociation and that zero time (× 100). Each value is the mean of triplicates.

guanyl nucleotide concentrations of 10^{-9}-10^{-8} M and was maximal at 10^{-4} M. As summarized in table II, only GTP, Gpp(NH)p and GDP accelerated the dissociation of peptide from membranes, demonstrating the specificity of the effect. Other purine nucleotides tested were inactive as they were in association experiments.

Adenylate cyclase-activation studies. VIP activated adenylate cyclase of liver

Table II. *Effect of purine nucleotides on dissociation of ^{125}I-VIP bound to liver plasma membranes.*
Liver membranes and ^{125}I-VIP were incubated for 90 min at 15° C; dissociation of tracer from membranes was induced by the addition of an excess (1 μM) of native VIP, as previously described (11). The effect of various nucleotides on dissociation is expressed as the ratio of the amount of ^{125}I-VIP bound after 60 min dissociation and that at zero time (×100). Each value is the mean of triplicates. Control value: 53 %.

Nucleotides	Concentration of nucleotides, M		
	10^{-9}	10^{-7}	10^{-5}
Gpp(NH)p	49	42	14
GTP	46	26	12
GDP	49	27	11
GMP	50	40	41
Cyclic GMP	48	41	40
ATP	58	51	36
ADP	49	48	37
AMP	52	50	50
Cyclic AMP	45	44	41

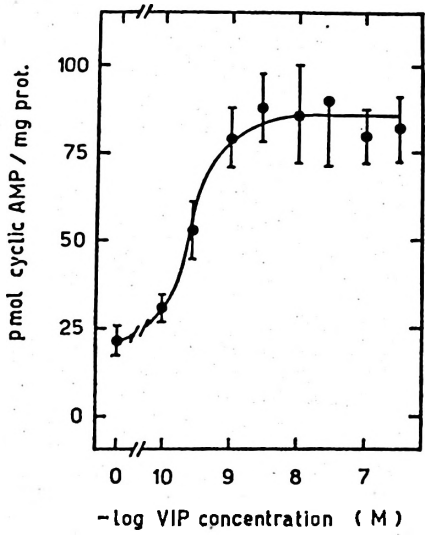

Fig. 3. *Effect of VIP on cyclic AMP production in liver membranes.* Membranes were incubated with increasing concentrations of VIP for 45 min at 15° C. The dose-effect curve is the mean ± S.E.M. of 3 experiments performed in triplicate.

plasma membranes in a dose-dependent manner (fig. 3). The concentration of VIP giving half-maximal stimulation was approximately 3×10^{-10} M. The maximal VIP-stimulated cyclic AMP production was about 4-fold higher than the basal enzyme activity and was observed at a VIP concentration as low as 10^{-9} M. VIP concentrations in the range 3×10^{-9} M-3×10^{-7} M induced cyclic AMP production extents similar to those obtained with 10^{-9} M VIP.

Both GTP and Gpp(NH)p at 10^{-5} M stimulated adenylate cyclase activity in the absence of VIP (fig. 4) since GTP induced a 4-fold and Gpp(NH)p a 7-fold increase of the basal cyclic AMP production. The effect of these two guanyl nucleotides was studied at a constant concentration (10^{-5} M) in the presence of increasing concentrations of VIP (10^{-10}-10^{-7} M). It was demonstrated that both nucleotides potentiated VIP stimulation of cyclic AMP production throughout the whole range of VIP concentrations. The effect was not additive since the three patterns of adenylate cyclase activation were clearly not parallel.

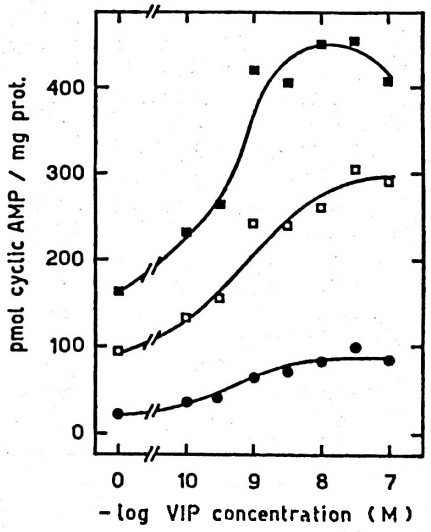

Fig. 4. *Effect of guanyl nucleotides on VIP-stimulated cyclic AMP production in liver membranes.*
Membranes were incubated with increasing concentrations of VIP for 45 min at 15° C. Experiments were performed in the absence (●) and in the presence of 10^{-5} M GTP (□) or Gpp(NH)p (■). Each point is the mean of triplicates.

Discussion

This study provides substantial evidence that guanyl nucleotides modify the binding of ^{125}I-VIP to liver membranes essentially by increasing the dissociation of the peptide-binding sites complex. This effect, observed at as low as 10^{-8}-10^{-7} M concentrations of GTP and Gpp(NH)p, was specific since, apart from GDP, other purine nucleotides tested were ineffective. These results are in good agreement with other reports on VIP-receptor interaction in brain (16) and intestinal epithelial (3) plasma membranes; an inhibition of ^{125}I-VIP binding to liver plasma membranes were reported previously (8) but it had not been studied in detail.

As expected, adenylate cyclase activity in liver plasma membranes was increased by VIP in a dose-dependent manner. Half-maximal stimulation was observed at a VIP concentration of 3×10^{-10} M, that corresponds closely to that (4.8×10^{-10} M VIP) needed for half-maximal inhibition of ^{125}I-VIP binding to the same membrane preparation (11). This finding suggests a functional coupling between VIP receptors and adenylate cyclase in liver plasma membranes. The calculated K_m value is practically identical to those reported for VIP in similar preparations (4, 9) but it is 10-fold lower than that in rat intestinal epithelial cell membranes (2) and 100-fold lower than that in guinea pig brain membranes (17). This fact demonstrates that adenylate cyclase in liver membranes is remarkably sensible to VIP and/or that the other membrane systems cited above conserve a relatively high quantity of VIP

throughout the isolation procedure. In this context, it is interesting to note that, for unknown reasons, VIP has been found to be ineffective in altering basal levels of cyclic AMP in isolated liver cells (12).

There is increasing evidence (6, 7) suggesting that peptide binding sites and adenylate cyclase are separate molecular entities that do not need to be present in stoichiometric amounts in the membrane or even normally juxtaposed within the plane of the membrane. In the case of VIP, this hypothesis has been demonstrated to be correct by the selective transfer of the VIP receptor from an intestinal cell line to an adenylate cyclase in another cell (13). The problem arises when it is intended to explain the functional coupling between both receptor and catalytic units. Present data show a guanyl nucleotide regulation of VIP-responsive adenyl cyclase in liver plasma membranes. Both GTP and Gpp(NH)p potentiated the increase in adenylate cyclase activity caused by VIP in that the increase in enzyme activity caused by nucleotides plus VIP was greater than the sum of the increase caused by each agent acting alone. The regulatory role of guanyl nucleotides upon VIP-stimulated cyclic AMP production has also been described in rat anterior pituitary (17) and pancreatic acinar (15) plasma membranes.

In conclusion, guanyl nucleotides simultaneously inhibit the binding of the peptide to its receptors and stimulate adenylate cyclase activity in rat liver plasma membranes. This is the first report that studies the opposite effect of guanyl nucleotides on VIP interaction at both levels of receptor and effector in a determinate plasma membrane preparation. Guanyl nucleotides appear to regulate the interconversion between the so called «high» and «low» affinity binding sites leading to different extents of adenylate cyclase activation. This explains why Gpp(NH)p was less effective than GTP in inhibiting ^{125}I-VIP but was more powerful than GTP in potentiating VIP-stimulated cyclic AMP production. Similar results were previously obtained for the interaction of glucagon with liver membranes (14, 18) as well as for other systems (19) in which the role of peptide and non-peptide receptors and GTP-regulatory proteins in membrane transduction were studied.

Resumen

Se muestra el papel regulador de los guanil nucleótidos sobre la interacción del péptido intestinal vasoactivo (VIP) con membranas plasmáticas de hígado de rata. La unión de I^{125}-VIP a las membranas fue inhibida parcialmente por GTP y por un análogo de GTP, Gpp(NH)p, dependiente de la dosis. Este efecto se debió principalmente a un espectacular aumento de la velocidad de disociación del complejo trazador-membranas en presencia de guanil nucleótidos. La especificidad de la inhibición del proceso de unión se demostró por la falta de actividad de otros nucleótidos de purina.

El VIP estimuló la producción de AMP cíclico por membranas plasmáticas hepáticas en el rango 10^{-10}-3×10^{-7} M. El 50 % de la estimulación máxima se observó a 3×10^{-10} M VIP y la estimulación máxima (4 veces el valor basal) a 10^{-9} M VIP. Tanto el GTP como el Gpp(NH)p potenciaron la producción de AMP cíclico estimulada por VIP, ya que el efecto del nucleótido más VIP fue mayor que la suma de efectos de los dos agentes actuando por separado. Por tanto, los guanil nucleótidos inhiben simultáneamente la unión del VIP sobre la actividad de la adenil ciclasa en membranas plasmáticas de hígado de rata.

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