Effects of Glucose, Amino Acids, and Insulin on Adaptation of Exocrine Pancreas to Diet[1] (35233)

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Adaptation of pancreatic enzyme synthesis to changes in composition of the diet is well recognized; however, mechanisms involved in this physiologic process are unknown. Grossman *et al.* (1) have suggested three distinct mechanisms whereby dietary adaptation might be obtained: a neural mechanism, perhaps mediated via the vagus nerve; a hormonal mechanism, possibly mediated via gastrointestinal hormones such as pancreozymin; and a humoral mechanism mediated by products of digestion. Since dietary adaptation has been demonstrated to occur in vagotomized animals, it seems that the vagus nerve plays little, if any, role in such a process (2).

The present experiments were performed to determine if parenteral glucose or essential amino acids might influence amounts of amylase or chymotrypsin synthesized by the exocrine pancreas in rats maintained on high protein or sucrose diets.

Methods. Male Wistar rats, average weight 170 g, were used for all experiments. Animals were fed diets with the following composition: for the protein-rich diets (%): casein, 69; sucrose, 20; olive oil, 4; salts, 4; cellulose, 2; and vitamins, 1; for the sugar-rich diets (%): casein, 15; sucrose, 74; olive oil, 4; salts, 4; cellulose, 2; and vitamins, 1. The animals showed the same weight gain on both diets.

The animals were maintained on the indicated diet for 21 days. They were fasted 24 hr prior to sacrifice to allow for accumulation of pancreatic zymogens. On the 23rd day of study, the rats were killed by guillotine, the pancreas was excised quickly and freed from mesenteric fat. Portions of pancreas 200–500 mg) were placed in 0.02 *M* sodium phosphate buffer (0–3°, pH 7.6) and homogenized with a Potter-Elvehjem homogenizer.

Methods used for determination of amylase and chymotrypsin have been described (2). Glucose and amino acids were given intraperitoneally; insulin was administered subcutaneously.

Data have been evaluated statistically by use of the Student's *t* test.

Glucose experiments. For the first series of studies, rats maintained on a protein-rich diet were given 21 daily injections of either water, 2.0 ml; or 300 mg of glucose in 2 ml water.

For the second series, rats maintained on a sucrose-rich diet were given 21 daily injections of either water or 300 mg of glucose in 2.0 ml water.

For the third series, rats maintained on a protein-rich diet were given 21 daily injections of either water or 600 mg of glucose in 2.0 ml water.

Amino acid experiments. Animals were fed sugar-rich diets; control groups received 5.0 ml of water ip daily; experimental groups received amino acids, 356.4 mg in 5.0 ml of water. The amino acid mixture consisted of the *l*-form of the following amino acids (mg): histidine, 26.4; isoleucine, 33.0; leucine, 52.0; lysine, 66.0; methionine, 26.4; threonine, 33.0; tryptophan, 13.2; valine, 46.2; phenylalanine, 46.2; and arginine 13.2. Selection of essential amino acids for rats was determined from Meister's data (3).

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TABLE I. Effect of Intraperitoneal Glucose on Pancreatic Enzymes of Rats Fed a Protein-Rich Diet.

	Amylase[b]	N[d]	p[e]	Chymotrypsin[c]	N[d]	p[e]
Control	109.6 ± 3.7[a]	7		147.0 ± 7.4[a]	7	
Glucose	210.6 ± 13.4	5	<0.001	153.2 ± 8.7	5	NS

[a] Values are means ± SE.
[b] Starch hydrolyzed (mg in 30 min/mg of pancreas wet wt).
[c] Acid liberated from ATEE (μEq/min/10 mg of pancreas wet wt).
[d] Number of animals.
[e] Significance between means of the two groups.

Insulin experiments. Rats were fed protein-rich diets. Four groups of animals were examined: Group C received 2.0 ml of water for 21 days; Group I received 1 unit of PZI-insulin every other day for 21 days; Group S received 600 mg of glucose daily for 21 days; Group S + I received 600 mg of glucose and 2 units of PZI-insulin subcutaneously each day. Preliminary studies revealed that rats fed a protein-rich diet and given 1 unit or more of PZI-insulin daily required supplemental glucose in order to prevent severe episodes of hypoglycemia and death.

Results. Table I shows effects of intraperitoneal glucose on pancreatic zymogen content in rats fed protein-rich diets. The increase in amylase activity of the glucose-treated groups when compared with control groups was significant ($p<0.001$). There was no difference between control and glucose-treated groups in chymotrypsin activity.

Table II shows effects of intraperitoneal glucose on pancreatic zymogen content in rats fed sugar-rich diets. No difference in amylase or chymotrypsin activities between control and glucose-treated groups was observed in these rats. Rats maintained on sugar-rich diets and greater amounts of amylase than rats fed protein-rich diets (Tables I and II).

Animals fed sugar-rich diets (Table III) and given daily injections of either water or amino acids had essentially the same amounts of pancreatic amylase and chymotrypsin.

Table IV shows effects of insulin (sc) and glucose (ip) on pancreatic zymogen content in rats fed protein-rich diets. The administration of 1 unit of PZI-insulin sc every other day was associated with decreased amylase activity (Group I compared with Group C; $p<0.001$). A significant increase ($p<0.001$) in pancreatic amylase was observed in rats given 600 mg of ip glucose daily (Group S compared with Group C). Daily administration of 2 units of PZI-insulin plus 600 mg glucose to rats fed protein-rich diets (Groups S + I) reversed the increase in amylase observed between Groups C and S ($p<0.001$). Insulin treatment was not associated with an effect on chymotrypsin activity except for the comparison between Groups S + I and Group C ($p<0.05$).

Discussion. The data presented in Table I

TABLE II. Effect of Intraperitoneal Glucose on Pancreatic Enzymes of Rats Fed a Sugar-Rich Diet.

	Amylase[b]	N[d]	p[e]	Chymotrypsin[c]	N[d]	p[e]
Control	1051.6 ± 21.7[a]	10		120.5 ± 4.1[a]	10	
Glucose	1079.3 ± 31.7	8	NS	134.1 ± 7.4	8	NS

[a] Values are means ± SE.
[b] Starch hydrolyzed (mg in 30 min/mg of pancreas wet wt).
[c] Acid liberated from ATEE (μEq/min/10 mg of pancreas wet wt).
[d] Number of animals.
[e] Significance between means of the two groups.

TABLE III. Effect of Intraperitoneal Amino Acids on Pancreatic Enzymes of Rats Fed a Sugar-Rich Diet.

	Amylase[b]	N^d	p^e	Chymotrypsin[c]	N^d	p^e
Control	892.2 ± 51.5[a]	9		110.8 ± 3.7[a]	9	
Amino acids	904.9 ± 30.0	9	NS	107.1 ± 2.7	9	NS

[a] Values are means ± SE.
[b] Starch hydrolyzed (mg in 30 min/mg of pancreas wet wt).
[c] Acid liberated from ATEE (μEq/min/10 mg of pancreas wet wt).
[d] Number of animals.
[e] Significance between means of the two groups.

show that the daily administration of intraperitoneal glucose to rats fed a protein-rich diet was associated with significant increases in pancreatic amylase activity. The same treatment to rats fed sugar-rich diets was not associated with such an increase. There was almost a 10-fold increase in amylase in the pancreas of rats maintained on sugar-rich diets (Table II) compared with those maintained on protein-rich diets (Table I). The intraperitoneal administration of glucose to rats maintained on protein-rich diets was associated with an increase in pancreatic amylase and presumably increased pancreatic amylase synthesis.

The mechanism whereby glucose initiates an adaptive increase in amylase content is unknown. It is possible that glucose may initiate an increase in pancreatic amylase by direct action on the pancreatic acinar cell. It is also possible that glucose may release some hormone from the duodenal mucosa which then effects an increase in pancreatic amylase synthesis and content. It seems from these studies that glucose treatment results in specific amylase adaptation as no change was observed in chymotrypsin activity when compared with control levels.

The data presented in Table III show that intraperitoneal administration of amino acids was not associated with changes in amylase or chymotrypsin. These data agree with the findings of Wang and Grossman (4), who demonstrated that single intravenous administration of 20 ml of a 5% solution of amino acids to dogs had no effect on either volume or enzyme output of pancreatic secretions. Thus, our data and those provided by Wang and Grossman indicate that parenterally administered amino acids do not act directly to alter pancreatic enzyme synthesis, at least in those doses so far utilized. Moreover, it has

TABLE IV. Effect of Subcutaneous Insulin and Intraperitoneal Glucose on Pancreatic Enzymes of Rats Fed a Protein-Rich Diet.

Treatment groups	Amylase[f]	N^g	p^h	Chymotrypsin[i]	N	p
C[b]	138.5 ± 8.4[a]	10		118.5 ± 4.9[a]	10	
I[c]	103.5 ± 6.8	10	<0.01	124.9 ± 4.2	10	NS
S[d]	181.7 ± 3.6	10	<0.001	127.9 ± 4.0	10	NS
S + I[e]	94.5 ± 3.4	8	<0.001	131.9 ± 3.4	8	<0.05

[a] Values are means ± SE.
[b] Control animals.
[c] Injection of 1 unit of insulin sc every other day for 21 days.
[d] Daily injection of 600 mg of glucose ip for 21 days.
[e] Daily injection of 600 mg of glucose ip and 2 units of insulin sc for 21 days.
[f] Starch hydrolyzed (mg in 30 min/mg of pancreas wet wt).
[g] Number of animals.
[h] Significance between control and indicated experimental groups.
[i] Acid liberated from ATEE (μEq/min/10 mg of pancreas wet wt).

been shown that amino acids such as valine, arginine, methionine, and histidine, when added to the diet, increase pancreatic proteolytic content (5–8). These observations suggest that amino acids must come in contact with the gut and possibly the duodenum to effect the increase in pancreatic protease activity. Several investigators have recently suggested that intracellular amino acid pools might have an effect in altering pancreatic protein biosynthesis in mammalian cells (9–11). The inability to demonstrate an increase in pancreatic protease content following parenteral amino acid administration suggests that intracellular amino acid pools play little, if any, role in enzyme adaptation.

Insulin administration to rats has been associated with at least two effects on synthesis of pancreatic digestive enzymes. One, insulin administration to rats fed protein-rich diets, or protein-rich diets plus supplemental glucose, was associated with decreased pancreatic amylase activity. Grossman *et al.* (1) also observed that insulin treatment of rats fed a balanced diet was associated with a decreased amylase content. Secondly, insulin administration to rats rendered diabetic by alloxan treatment was associated with increased pancreatic amylase synthesis (12, 13) and content (14, 15). The latter effect was consistent with the well-recognized "permissive effect" of insulin on protein synthesis. These data, as well as those presented by Palla *et al.* (16), suggest that the hormone, insulin, is not directly involved in the adaptive process (17).

Summary. Rats fed a high protein diet and given intraperitoneal glucose showed an increase in pancreatic amylase content when compared with rats fed a high protein diet alone. Rats fed high sucrose diets did not evidence increased pancreatic amylase content when given intraperitoneal glucose. Rats fed high sucrose diets did not show changes in amylase or chymotrypsin content following intraperitoneal administration of amino acid mixtures. Insulin administration to rats fed high protein diets was associated with decreases in amylase content but no change in chymotrypsin content. Greater amounts of amylase were present in the pancreases of rats fed sucrose-rich diets compared with rats fed protein-rich diets.

1. Grossman, M. I., Greengard, H., and Ivy, A. C., Amer. J. Physiol. **141**, 38 (1944).

2. Morisset, J., and Dunnigan, J., Rev. Can. Biol. **26**, 11 (1967).

3. Meister, A., *in* "Biochemistry of the Amino Acids," p. 204. Academic Press, New York (1965).

4. Wang, C. C., and Grossman, M. I., Amer. J. Physiol. **164**, 527 (1951).

5 Hong, S. S., and Magee, D. F., Amer. J. Physiol. **191**, 71 (1957).

6. Magee, D. F., and Anderson, E. G., Amer. J. Physiol. **181**, 79 (1955).

7. Magee, D. F., and Hong, S. S., Amer. J. Physiol. **184**, 449 (1956).

8. Magee, D. F., and Hong, S. S., Amer. J. Physiol. **197**, 27 (1959).

9 Gall, O., Beney, L., and Szekely, M., Acta Physiol. **28**, 31 (1965).

10. Kipnis, D. M., Reiss, E., and Helmreich, E., Biochem. Biophys. Acta **51**, 519 (1961).

11. Snook, J. T., J. Nutr. **87**, 297 (1965).

12. Soling, H. D., Unger, K. O., Eur. Pancreatic Club IV Symp., Goettingen, West-Germany **1969**, p. 7.

13. Unger, K. O., Schmidt, H., and Soling, H. D., Eur. Pancreatic Club IV Symp., Goettingen, West-Germany **1969**, p. 6.

14. Ben Abdeljlil, A., Palla, J. C., and Desnuelle, P., Biochem. Biophys. Res. Commun. **18**, 71 (1965).

15. Palla, J. C, Ben Abdeljlil, A., and Desnuelle, P., Gut **9**, 254 (1968).

16. Palla, J. C., Ben Abdeljlil, A., and Desnuelle, P., Biochim. Biophys. Acta **158**, 25 (1968).

17. Snook, J. T., Amer. J. Physiol. 215, 1329 (1968).

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