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DIFFERENTIAL EFFECTS OF GUANINE NUCLEOTIDES ON THE FIRST STEP OF VIP AND GLUCAGON ACTION IN MEMBRANES FROM LIVER CELLS.

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SUMMARY: Despite the presence of a similar number of glucagon and VIP receptors in liver membranes, VIP induces a negligeable stimulation of adenylate cyclase when compared with glucagon effect. In order to elucidate these discrepancies, the effects of guanine nucleotides on the VIP and glucagon-responsive adenylate cyclase of liver were compared using pure ATP as substrate. 10^{-8} M VIP accounted for a 1.5-fold increase of basal activity. In the presence of GTP or Gpp(NH)p (10^{-9} to 10^{-5} M), the level of cAMP production induced by VIP was no more than additive. In contrast, Gpp(NH)p potentiated the effect of glucagon on liver adenylate cyclase. These discrepancies are not explained by a difference in the peptide binding process. These data suggest that, in liver membranes, a GTP-binding protein N_2 is associated with the glucagon-sensitive adenylate cyclase, but is not detected for VIP. It is suggested that N_2 appears to be specific for the peptidic receptor.

Specific binding sites for vasoactive intestinal peptide (VIP) were discovered in liver plasma membranes (1, 2) and further characterized in liver cells (3). The number of specific binding sites for VIP and glucagon was found similar and their respective affinity was higher for VIP than for glucagon (3). These features were not reflected in the pattern of membrane-bound adenylate cyclase stimulation by either peptide since VIP was shown to elicite a response which was 15% that of maximal response induced by glucagon (1, 2). Similarly, in the same preparation of liver cells, glucagon increased basal cyclic AMP level about 15 times, whereas VIP was very poorly effective (3). These data indicate that VIP binding sites are not coupled to liver adenylate cyclase as are glucagon binding sites.

Since binding of VIP to several other tissues have been shown to result in a remarkable stimulation of membrane adenylate cyclase (4), it appears that in liver, the coupling of VIP binding sites to adenylate cyclase is prevented. Whether this is related to a GTP-regulatory component might be suggested. Indeed, as first described

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by Rodbell et al. (5) for the glucagon-sensitive adenylate cyclase of liver, guanine nucleotides are obligatorily implicated in the coupling process between hormone binding and adenylate cyclase stimulation. Such an essential role of guanine nucleotides has been demonstrated in the first step of VIP action on intestinal epithelial cell (6, 7) but is not reported for the mechanism of VIP action on liver.

The present study was designed to investigate the role of guanine nucleotides on the first step of VIP action in liver plasma membranes, i.e. the interaction of VIP with its binding sites and the activation of adenylate cyclase. Data are compared with those obtained in the presence of glucagon, as control of the experiment.

MATERIALS AND METHODS

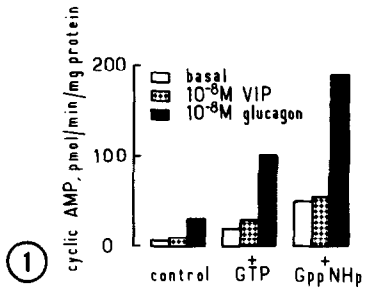
GTP and the non hydrolyzable nucleotide, Gpp(NH)p were from Sigma. Porcine VIP was a gift of the National Institute of Health (USA) and Professor V. Mutt (Karolinska Institute, Stockholm). Pancreatic porcine glucagon was a gift from R.J. Schlichtkrull (Novo Research Institute, Copenhagen).

The purified liver plasma membranes fraction from rat (stepp 11 in reference 8) was used in all studies, Adenylate cyclase was assayed as described (9) with the following modifications previously reported (7): 0.3 mM pure ATP was used as substrate and 0.2 mM 3-isobutyl-1-methylxanthine was used as inhibitor of phosphodiesterase. Studies of binding of ^{125}I-peptide to membranes were conducted as described (2).

RESULTS

Effects of guanine nucleotides on liver adenylate cyclase (Fig.1).

GTP and Gpp(NH)p (10^{-5} M) induced a 3 and 9 fold increase of basal activity, respectively. VIP, tested alone at 10^{-8} M, induced a significant 1.5 fold stimulation of cyclic AMP production over basal activity. When VIP is tested in the presence of GTP and/or Gpp(NH)p, the stimulation evoked by the combination of the nucleotide and VIP (30.6 $\pm$ 1.24 or 57.88 $\pm$ 0.7 pmol cAMP/min/mg protein, respectively) was not significantly different from the sum of the effects of each compound added separately (26.34 $\pm$ 1.8 or 59.22 $\pm$ 1.7 pmol cAMP/min/mg protein, respectively) (NS). This was observed whatever the concentration of VIP tested, in the range of 10^{-10} M - 10^{-7} M(data not shown). In contrast, the amount of cyclic AMP produced by glucagon in the presence of GTP or Gpp(NH)p (99.5 $\pm$ 2.7 or 185.5 $\pm$ 2.2 pmol cAMP/min/mg protein, respectively) was significantly higher than the sum of the effects of each substance added separately (50.1 $\pm$ 2.3 or 91.3 $\pm$ 1.6 pmol cAMP/min/mg protein, respectively) ($p<0.001$),



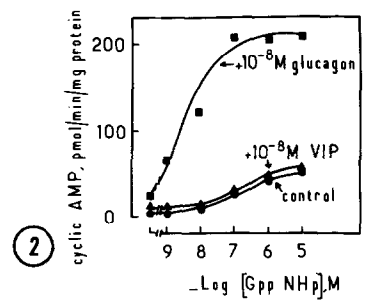


Figure 1: Effects of 10^{-5} M GTP and Gpp(NH)p on the basal, VIP and glucagon-sensitive adenylate cyclase of liver membranes. Results are mean of triplicate. Another experiment gave similar results.

Figure 2: Effects of increasing concentrations of Gpp(NH)p on liver adenylate cyclase measured in the absence (●) and in the presence of 10^{-8} M VIP (▲) or 10^{-8} M glucagon (■). Results are mean of triplicate. Another experiment gave similar results.

indicating the potentiating effect of guanine nucleotides on the action of glucagon on liver adenylate cyclase, in agreement with Rodbell et al. (10).

Differential effects of guanine nucleotides on VIP and glucagon action are confirmed by the study of the dose-response of Gpp(NH)p on the basal and VIP or glucagon-stimulated liver adenylate cyclase (Fig.2). Gpp(NH)p increased adenylate cyclase of liver in a large range of concentrations from 10^{-9} to 10^{-5} M; the effect of 10^{-8} M VIP in stimulating adenylate cyclase was only additive of that of Gpp(NH)p. At the opposite, potentiation of the effect of 10^{-8} M glucagon by Cpp(NH)p was remarkable at all the concentrations tested and significant at a dose of Gpp(NH)p as low as 10^{-9} M.

These results indicate that a GTP-binding protein is associated with the glucagon-responsive adenylate cyclase of liver membranes as previously described (5), but is not detected for the VIP-responsive adenylate cyclase.

Effects of guanine nucleotides on binding of glucagon and VIP to liver membranes. Further experiments were carried out to verify if the lack of potentiating effects of guanine nucleotides on the VIP-sensitive adenylate cyclase was not related to an alteration of GTP action on the VIP binding process (Fig.3). GTP and Gpp(NH)p from 10^{-7} to 10^{-4} M inhibited binding of labelled VIP, 50% inhibition of total binding measured at equilibrium being induced by about 3.10^{-5} M nucleotides. GTP was found slightly more efficient than Gpp(NH)p. Binding of glucagon

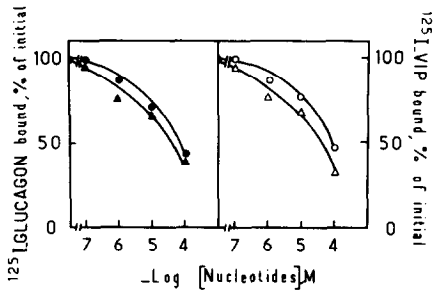


Figure 3: Effects of increasing concentrations of guanine nucleotides on binding of ^{125}I-glucagon (closed symbols) and ^{125}I-VIP (open symbols) to liver membranes. Binding of labeled peptides was tested at 30° C after 30 minutes incubation, in the presence of GTP (▲ △) or Gpp(NH)p (● ○). Results are mean of two experiments performed in triplicate.

was inhibited by nucleotides in the same range of concentrations than those inhibiting binding of VIP.

These results suggest that as for the glucagon receptor, a GTP-binding protein is associated with the VIP receptors of liver membranes.

DISCUSSION

Our data indicate that guanine nucleotides exert an inhibitory effect on the binding of VIP to liver membranes but they do not influence the action of the peptide on adenylate cyclase. In the same liver membranes preparation, as previously described (5), binding of glucagon was inhibited and glucagon-induced adenylate cyclase was highly enhanced by guanine nucleotides. Thus, the different efficiencies of VIP and glucagon in stimulating the liver adenylate cyclase (1, 2) is associated with a differential potentiating effect of GTP on the peptides-responsive enzyme.

Further features indicate that the modulation of the adenylate cyclase activation by VIP does not depend only on the characteristics of the VIP binding sites. Indeed, in the same species, intestinal epithelial and liver cells exhibit VIP binding sites that are identical by their number, affinity and specificity (3, 11), but interaction of VIP with binding sites of intestinal membranes leads to activation of adenylate cyclase (12, 13) whereas this process does not occur in liver membranes.

By cell fusion experiment, it was recently demonstrated that the VIP binding site could be transferred from a human colonic carcinoma cell line and could activate the adenylate cyclase of another cell line (14). This result, in agreement with the mobile receptor concept proposed by

the group of Cuatrecasas (15) indicated that the VIP binding site and adenylate cyclase could be physically separated and consequently regulated by different porcesses. Our results indicate that the discrepancies in the effect of VIP on adenylate cyclase of liver and intestinal membranes, may be related to the pattern of guanine nucleotides regulatory action at the level of the enzyme. Indeed, in contrast with the VIP-responsive adenylate cyclase of liver membranes, the activation by VIP of intestinal adenylate cyclase was shown to be highly regulated by guanine nucleotides: GTP potentiated the effect of VIP on adenylate cyclase and increased the sensitivity of the enzyme for the VIP binding sites (7). This indicated the presence of a guanine nucleotide regulatory site associated with the catalytic unit of the VIP-sensitive intestinal adenylate cyclase. This site termed N_2 according to the nomenclature of Rodbell (5) does not appear to be present or effective in the liver membranes.

Whether this component N_2, which is implicated in the coupling process of peptide binding site with adenylate cyclase,as strongly suggested (16) is specific for the peptidic receptor is to elucidate. Nevertheless, the fact that liver adenylate cyclase is preferentially stimulated by glucagon or catecholamines (17) and poorly regulated by VIP provides strong arguments in favor of such a specificity. This could explain why the N_2 component which determines activation of liver adenylate cyclase by glucagon does not fit in with the VIP binding sites and thus, does not interfere with the VIP-responsive adenylate cyclase.

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