Effect of Intestinal Osmolality on Insulin Secretion to Subsequent Intravenous Glucose or Arginine in the Rat (43106)

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Abstract. **To elucidate the effect of intestinal osmolality on insulin secretion, we investigated insulin response to a subsequent intravenous infusion of glucose or arginine after intragastric or intraduodenal mannitol or NaCl instillation in the rat. After anesthesia with intraperitoneal pentobarbital sodium, mannitol solution (10% or 20%) or 2.7% NaCl was instillated into the stomach or duodenum for 5 min at a flow rate of 0.5 ml/min, and 20% glucose (0.5 g/kg) or 10% L-arginine (0.5 g/kg) was infused bolus into the femoral vein 45 min after intestinal instillation. Insulin response to intravenous glucose was significantly higher in the rat with intragastric or intraduodenal mannitol or NaCl infusion than in control rats with intragastric or intraduodenal instillation of distilled water. Insulin response to intravenous arginine was almost the same in all groups. Subcutaneous preadministration of propranolol (0.4 mg/kg), atropine (1.2 mg/kg), or phentolamine (0.8 mg/kg) did not alter the present phenomenon.**

These results suggest that intestinal osmolality may enhance insulin release to intravenous glucose, but not to arginine in the rat. [P.S.E.B.M. 1990, Vol 194]

It is well known that the insulin secreted after an oral glucose administration is influenced by gastrointestinal factors, and several investigators (1–4) have reported that several gastrointestinal hormones had some insulinotropic effects through an endocrine transmission. Goldberg *et al.* (5), Shima *et al.* (6), and we (7) have reported that ingestion of a non-nutrient drink augments insulin secretion in response to a subsequent intravenous glucose load in humans and rats, suggesting that intestinal mechanical stimulation and/or osmolality may play a component role in insulin release. However, the effect of intestinal osmolality on insulin secretion remains to be further elucidated. In the present study, we investigated insulin, glucagon, and gastrin response to a subsequent intravenous infusion of glucose or arginine after intragastric or intraduodenal infusion of mannitol or sodium chloride solution in the rat.

Received November 15, 1989. [P.S.E.B.M. 1990, Vol 194]
Accepted April 16, 1990.

0037-9727/90/1944-0342$2.00/0

Materials and Methods

Animals and Methods. Male Wistar albino rats weighing approximately 200 g were used in this study. After overnight fast, the rats were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg), and the femoral veins were exposed. After the abdomen was opened, a polyethylene tube was inserted into the stomach or duodenum. Mannitol solution (10% or 20%, dissolved in 0.9% NaCl) or 2.7% NaCl was instillated into the stomach or duodenum for 5 min at a flow rate of 0.5 ml/min. In control rats, distilled water was instillated into the stomach or duodenum in the same manner. Forty-five minutes after the intragastric or intraduodenal instillation, 0.5 g/kg glucose in 20% solution or 0.5 g/kg L-arginine in 10% solution was infused bolus into the right femoral vein. Blood specimens were drawn from the protal (0.5 ml) and left femoral vein (0.5 ml) via venopuncture at 0, 15, and 30 min. In one series of a glucose or arginine infusion test, three rats were used to avoid the influence of hypovolemia. Blood specimens were drawn from the different rats at 0, 15, or 30 min. The blood samples were collected in a chilled tube containing aprotinin and EDTA and immediately centrifuged at 4°C and stored at −20°C until the time of assay. Under the same

conditions as described above, glucose infusion test was performed in the rat subcutaneously injected with propranolol (0.4 mg/kg; ICI Pharma, Osaka, Japan), atropine sulfate (1.2 mg/kg; Tanabe Seiyaku, Osaka), or phentolamine (0.8 mg/kg; Ciba Geigy, Takarazuka, Japan) 30 min before the experiment.

Measurements. Plasma glucose was measured by glucose oxidase method. Plasma insulin, glucagon, and gastrin were assayed by respective radio immunoassay (8–10). Hematocrits were determined by the microcapillary method. Total plasma protein and albumin levels were measured by the bromocresyl-green and biuret method.

Statistical Analysis. The data are expressed as the mean ± SD. Analysis of variance and Student's unpaired two-tailed *t* test were used for statistical evaluation.

Results

Hematocrits and Plasma Protein Levels. As shown in Table I, there were no significant differences in hematocrits and plasma protein levels in all groups.

Percentile Changes in Plasma Glucose, Insulin, Glucagon, and Gastrin Response to Intravenous Glucose in the Rat with Intragastric or Intraduodenal Instillation of Mannitol or NaCl. As shown in Figures 1 and 2, plasma glucose responses in rats with intragastric or intraduodenal mannitol or NaCl instillation were not different from those in controls. As shown in Figure 1, the percentile increment in femoral and portal insulin in rats with intragastric instillation of 10% mannitol (413 ± 47 and 552 ± 88%), 20% mannitol (467 ± 67 and 572 ± 76%), or NaCl (408 ± 45 and 538 ± 80%) was significantly higher than that in controls (320 ± 40 and 380 ± 60%) at 15 min, respectively. As shown in Figure 2, the percentile increment in femoral and portal insulin in rats with intraduodenal instillation of 10% mannitol (427 ± 47 and 572 ± 87%), 20% mannitol (493 ± 60 and 596 ± 72%), or NaCl (410 ± 46 and 555 ± 79%) was significantly higher than that in controls (346 ± 53 and 396 ± 48%) at 15 min, respectively. The percentile increment in portal insulin in rats with intragastric (500 ± 92%) or intraduodenal (520 ± 80%) instillation of 20% mannitol was significantly higher than that in controls at 30 min.

Plasma glucagon was similarly decreased after intravenous glucose in all groups. Gastrin response was also similar in all groups.

As shown in Table II, the stimulating effect of intestinal mannitol or NaCl on insulin release to subsequent glucose was not altered in the rat treated with propranolol, atropine, or phentolamine.

Percentile Changes in Plasma Glucose, Insulin, Glucagon, and Gastrin, Response to Intravenous Arginine in the Rat with Intragastric or Intraduodenal Instillation of Mannitol or NaCl. As shown in Figures 3 and 4, percentile changes in plasma glucose, insulin, glucagon, and gastrin in response to subsequent arginine were not altered in rats with intragastric or intraduodenal instillation of mannitol or NaCl.

Discussion

Femoral and portal insulin levels after intravenous glucose load were significantly increased by intragastric or intraduodenal mannitol or NaCl instillation compared with controls, although plasma glucose levels were similar to controls. This result confirmed the previous reports that ingestion of a non-nutrient drink augments insulin secretion in response to a subsequent intravenous glucose (5–7). Because hematocrits and plasma protein levels were not changed by intestinal instillation of mannitol, NaCl, or distilled water, the shift of fluid did not influence the present phenomenon. Gastrointestinal osmolality rather than intestinal filling may enhance glucose-induced insulin release, because insulin release to glucose was enhanced by intestinal instillation of mannitol or NaCl but not by distilled water. Insulin secretion was enhanced similarly by intragastric or intraduodenal mannitol or NaCl instillation, suggesting that duodenointestinal osmolality may play a component role in insulin release. Shima *et al.*

Table I. Hematocrits, Plasma Total Protein, and Albumin Levels in Rats with Intravenous Glucose Infusion Test

		Before	15 min	30 min
Hematocrits (%)	IG[a] and ID water (n = 12)	43 ± 4[b]	41 ± 4	42 ± 5
	IG and ID mannitol and NaCl (n = 36)	41 ± 4	42 ± 3	42 ± 3
Total protein (g/dl)	IG and ID water (n = 12)	5.0 ± 0.4	5.1 ± 0.5	5.1 ± 0.4
	IG and ID mannitol and NaCl (n = 36)	4.9 ± 0.5	5.0 ± 0.4	5.0 ± 0.3
Serum albumin (g/dl)	IG and ID water (n = 12)	2.6 ± 0.4	2.6 ± 0.3	2.7 ± 0.3
	IG and ID mannitol and NaCl (n = 36)	2.6 ± 0.3	2.5 ± 0.2	2.6 ± 0.2

[a] IG, intragastric instillation; ID, intraduodenal instillation.
[b] Data are expressed as the mean ± SD.

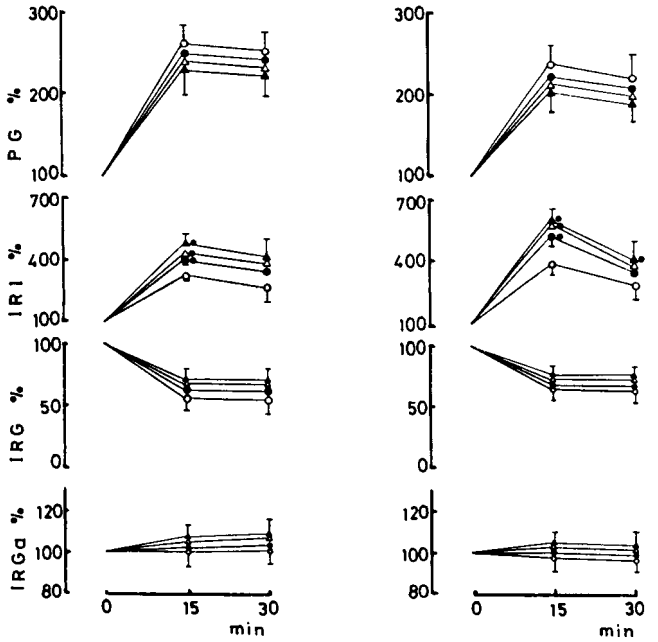

Figure 1. Percentile changes in plasma glucose, insulin, glucagon, and gastrin response to intravenous glucose in the rat with intragastric instillation. The bars represent SD. Basal level was expressed as 100. PG; plasma glucose; IRI, plasma insulin; IRG, plasma glucagon, and IRGa, plasma gastrin. Left panel, femoral vein; right panel, portal vein ○, intragastric water (n = 6); ●, intragastric NaCl (n = 6); △, intragastric 10% mannitol (n = 6); ▲, intragastric 20% mannitol (n = 6). ·$P < 0.05$, significantly different from control.

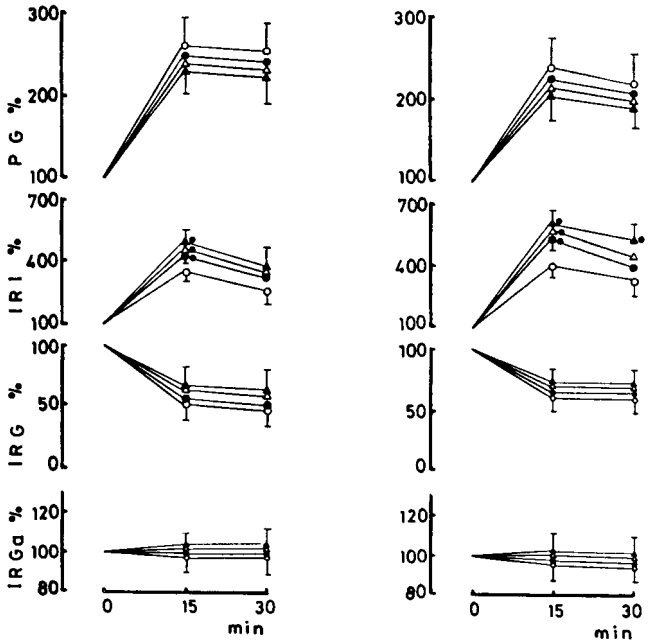

Figure 2. Percentile changes in plasma glucose, insulin, glucagon, and gastrin response to intravenous glucose in the rat with intraduodenal instillation. For legend, see Figure 1.○ intraduodenal water (n = 6); ●, intraduodenal NaCl (n = 6); △, intraduodenal 10% mannitol (n = 6); ▲, intraduodenal 20% mannitol (n = 6).

(6) have reported that mechanical stimulation of the digestive tract (using nonabsorbable konnyaku) might have enhanced insulin release to intravenous glucose. However, konnyaku included 2.2% sugar and small amounts of minerals. Although a significant dose-dependent effect of intestinal osmolality on insulin release was not observed, insulin release to glucose was slightly higher in the rat with intraintestinal instillation of 20% mannitol than in the rat with 10% mannitol. Mannitol or NaCl was instillated into the stomach or duodenum, and then intravenous glucose was administered in the present study. The sequence of intraintestinal mannitol or NaCl and intravenous glucose may have an influence on insulin secretion. Further studies are necessary to elucidate more precisely the role of intestinal osmolality in insulin secretion. Because propranolol, atropine, or phentolamine did not influence the stimulating effect of intestinal mannitol or NaCl on insulin release to glucose, local sympathetic or parasympathetic autonomic factors (adrenergic or cholinergic) were not responsible for the phenomenon. However, this does not rule out the possible influence of autonomic peptidergic factors.

Glucagon is known to augment insulin secretion (11, 12). However, glucagon levels were not significantly different in all groups in the present study. This result was in agreement with the report of Goldberg *et al.* (5) that glucose-induced suppression of glucagon was not affected by ingestion of the drink (3% mannitol) in the human. Gastrin is also known to enhance glucose-induced insulin release (1, 3). Gastrin response after intravenous glucose was not altered by intestinal mannitol or NaCl instillation. Thus, it is unlikely that gastrin influenced the insulin response.

On the other hand, intestinal mannitol or NaCl had no influence on insulin release to subsequent intravenous arginine. In the rat with intravenous arginine, plasma glucose level was increased to 125 mg/dl. This plasma glucose level was lower than that in the rat with intravenous glucose, suggesting that glucose-dependent insulinotropic factor may be responsible for the present phenomenon. Gastric inhibitory polypeptide (GIP) was not measured in the present study. Although orally administered mannitol has been reported not to stimulate GIP release (5, 13) and Ebert and Creutzfeldt (14) have observed an incretin effect in rats fed glucose despite the administration of large amounts of anti-GIP antibodies, the role of GIP in the present phenomenon cannot be completely excluded. Further studies are needed to clarify the role of GIP and/or other insulinotropic intestinal hormones in the present phenomenon.

Intestinal osmolality increases insulin secretion to glucose, but not to arginine, probably through glucose-dependent insulinotropic factor(s) in the rat.

Table II. Percentile Increment in Plasma Insulin at 15 Min in Intravenous Glucose Infusion Test

	Propranolol		Atropine		Phentolamine	
	Femoral	Portal	Femoral	Portal	Femoral	Portal
IG,[a] 10% mannitol (n = 6)	360 ± 50[b,c]	386 ± 51[c]	359 ± 46[c]	367 ± 51[c]	459 ± 53[c]	473 ± 60[c]
IG, 20% mannitol (n = 6)	383 ± 50[c]	402 ± 53[c]	362 ± 44[c]	381 ± 56[c]	471 ± 58[c]	495 ± 71[c]
IG, 2.7% NaCl (n = 6)	345 ± 42[c]	357 ± 45[c]	352 ± 43[c]	363 ± 48[c]	453 ± 60[c]	468 ± 58[c]
IG, water (n = 6)	250 ± 35	270 ± 39	260 ± 40	268 ± 37	355 ± 51	372 ± 55
ID, 10% mannitol (n = 6)	366 ± 45[c]	386 ± 46[c]	380 ± 46[c]	391 ± 52[c]	450 ± 50[c]	483 ± 52[c]
ID, 20% mannitol (n = 6)	373 ± 41[c]	395 ± 49[c]	380 ± 50[c]	401 ± 53[c]	463 ± 48[c]	501 ± 74[c]
ID, 2.7% NaCl (n = 6)	361 ± 42[c]	362 ± 43[c]	365 ± 43[c]	375 ± 44[c]	445 ± 52[c]	488 ± 49[c]
ID, water (n = 6)	261 ± 36	269 ± 40	265 ± 37	282 ± 40	370 ± 52	391 ± 57

[a] IG, intragastric instillation; ID, intraduodenal instillation.
[b] Data are expressed as the mean (%) ± SD. Propranolol, rats pretreated with propranolol; atropine, rats pretreated with atropine; and phentolamine, rats pretreated with phentolamine. Femoral, insulin increment in femoral vein and portal, insulin increment in portal vein.
[c] $P < 0.05$, significantly different from IG or ID water.

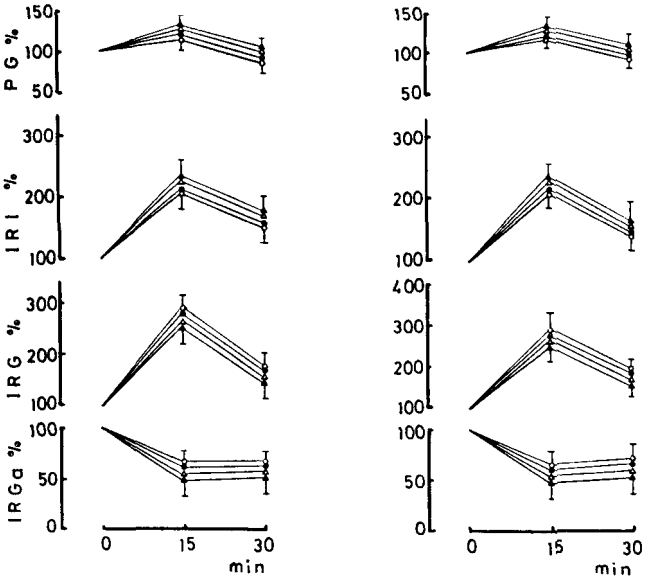

Figure 3. Percentile changes in plasma glucose, insulin, glucagon, and gastrin response to intravenous arginine in the rat with intragastric instillation. For legend, see Figure 1.

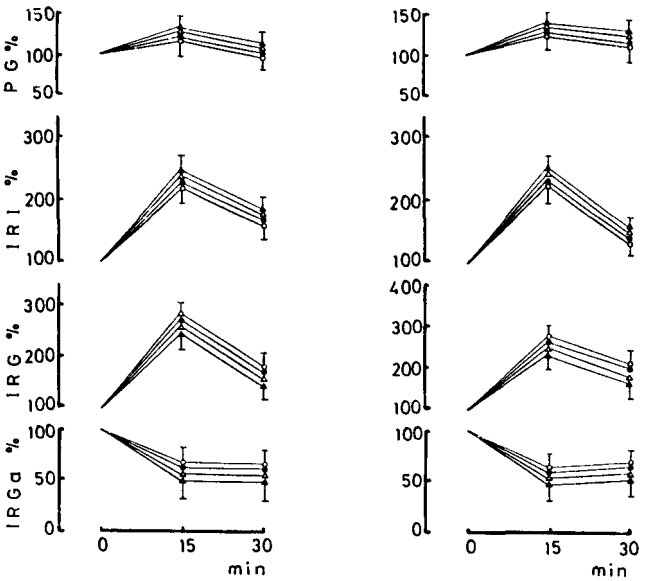

Figure 4. Percentile changes in plasma glucose, insulin, glucagon, and gastrin response to intravenous arginine in the rat with intraduodenal instillation. For legend, see Figures 1 and 2.

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