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# THE GIP RECEPTOR ON PANCREATIC BETA CELL TUMOR : MOLECULAR IDENTIFICATION BY COVALENT CROSS-LINKING

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$^{125}$I-GIP binds reversibly to a high affinity binding site in crude plasma membranes prepared from a hamster pancreatic ß cell tumor. The treatment of labeled membranes with the cross-linker dithiobis (succinimidylpropionate) prevents, to a great extent, the rapid dissociation of $^{125}$I-GIP-membrane complexes which is observed when $10^{-6}$ M native GIP is added. Polyacrylamide gel electrophoresis of membrane proteins reveals a major $^{125}$I-GIP-protein complex of Mr 64,000. This labeling decreases when increasing concentrations ($10^{-9}$-$10^{-6}$ M) of native GIP are added but is not altered by other peptide hormones (tested at $10^{-6}$ M) including glucagon, VIP and insulin. The Mr 64,000 complex is not observed in tissues which have no specific binding sites for GIP such as intestinal epithelium. Assuming one molecule of $^{125}$I-GIP is bound per molecule of protein, one protein with Mr 59,000 is identified as the specific GIP binding site.

The Gastric Inhibitory Polypeptide (GIP) is released from the gastrointestinal tract after a meal, especially glucose ingestion (1) and enhances pancreatic insulin release (2). GIP is thus considered as the strongest incretin candidate among gastrointestinal hormones (2). We recently reported that GIP possesses highly specific binding sites in membranes from a hamster pancreatic ß cell tumor (3), strongly argueing for a direct action of GIP on the pancreatic ß cell. The present study was designed to further investigate the nature of GIP receptors. By using the bifunctional cross-linking reagent dithiobis (succinimidylpropionate) (4), the GIP receptor was covalently labeled with $^{125}$I-GIP and its molecular size was determined.

## MATERIAL AND METHODS

### Materials

Porcine GIP and Vasoactive Intestinal Peptide (VIP) were provided by V. Mutt (Karolinska Institutet, Stockholm, Sweden). Porcine pancreatic glucagon and monocomponent insulin were purchased from Novo Research Institute (Copenhagen, Denmark). Dithiobis (succinimidyl propionate) (DTSP) was from Pierce Chemical Company (Rockford, USA). The calibrating proteins were obtained from Bethesda Research Laboratories (Gaithersburg, USA). Other chemicals were of the highest purity commercially available.

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Membranes preparation

Membranes were prepared from β cell pancreatic tumor serially transplanted in Syrian hamster (5). Tumors were harvested and homogenized in a ice-cold solution containing 0.25 M sucrose, 0.01 M triethanolamine, 5 mM EDTA, pH 7.5, using an electric blendor (Waring, USA). The preparation was centrifuged for 15 minutes at 2,000 g. The supernatant was collected and centrifuged for 20 minutes at 20,000 g. The final pellet containing the membranes was washed three times in 60 mM HEPES buffer and kept at -80°C until use.

Binding of $^{125}$I-GIP to pancreatic β cells plasma membranes

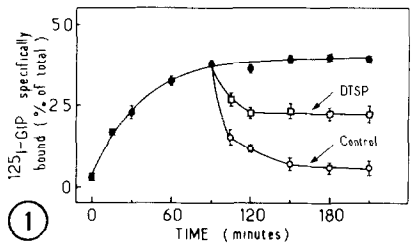
Pure natural porcine GIP was iodinated at a specific activity of 160 Ci/ g with the chloramine T method (6) and purified by gel filtration on Sephadex G-50. $^{125}$I-labeled GIP and native GIP display the same apparent affinity for GIP binding sites in a pancreatic β cell line in culture (7). Plasma membranes (200 µg protein/ml) were incubated for 90 minutes at 15°C with $^{125}$I-GIP (2. $10^{-9}$M) and when necessary other compounds in 2.5 ml of 60 mM HEPES buffer, pH 7.5 containing 2% (w/v) bovine serum albumin and 0.1% (w/v) bacitracin. The reaction was stopped by adding 35 ml of ice-cold 60 mM HEPES buffer, pH 7.5. Membrane-bound labeled GIP was separated by centrifugation at 20,000 g for 15 minutes at 4°C.

Affinity cross-linking of GIP receptor

The cross-linking reaction was performed essentially as previously described for the covalent labeling of VIP receptor in intestinal epithelial membranes (8). Membranes containing bound $^{125}$I-GIP were suspended in 1 ml of 60 mM HEPES buffer, pH 7.5, then incubated for 15 minutes at 4°C in the presence of 1 mM DTSP. The reaction was stopped by addition of 60 mM lysine as a reagent quench. Finally, the cross-linked materials were pelleted at 20,000 g for 15 minutes, resuspended in 60 mM Tris, 3% sodium dodecyl sulfate, 10% glycerol at pH 6.8 and submitted to electrophoresis. Electrophoresis was performed using a 5-20% polyacrylamide slab gel according to the Laemmli method (9) with a 3% stacking gel as described in details elsewhere (8). Dried gel was exposed to a Trimax type XM film (3M) with a 3M Trimax intensifying screen

RESULTS

The specific binding of $^{125}$I-GIP to pancreatic β cell tumor membranes is time-dependent and reaches a plateau from 90 to 120 min (Fig.1). When cold GIP ($10^{-6}$M) is added at the plateau, a rapid and important dissociation of $^{125}$I-GIP-receptor complexes is observed. The treatment of $^{125}$I-GIP labeled membranes by the cross-linking reagent DTSP prior to the addition of $10^{-6}$M GIP, reduces to a great extent this dissociation (Fig.1). These data support that DTSP treatment of labeled membranes induces the formation of a significant amount of irreversible $^{125}$I-GIP-receptor complexes. When labeled membranes are treated with DTSP and subjected to electrophoresis on a polyacrylamide gel (Fig.2), resulting autoradiograph clearly reveals that $^{125}$I-GIP has covalently cross-linked to a high molecular weight component (Fig.2, lane A). One major band is observed, corresponding to a $^{125}$I-GIP-protein complex with a molecular weight of 64,000. Two minor bands corresponding to Mr 46,000 and 32,000 components are also observed. Without DTSP treatment of membranes, these bands are no longer observed (not shown). The specificity of labeling of the major Mr 64,000 component



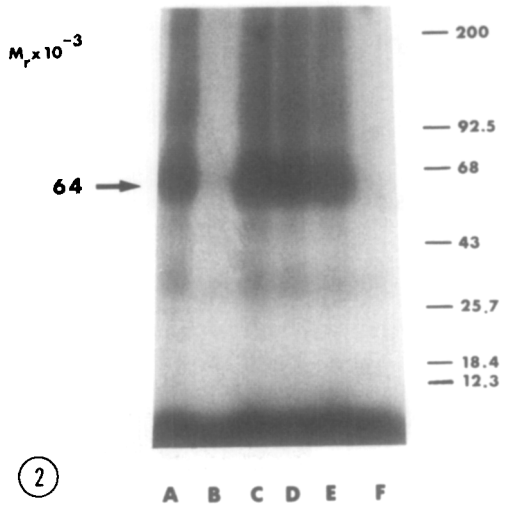
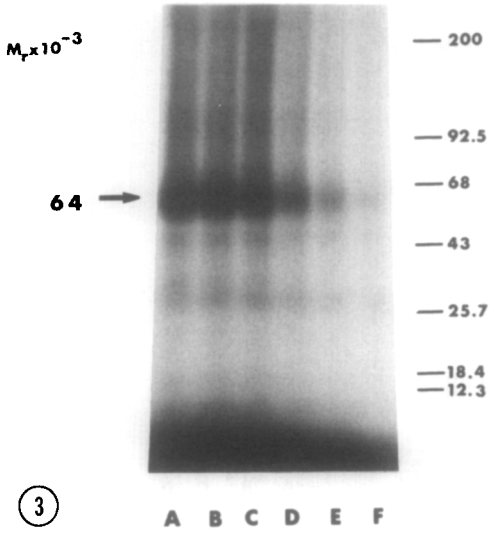




Figure 1: Effect of DTSP treatment of $^{125}$I-GIP labeled membranes on the dissociation of $^{125}$I-GIP receptor complexes. The specific binding of $^{125}$I-GIP to pancreatic ß cell tumor membranes is measured at various times (●) as described under Methods. After 90 min of incubation membrane-bound $^{125}$I-GIP is treated (□) or not (control; ○) with 1 mM DTSP as described under Methods and resuspended in a fresh medium containing 1 µM native GIP. The subsequent dissociation is followed for various times as indicated in the figure. Each point is the mean ± SEM of triplicate determinations.

Figure 2: Peptide and tissue specificity of covalent labeling by $^{125}$I-GIP of membrane proteins as seen by SDS/Polyacrylamide gel electrophoresis. Conditions are as described under Methods. Membranes were incubated with $^{125}$I-GIP alone or in the presence of native GIP or other peptide hormones. They were then treated with 1 mM DTSP. Lane A shows the autoradiographic pattern of pancreatic ß cell tumor membranes incubated with $^{125}$I-GIP alone; lane B, with 1 µM GIP; lane C, with 1 µM glucagon; lane D, with 1 µM VIP; lane E, with 1 µM insulin; lane F shows the autoradiographic pattern of intestinal epithelial membranes incubated with $^{125}$I-GIP alone. Membranes from rat small intestinal epithelial cells were prepared as described in details elsewhere (17).

is tested at first by adding unlabeled GIP and other peptide hormones together with $^{125}$I-GIP. As shown in Fig.2 (lane B) covalent labeling of the Mr 64,000 component is completely inhibited by 1 µM GIP while unaffected by 1 µM glucagon (lane C), VIP (lane D) or insulin (lane E). When rat intestinal plasma membranes which do not exhibit GIP binding sites (7) are used instead of pancreatic membranes, the Mr 64,000 component is absent (Fig.2, lane F). These observations argue for the specificity of the Mr 64,000 component with respect to peptide recognition and cellular expression.

The labeling of the Mr 64,000 component is abolished by low GIP concentrations between $10^{-9}$ and $10^{-6}$M (Fig.3, lanes A to F). This indicates the high affinity of the binding site for GIP and corroborates the competitive inhibition of $^{125}$I-GIP binding to pancreatic tumor membranes by native GIP which occurs in the same concentration range (Fig.4).



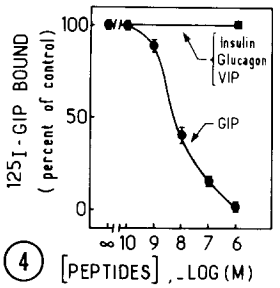




Figure 3: Dose response of native GIP in inhibiting the covalent labeling by $^{125}$I-GIP of proteins from pancreatic β cell tumor membranes. Conditions are as described under Methods. Membranes were incubated with $^{125}$I-GIP and various concentrations of native GIP, then treated with 1 mM DTSP. Lane A shows the autoradiographic pattern of membranes incubated with $^{125}$I-GIP alone; lane B, with $10^{-10}$M GIP; lane C, with $10^{-9}$M GIP; lane D, with $10^{-8}$M GIP; lane E, with $10^{-7}$M GIP; lane F, with $10^{-6}$M GIP.

Figure 4: Competitive inhibition by native GIP ( ● ) or other peptide hormones ( ■ ) (insulin, glucagon, VIP) of specific $^{125}$I-GIP binding to hamster pancreatic β cell tumor membranes. Results are expressed as the percentage of radioactivity specifically bound in the absence of added unlabeled peptide. Each point is the mean ± SEM of 3 separate experiments.

## DISCUSSION

The cross-linking reagent dithiobis (succinimidylpropionate) is found suitable to covalently link $^{125}$I-GIP to its binding sites in membranes prepared from pancreatic β cell tumor. Polyacrylamide gel electrophoresis of labeled membranes treated by the cross-linker indicates the presence of a major labeled component with Mr 64,000. If we assume that one molecule of GIP (Mr ≃ 5,100) is linked per protein, the Mr of the membrane protein that is covalently linked to $^{125}$I-GIP is about 59,000. This size compares well with the size of receptors for other peptide hormones including the receptor for VIP in intestine (8), octapeptide of cholecystokinin in pancreas (10), prolactin (11) and glucagon (12) in liver. It is however smaller than the size of receptors for insulin (13) and growth factors such as EGF (14) and IGF (15). The Mr 59,000 protein characterized here in pancreatic β cell tumor membranes displays many characteristics of a GIP receptor. (a) Cross-linking of $^{125}$I-GIP to this protein is inhibited by native GIP at concentrations that are within the dose-response curves for stimulating insulin release in a pancreatic β cell line in culture as reported recently by our group (7) and for saturation of the

GIP binding sites in pancreatic β cell tumor membranes (see Fig. 4). (b) The labeling of this protein is highly specific since it is not altered by various peptide hormones including VIP, glucagon and insulin (see Fig.2). (c) Finally, this Mr 59,000 protein is tissue specific since it is not found in tissues which are not able to bind $^{125}$I-GIP specifically such as intestinal epithelium (see Fig.2).

A prerequisite for the use of the cleavable cross-linker DTSP which contains a disulfide bridge (4), is to work under non-reducing conditions. This feature does not permit the GIP binding site to be analyzed further by testing the presence of S-S bonds within its structure. The recent synthesis of hydrophilic non cleavable analogue of DTSP (16) will perhaps provide the chemical tool necessary to characterize further the molecular structure of GIP binding sites.

In conclusion, we have identified a Mr 59,000 protein responsible for the specific binding of GIP in a pancreatic β cell tumor. The implication of this newly discovered GIP binding site in the physiological regulation of insulin secretion is currently under investigation in our group. Preliminary results suggest that a cultured pancreatic β cell line where GIP triggers insulin release (7) also expresses this Mr 59,000 GIP binding site.

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REFERENCES

1. Brown, J.C., Dryburgh, J.R., Ross, S.A., Dupré, J. (1975) Rec. Progr. Horm. Res. 31, 487
2. Creutzfeldt, W. (1979) Diabetologia 16, 75
3. Maletti, M., Amiranoff, B., Laburthe, M. and Rosselin, G. (1983) C.R. Acad. Sci. Paris 297, 563
4. Lomant, A.J. and Fairbanks, G. (1976) J. Mol. Biol. 104, 243
5. Grillo, T.A.I., Whithy, A.J., Kirkman, H., Foa, P.P., Kobernick, S.D. and Green, R. (1967) Diabetes 16, 409
6. Greenwood, F.C., Hunter, W.M. and Glover, J.S. (1963) Biochem. J. 89, 114
7. Amiranoff, B., Vauclin-Jacques, N., Laburthe, M. (1984), submitted
8. Laburthe, M., Bréant, B. and Rouyer-Fessard, C. (1984) Eur. J. Biochem. 139 181
9. Laemmli, U.K. (1970) Nature (London) 227, 680
10. Sakamoto, C., Williams, J.A., Wong, K.Y. and Goldfine, I.D. (1983) FEBS Lett. 151, 63
11. Borst, D.W. and Sayare, M. (1982) Biochem. Biophys. Res. Commun. 105, 194
12. Johnson, G.L., Mc Andrew, V.I. and Pilch, P.F. (1981) Proc. Natl Acad. Sci. USA 78, 875

13. Pilch, P.F. and Czech, M.P. (1979) J. Biol. Chem. 254, 3375
14. Kasuga, M., Van Obberghen, E., Nissley, S.P. and Rechler, M.M. (1982) Proc. Natl Acad. Sci. USA 79, 1864
15. Hock, R.A., Nexo,E. and Hollenberg, M.D. (1979) Nature (London) 277, 403
16. Staros, J.V. (1982) Biochemistry 21, 3950
17. Amiranoff, B., Laburthe, M., Dupont, C. and Rosselin, G. (1978) Biochim. Biophys. Acta 544, 474