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Gastric Inhibitory Polypeptide, Cholecystokinin, and Secretin Effects on Insulin and Glucagon Secretion by Islet Cultures[1] (40448)

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Gastric inhibitory polypeptide (GIP) stimulated both insulin and glucagon secretion by rat pancreatic islet cell cultures (1). The present investigation was undertaken to further examine the effects of GIP as well as those of the carboxy-terminal octapeptide of cholecystokinin (CCK-OP) and synthetic secretin on insulin and glucagon release in these cultures.

Materials and methods. Monolayer cultures of the pancreas of 2- to 5-day old Wistar rats were established as described previously (2, 3). For each experiment, one pool of neonatal rat pancreases was enzymatically dispersed. From the resulting cell suspension, equal aliquots were added to culture dishes. Thus, the numbers of cells added to each dish from such a pool were considered equivalent, and within each experiment, the culture dishes were considered true replicates. However, results from one experiment are not necessarily comparable to those from other experiments since different pools of neonatal rat pancreases were used and the number of cells dispersed to each culture dish could differ between experiments. In general, an attempt was made to set up approximately as many culture plates (60 mm) as neonatal rat pancreases used. When smaller culture plates (35 mm) were used, twice as many cultures were set up as neonatal rat pancreases utilized. However, even under these circumstances when equivalent numbers of cells were apparently seeded into dishes, basal hormonal release sometimes differed significantly—even by several-fold between experiments.

Experiments were performed either in Earle's balanced salt solution (EBSS) (4) supplemented with bovine serum albumin (BSA, 0.25% w/v), or in Eagle's minimal essential medium (MEM) supplemented with nonessential amino acids (5) and fetal bovine serum (0.5% v/v). The latter represents a more physiologic incubation medium. A 2-hr preincubation period (glucose 5.5 mm) was followed by a 1-hr incubation in test medium, and immunoreactive insulin (IRI) and immunoreactive glucagon (IRG) released during the 1-hr period were measured by radioimmunoassay (6, 7). Thus total islet hormone released during the 1-hr incubation period was determined, but the pattern of release (first phase and second phase of release) was not determined. Therefore, this *in vitro* system will not distinguish whether the test substances affected first phase, second phase, or both phases of islet hormone release. On the other hand, this system allows the testing of a large number of test substances under identical (albeit static) conditions. Results are expressed as mean uU/ml (IRI) or mean pg/ml (IRG), ± 1 SD. At the peptide concentrations tested, neither the insulin nor the glucagon immunoassays were affected. For each set of incubation conditions, four culture dishes were used. Therefore, in Tables I–IV, for each group (each line in these Tables), $n = 4$. Significance was determined by two-tailed Student's t test.

GIP was purchased from the University of British Columbia (Dr. J. Brown), and CCK-OP and synthetic secretin were obtained from Squibb (Dr. M. Ondetti).

Results. It was previously observed that a mixture of amino acids modulated the insulinogenic and glucagonogenic actions of GIP (1). Therefore, in these present studies the effects of the single amino acids, arginine and leucine, on IRI secretion in the absence and presence of glucose, and the insulinogenic response to GIP were examined. This was accomplished by incubation of islet cell cultures in EBSS supplemented only with BSA

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(0.25% w/v). In the presence of glucose 8.3 m*M*, IRI release was significantly enhanced by arginine and leucine added either singly or in combination (Tables I, II). When added in combination, arginine plus leucine appeared to exert a significant additive effect. Under these conditions (glucose 8.3 m*M*), GIP at a concentration as low as 1 ng/ml significantly increased IRI secretion (Table I). In the absence of glucose, leucine in two experiments and arginine in one of two experiments were insulinogenic (Tables I, II). Under these conditions (glucose absent but arginine or leucine present), GIP also stimulated IRI secretion, but a 10- to 100-fold higher supraphysiologic peptide concentration was necessary than when glucose was also present (Table I).

The effects of GIP, CCK-OP, and secretin on IRI and IRG secretion were compared in islet cell cultures incubated in EBSS supplemented only with BSA and glucose 16.5 m*M* (Table III). At a peptide concentration of 1.0 ng/ml, GIP is about 0.2 n*M*, secretin 0.3 n*M*, and CCK-OP 0.9 n*M*. Under these conditions, GIP not only was a more potent stimulator of IRI release than was CCK, but also

TABLE I. EFFECT OF GIP, ARG, AND LEU ON IRI SECRETION (μU/ml ± SD).[a]

Glucose (mM)	GEP (ng/ml)	Amino Acid (mM)	Expt. 1 (Arg)	Expt. 2 (Leu)
0	0	0	11 ± 1	18 ± 3
0	0	5	14 ± 9	30 ± 6** [b]
0	1	5	13 ± 1	38 ± 6
0	10	5	19 ± 5	85 ± 11*** [c]
0	100	5	30 ± 3** [c]	183 ± 13*** [c]
8.3	0	0	24 ± 4	58 ± 5
8.3	0	5	53 ± 4*** [b]	101 ± 27** [b]
8.3	1	5	65 ± 7* [c]	191 ± 15*** [c]
8.3	10	5	129 ± 11*** [c]	491 ± 63*** [c]
8.3	100	5	197 ± 6*** [c]	773 ± 106*** [c]

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$.

[a] Incubated in EBSS supplemented with BSA (0.25% w/v).

[b] Compared to −amino acid, −GIP.

[c] Compared to +amino acid, −GIP.

TABLE II. EFFECT OF Arg AND Leu ON IRI SECRETION (μU/ml ± SD).[a]

Expt.	Glucose	Amino Acid	IRI
3	0	—	72 ± 8
	0	Arg 5 m*M*	113 ± 7**
	0	Leu 5 m*M*	107 ± 14*
	8.3 m*M*	—	128 ± 19
	8.3 m*M*	Arg 5 m*M*	193 ± 17***
	8.3 m*M*	Leu 5 m*M*	241 ± 26***
4	8.3 m*M*	—	22 ± 4
	8.3 m*M*	Arg 5 m*M*	52 ± 4***
	8.3 m*M*	Leu 5 m*M*	41 ± 9***
	8.3 m*M*	Arg 5 m*M* + Leu 5 m*M*	74 ± 7***
5	8.3 m*M*	—	26 ± 3
	8.3 m*M*	Arg 5 m*M*	45 ± 4***
	8.3 m*M*	Leu 5 m*M*	41 ± 4***
	8.3 m*M*	Arg 5 m*M* + Leu 5 m*M*	65 ± 2***

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$ compared to IRI release in the absence of the amino acid.

[a] Incubated in EBSS supplemented with BSA (0.25% w/v).

TABLE III. EFFECT OF GIP, CCK-OP, AND SECRETIN ON IRI (μU/ml ± SD) AND IRG (pg/ml ± SD) SECRETION IN THE PRESENCE OF GLUCOSE 16.5 m*M*.[a]

Expt	Additions	IRI	IRG
6	None	60 ± 3	143 ± 30
	Secretin 0.1 ng/ml	60 ± 6	125 ± 7
	Secretin 0.5 ng/ml	60 ± 6	126 ± 11
	Secretin 1.0 ng/ml	51 ± 6	149 ± 40
7	None	111 ± 12	100 ± 12
	CCK-OP 0.1 ng/ml	132 ± 25	80 ± 12
	CCK-OP 0.5 ng/ml	170 ± 22***	80 ± 24
	CCK-OP 1.0 ng/ml	172 ± 9***	83 ± 10
8	None	36 ± 2	69 ± 19
	GIP 0.5 ng/ml	78 ± 4***	128 ± 3***
	CCK-OP 0.5 ng/ml	51 ± 6***	54 ± 11
	Secretin 0.5 ng/ml	37 ± 3	61 ± 9
	GIP 0.5 ng/ml + CCK-OP 0.5 ng/ml	98 ± 12***	128 ± 12***
	GIP 0.5 ng/ml + Secretin 0.5 ng/ml	104 ± 15***	123 ± 22***
	CCK-OP 0.5 ng/ml + Secretin 0.5 ng/ml	60 ± 4***	75 ± 14
	GIP 0.5 ng/ml + CCK-OP 0.5 ng/ml + Secretin 0.5 ng/ml	113 ± 14***	139 ± 10***

*** $p < 0.01$ compared to hormone secretion in the absence of the peptide(s).

[a] Incubated in EBSS supplemented with BSA (0.25% w/v).

TABLE IV. EFFECT OF GIP, CCK-OP, AND SECRETIN ON IRI (μU/ml ± SD) AND IRG (pg/ml ± SD) SECRETION IN THE PRESENCE OF GLUCOSE 16.5 m*M* AND COMPLETE MEM.[a]

Additions	IRI	IRG
None	152 ± 13	77 ± 5
GIP 1 ng/ml	170 ± 9	85 ± 11
GIP 10 ng/ml	259 ± 22***	159 ± 14***
CCK-OP 1 ng/ml	258 ± 21***	66 ± 5*
CCK-OP 10 ng/ml	585 ± 59***	94 ± 9*
Secretin 1 ng/ml	162 ± 10	78 ± 8
Secretin 10 ng/ml	199 ± 17**	106 ± 6**

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to hormone secretion in the absence of the peptide.

[a] Supplemented with nonessential amino acids and fetal bovine serum (0.5% v/v).

stimulated IRG secretion whereas CCK-OP did not (Table III, Experiments 7 and 8). Secretin had no effect on either IRI or IRG secretion, except for further enhancing IRI release when added along with GIP (Table III, Experiments 6 and 8). The effects of GIP and CCK-OP on IRI release were also additive (Table III, Experiment 8).

In a final study, higher concentrations of the peptides were tested in islet cell cultures incubated in complete MEM supplemented with fetal bovine serum (0.5% v/v) and glucose 16.5 m*M* (Table IV). As reported earlier, the stimulatory effect of GIP 1.0 ng/ml on both IRI and IRG secretion was obscured when the mixture of amino acids was present (1). The total concentration of these amino acids ranges from roughly equal to fivefold greater than the average concentration of amino acids observed in normal human serum or plasma (5). In this more physiologic incubation medium, CCK-OP 1.0 ng/ml (0.9 n*M*) had an insulinogenic effect similar to that seen with GIP 10 ng/ml (2.0 n*M*). At 10 ng/ml (3.0 n*M*), secretin had a modest stimulatory action on IRI secretion, which was less than that seen with a much lower concentration of CCK-OP (0.9 n*M*). GIP had the most potent glucagonogenic action. However, CCK and secretin at a peptide concentration of 10 ng/ml, also slightly stimulated IRG secretion.

Discussion. Insulin secretion is greater when glucose is given orally than when an identical amount of glucose is given intravenously (8). This difference has been attributed to the insulinotropic action of one or more gastrointestinal hormones released by oral glucose, an effect called the enteroinsular axis (9). These hormones include CCK, GIP, gastrin, glucagon, secretin, and vasoactive intestinal polypeptide, as well as a hypothetical intestinal hormone designated as "incretin" or "intestinal insulin releasing polypeptide" (10–17). Recent evidence suggests that of the identified gastrointestinal hormones, GIP is the most likely intestinal factor responsible for this observation since GIP is secreted after oral but not intravenous glucose, and it is a potent insulin secretagogue (18, 19). Further support for this view comes from the recent report that gastrin, pentagastrin, and cholecystokinin all enhance the amount of GIP that is secreted following intraduodenal glucose (20). However, as shown here *in vitro*, CCK-OP is nearly as potent an insulinotropic agent as GIP, and under certain circumstances, using a more physiologic incubation medium, appears to be more potent than GIP. Furthermore, GIP plus CCK-OP, and GIP plus secretin may have synergistic insulinogenic actions.

From studies utilizing the isolated perfused rat pancreas, it has been reported that GIP 5 ng/ml had no insulinotropic action when glucose concentrations were low (less than 5.5 m*M*) (19, 21). However, it was observed here and in a previous report (1) that GIP 10 and 100 ng/ml will stimulate insulin secretion when glucose is low or absent. However, these are clearly supraphysiologic levels, since peak plasma GIP concentrations are about 1 ng/ml. From studies in fasted, normoglycemic dogs, it has been reported that GIP was insulinogenic and hypoglycemic (22). In our case when glucose was absent, either arginine or leucine were present, both of which may have an insulinotropic action even in the absence of glucose. Of particular interest is our observation that when glucose is absent but leucine is present and GIP is added at increasing concentrations, leucine has a weak glucose-like effect on the GIP dose-insulin response relationship.

The effect of gastrointestinal hormones on glucagon secretion is less well described. A glucagonogenic effect of CCK-pancreozymin has been described previously, but the peptide preparation used was not completely purified, and may have contained GIP (23). As already reported, GIP stimulates glucagon

release both *in vivo* and *in vitro* (1, 24–26). Furthermore, CCK-OP at the lower concentrations tested (1.0 ng/ml or less) does not increase glucagon secretion, although a slight glucagonotropic action was observed at CCK-OP 10 ng/ml (9 n*M*). This is in contrast to significant glucagonotropic effects of GIP at concentrations as low as 0.5 ng/ml (0.1 n*M*). Thus, the previously reported glucagonogenic action of CCK-pancreozymin appears to be due to the presence of contaminating material, most likely to have been GIP. Stimulation by CCK-OP of *in vivo* glucagon secretion has been recently described in the dog (27). At a dose of 480 ng/kg/h, IRG rose by 52 pg/ml. The corresponding serum level of CCK-OP was not measured. The normal plasma levels of CCK are somewhat controversial due to problems with the CCK radioimmunoassay (28). In humans, basal serum levels of about 500–700 pg/ml have been reported, with peak serum levels about 2–3 times as great (29). Thus, since high concentrations are necessary to elicit any glucagonotropic effect, CCK is unlikely to have any physiologic role in glucagon release. Basal serum secretin levels are about 20 pg/ml in humans, with peak levels about 10 times as great (30, 31). Thus, secretin does not appear to be a significant physiologic glucagonotropic agent since high concentrations (10 ng/ml or about 3 n*M*) are required. This confirms previous *in vitro* and *in vivo* observations (32, 33) and is in contrast to the reported suppression of glucagon secretion *in vivo* in the dog (34, 35). In the isolated perfused dog pancreas, however, secretin had no effect on glucagon secretion (36).

Summary. Using an *in vitro* islet cell culture assay system, GIP and CCK-OP appear to be about equally potent insulinotropic agents. Secretin has only a very slight stimulatory effect on insulin release, and only at a very high peptide concentration. Furthermore, GIP plus CCK-OP and GIP plus secretin appear to have synergistic insulinogenic actions. If supraphysiologic GIP concentrations are tested, glucose is not necessary at an insulin stimulatory concentration for GIP to stimulate insulin secretion.

Glucagon release is enhanced only by GIP at physiologic peptide concentrations. Both secretin and CCK-OP have only very slight glucagonotropic actions even at high concentrations.

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