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Stimulation by Gastric Inhibitory Polypeptide of Insulin and Glucagon Secretion by Rat Islet Cultures[1] (39997)

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Insulin secretion is greater when glucose is given orally compared to intravenously (1). This has been attributed to the insulinotropic effect of certain gastrointestinal hormones that are released by oral glucose. Recently, evidence suggests that a leading candidate for this role is gastric inhibitory polypeptide (GIP), which is secreted after oral but not intravenous glucose (2, 3).

Stimulation of insulin release by GIP administration has been shown *in vivo* (2, 4). An *in vitro* stimulatory effect of GIP on insulin secretion by the isolated perfused rat pancreas has been demonstrated at a physiologic concentration of GIP (1 ng/ml), but very high concentrations (1–10 μg/ml) were necessary to elicit a modest rise in insulin release by isolated rat islets (5, 6). Since the collagenase method of isolating islets may alter the plasma membrane and thus affect the responsiveness of these cells, we investigated GIP action on cultured islet cells.

Materials and methods. Monolayer cultures of the pancreas of 2- to 5-day-old Wistar rats were established as previously described (7, 8). Four days following initiation of the cultures, experiments were performed.

Experiments were done in Earle's balanced salt solution (EBSS) (9) supplemented with bovine serum albumin (0.25%, w/v). A 2-hr preincubation interval was followed by a 1-hr incubation in the test medium, which consisted of EBSS with bovine serum albumin and varying amounts of glucose (0, 1.7, 5.5, 8.3, or 16.5 m*M*), with or without essential and nonessential amino acids (10), and with gradients of GIP (0, 1, 10, 100, or 1000 ng/ml; purchased from Dr. J. C. Brown). All incubations were performed at 37° in a humidified automatic CO_2 tissue culture incubator with an atmosphere of 95% air and 5% CO_2.

Immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) were measured by cellulose radioimmunoassay (11, 12). Antiserum 30K was used to measure IRG. All samples for IRG were collected in benzamidine (final concentration, 0.05 *M*) to reduce hormone degradation, as previously reported (12). Results are expressed as mean microunits per milliliter (IRI) or mean picograms per milliliter (IRG) ± 1 SD. Significance was determined by two-tail Student's *t* test.

The binding of GIP to cells was assessed by an immunoperoxidase technique (13). Cultures were fixed in Bouin's solution for 2 hr, then sequentially incubated at 4° for 30 min each with GIP (10 ng/ml), rabbit anti-GIP serum, and horseradish peroxidase-labeled sheep anti-rabbit γ-globulin, and then incubated with 3,3′-diaminobenzidine (which served as substrate for the peroxidase reaction) and hydrogen peroxide for 45 min at room temperature. Control experiments were done in which one or more of the components needed was omitted, and these specimens were negative for

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staining. In addition, when rabbit anti-GIP was first incubated with GIP and then used, no staining occurred.

Results. At 16.5 m*M* glucose, a concentration of GIP as low as 1 ng/ml significantly enhanced IRI release (Table I). However, when glucose was 1.7 m*M*, GIP concentration of 10 ng/ml or greater were required to increase IRI release significantly. The relative stimulatory effect of GIP on IRI secretion was greater when glucose was elevated or when amino acids were absent. However, as shown in Table II, since the amino acid mixture itself enhanced glucose-stimulated IRI secretion, this may have obscured any potential additional stimulatory activity of GIP.

GIP at 1 ng/ml significantly increased IRG secretion (Table III). Since stimulation was seen even at glucose m*M* 16.5, this action apparently overrides any suppressive effect that high glucose levels may have. Since IRG secretion was enhanced by the presence of the amino acid mixture, when amino acids were also present they may have obscured any stimulatory action of GIP at a concentration of 1 ng/ml (Table IV).

Binding of GIP to a minority of the islet cell population was demonstrated in cultures stained with the immunoperoxidase method (13) following incubation of the culture with GIP (Fig. 1). Many of the islet cells and all of the fibroblasts remained unstained.

Discussion. Since both IRI and IRG secretion by these islet cultures were increased by physiologic amounts of GIP, a direct *in vitro* stimulatory effect of GIP on the pancreatic islet has been demonstrated. Previously, physiologic concentrations of GIP were shown to stimulate IRI secretion by the perfused rat pancreas, but very high and excessive concentrations were needed to increase IRI release by isolated islets (5, 6). This difference may be attributable to damage to GIP receptors when islets are isolated by the collagenase method. This is based upon the reported lack of inhibition of IRI secretion by somatostatin when examined in islets isolated by the collagenase method (14).

In preliminary reports, stimulation by GIP of IRG secretion has been found both *in vivo* in man and *in vitro* with perfused rat pancreas (15, 16). Our findings also support these observations. Furthermore, this effect was not suppressible by high glucose concentrations. Both IRI and IRG release are modulated by the presence of an amino acid mixture, which also affects the relative stimulatory effect of GIP on these cultures. Interaction between glucose and amino acids has been previously shown for both IRI and IRG secretion by the perfused pancreas (17). Furthermore, we have indirect evidence from the studies reported here that GIP may interact with a minority subpopulation of islet cells (Fig. 1).

In pancreatic monolayer cultures, the secretion and/or localization of several hormones has been reported. These include, in addition to insulin (B cell) and glucagon (A cell), gastrin (D cell?) (18), somatostatin

TABLE I. EFFECT OF AMINO ACIDS ON GIP-STIMULATED IRI RELEASE (μU/ml ± SD).[a]

Glucose (m*M*)	GIP (ng/ml)	With amino acids[b]	Without amino acids[c]
1.7	0	104 ± 8	79 ± 10
1.7	1	106 ± 13	89 ± 10
1.7	10	148 ± 19**	117 ± 22*
1.7	100	214 ± 43**	181 ± 16***
1.7	1000	283 ± 36***	–
16.5	0	141 ± 13	130 ± 29
16.5	1	173 ± 21*	220 ± 29**
16.5	10	309 ± 47***	381 ± 38***
16.5	100	536 ± 76***	757 ± 93***
16.5	1000	741 ± 65***	–

[a] *P* (compared to GIP, 0 ng/ml): (*) <0.05, (**) <0.01, (***) <0.001. *N* = 4 in all groups.
[b] EBSS supplemented with Eagle's amino acids (10).
[c] EBSS only.

TABLE II. EFFECT OF AMINO ACID SUPPLEMENTATION ON GLUCOSE-STIMULATED IRI RELEASE.

Glucose (m*M*)	IRI release with amino acids[a]/IRI release without amino acids[b]	*P*[c]
1.7	1.15	NS
5.5	1.11	NS
8.3	1.33	<0.01
16.5	1.50	<0.01

[a] EBSS supplemented with Eagle's amino acids (10).
[b] EBSS only.
[c] With amino acids vs without amino acids.

TABLE III. EFFECT OF GIP ON IRG RELEASE (pg/ml±SD).[a]

GIP (ng/ml)	Experiment 1[b] 1.7 m*M* Glucose	Experiment 1[b] 16.5 m*M* Glucose	Experiment 2[c] 1.7 m*M* Glucose	Experiment 3[c] 16.5 m*M* Glucose
0	547 ± 110	400 ± 0	230 ± 38	230 ± 12
1	653 ± 162	547 ± 55*	420 ± 78*	305 ± 78*
10	862 ± 48*	852 ± 33**	568 ± 125**	455 ± 30**

[a] *P* (compared to GIP, 0 ng/ml): (*) <0.01, (**) <0.001.
[b] *N* = 3 in all groups.
[c] *N* = 4 in all groups.

TABLE IV. EFFECT OF AMINO ACIDS ON GIP-STIMULATED IRG RELEASE (pg/ml±SD) AT 8.3 m*M* GLUCOSE.[a]

GIP (ng/ml)	*N*	No amino acids[b]	*N*	With amino acids[c]
0	3	110 ± 15	4	173 ± 13
1	4	146 ± 16*	4	193 ± 13
10	4	207 ± 15**	4	340 ± 36**

[a] *P* (compared to GIP, 0 ng/ml): (*) <0.05, (**) <0.01.
[b] EBSS only.
[c] EBSS supplemented with Eagle's amino acids (10).

(D cell) (10), secretin (cell type?) (20), and pancreatic polypeptide (cell type?) (21). When stained by specific immunocytochemical methods, the majority of the islet cells in these cultures appears to be B cells. However, when localization of exogenous GIP was assessed by the methods used in this investigation, only a small minority of the islet cells was stained, suggesting that these cells are either A or D cells, although binding by a subgroup of B cells is not excluded. If the former is correct, then the effect of GIP on IRI secretion may not occur via a direct action upon the B cells. These findings suggest, therefore, that GIP may act first on a cell type other than the B cell, and its action on IRI release is, in turn, mediated through this effect. This may be through direct cell-to-cell gap junctions (22), or may be humorally mediated.

The immunoperoxidase method used in the studies reported here is less sensitive than the peroxidase-antiperoxidase technique of Sternberger *et al.* (23). Using the latter method and its much greater sensitivity, we have recently observed the presence of GIP in the A cells of the unperfused neonatal and adult rat pancreas as well as in islet cells of 4-day-old monolayer cultures of pancreas from 2- to 5-day-old neonatal rats (24). Although GIP has been recently reported to inhibit the action of glucagon in rat adipocytes by competing with binding of glucagon to rat adipocyte receptors (25), we have observed in the studies using the peroxidase-antiperoxidase method that when GIP antiserum was incubated with glucagon prior to use, staining was still seen. Although the immunocytochemical findings reported here and our more recent observations with the peroxidase-antiperoxidase method may appear contradictory, there is a plausible explanation. Since the relative sensitivities of the two methods are so different, when islets are maintained in tissue culture for several days, much of the GIP within the A cells may become depleted to levels undetectable by the less sensitive procedure, but upon addition of exogenous GIP, that which is bound can be shown. However, with the peroxidase-antiperoxidase technique, we can demonstrate even low amounts of GIP. Thus, a tentative explanation for these findings is that exogenous GIP may not only bind to A cells but may also be taken up by these cells, presumably by endocytosis (26). An alternative explanation is that A cells may actually synthesize GIP.

Summary. Physiologic amounts of GIP stimulated the secretion of IRI and IRG by monolayer cultures of neonatal rat pancreatic islets. Localization of exogenous GIP to a minority subpopulation of the islet cells was observed. The results may be interpreted to indicate that the effect of GIP on IRI release by the B cell is not direct, but rather, is mediated through its action on another islet cell type. Conversely, the action of GIP on IRG release may be directly mediated through an effect of GIP on the A cell. The action of GIP on

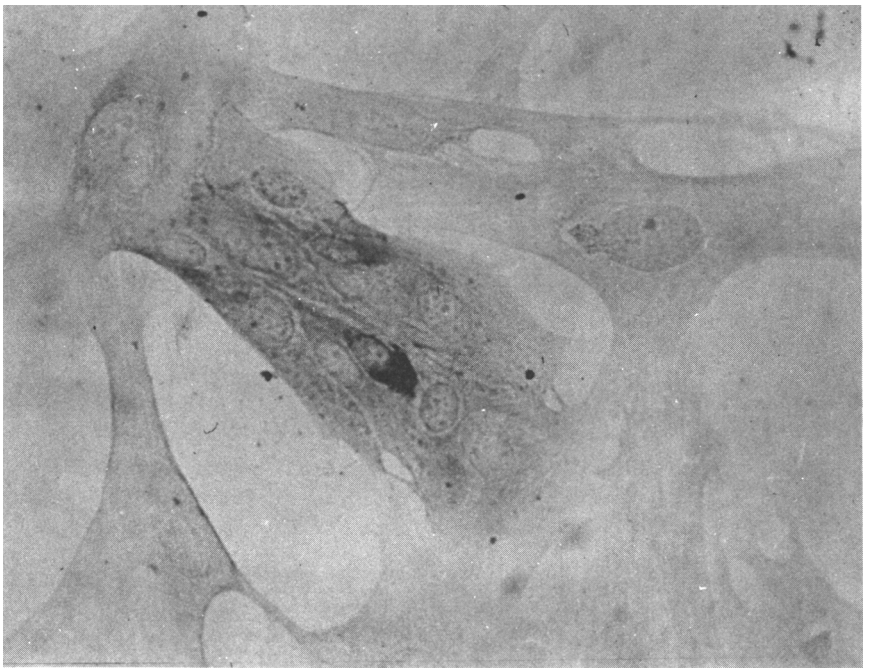

FIG. 1. Photomicrograph showing staining with the immunoperoxidase technique (13) following incubation of the culture with GIP. Strong staining is seen over one cell within the islet group, and lighter staining over two additional cells. The surrounding fibroblasts as well as the remaining islet cells are unstained. × 400.

IRG release apparently overrides any suppressive effect that high glucose levels may exert. The stimulation by GIP of both IRI and IRG release was greater when a mixture of amino acids was omitted.

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