Starvation-induced Changes of Somatostatin, Glucagon, and Insulin Secretion from the Isolated Perfused Rat Pancreas

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SUMMARY

The effect of 16- and 48-h fasting on pancreatic somatostatin, insulin, and glucagon secretion was studied, using the isolated perfused rat pancreas. In the presence of 4.4 mM glucose, basal somatostatin and insulin concentrations in the perfusate were significantly lower in 48-h fasted rats than in fed animals, whereas basal glucagon secretion was significantly elevated in fasted rats. The infusion of 19 mM arginine significantly augmented secretion of somatostatin and glucagon and attenuated insulin secretion in 48-h fasted rats. It is concluded that fasting causes a decrease in basal pancreatic somatostatin secretion in vitro, although the response to arginine is rather exaggerated. Insulin and glucagon secretion also changed during the fasting. These results suggest that not only insulin and glucagon, but also somatostatin contribute to nutrient homeostasis. DIABETES 29:323–325, April 1980.

Recent studies have demonstrated that pancreatic D-cells, secreting somatostatin, are located closely to the A-cells and B-cells within the pancreatic islet.[1,2] Orci and Unger[3] suggested the role of pancreatic somatostatin as a "paracrine" substance, acting locally on neighboring cells to regulate hormone secretion. On the other hand, several investigators have postulated that somatostatin plays an important role in the regulation of nutrient homeostasis,[3–7] by affecting gastrointestinal function and nutrient absorption. In this respect, somatostatin secretion under various nutritional conditions is of great interest. Starvation is known to cause changes in insulin and glucagon secretion.[8–12] The present study was designed to investigate the effect of fasting on somatostatin, glucagon, and insulin secretion from the isolated perfused rat pancreas.

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MATERIALS AND METHODS

Male Wistar rats weighing 250–300 g were housed for at least a week in a conditioned room and divided into three groups: fed ad libitum, and 16- and 48-h fasting before the experiment. Body weights in 16- and 48-h fasted rats were significantly lower than those of fed rats, with the mean loss of 5.9% and 12%, respectively. The pancreas was isolated and perfused by the procedure described by Grodsky et al.[13] with minor modifications;[14] the preparation includes the attached segment of duodenum and the splenic veins were ligated.

All perfusions were accomplished with 4.4 mM glucose with Krebs-Ringer bicarbonate buffer containing 0.25% bovine serum albumin and 4.6% dextran (mean mol. wt. 70,000). The medium was gassed with 95% O_2–5% CO_2 and maintained at pH 7.4 and 37°C.

The flow rate was kept constant at 1.9 ml/min. After an equilibration period of 20 min, L-arginine hydrochloride, to provide a final concentration of 19 mM, was introduced over 15 min through a side arm pump. Each 1-min effluent from the portal vein was collected in chilled tubes containing 1000 U of Trasylol, frozen immediately, and stored at −20°C until assayed.

Radioimmunoassays. Immunoreactive somatostatin was measured by a specific radioimmunoassay with a modification of the method described by Arimura et al.,[15] using antiserum T-316, which is specific for somatostatin. Antiserum T-316 was produced in a rabbit by repeated immunizations with synthetic cyclic somatostatin (Takeda Pharmaceutical Co., Osaka) coupled to crystallized bovine serum albumin. T-316 had no cross-reactivity with insulin, glucagon, gastrin, motilin, VIP, secretin, and substance-P. N-tyrosyl somatostatin was iodinated with ^{125}I-Na by the chloramine-T method[16] and subsequently purified on Sephadex G-25 (fine) column. The minimum detectable quantity of the assay was 10 pg/ml and serial dilutions of the perfusate gave a parallel curve to that of standard somatostatin. Im-

munoreactive insulin was measured by radioimmunoassay with the polyethylene glycol method.[17] Immunoreactive glucagon was determined by radioimmunoassay with a talcum absorption technique of Sakurai et al.,[18] using antiserum (Otsuka Assay Laboratory, Tokushima), which is specific for pancreatic glucagon.[19]

Statistical analysis was performed using Student's *t* test for unpaired groups.

RESULTS

Basal perfusate somatostatin concentrations in the presence of 4.4 mM glucose were significantly lower in 48-h fasted rats ($P < 0.005$) and tended to be lower, although not significantly, in 16-h fasted rats than in those of fed controls, as shown in Table 1. Basal perfusate insulin levels also decreased to the undetectable value in 48-h fasted rats compared with the mean value of 6 ± 1 μU/ml in fed animals. Conversely, basal perfusate glucagon concentrations were significantly higher in 48-h fasted rats ($P < 0.05$) and higher, although not significantly, in 16-h fasted rats than those in fed controls.

Arginine (19 mM) infusion in addition to 4.4 mM glucose promptly evoked somatostatin release in all three groups with the mean (± SEM) peak values of 55 ± 5 pg/ml, 55 ± 4 pg/ml, and 55 ± 4 pg/ml in fed, 16-h fasted, and 48-h fasted rats, respectively, which were not significantly different from each other (Figure 1).

However, when arginine-induced somatostatin response is expressed as the sum of increments above the basal level during the 15-min period (ΣΔIRS), somatostatin secretion in rats fasted for 48-h significantly increased, compared with that in the fed rats ($P < 0.05$), as shown in Figure 2. On the contrary, the integrated insulin secretion induced by arginine (ΣΔIRI) was reduced by starvation for 48 h compared with that in the fed group ($P < 0.005$), whereas glucagon secretion (ΣΔIRG) was markedly higher in the 48-h fasted than in the fed group ($P < 0.005$).

DISCUSSION

The present study demonstrates that 48-h fasting decreased basal somatostatin release from the isolated perfused rat pancreas, whereas arginine-induced somatostatin release was rather enhanced by starvation, considering the decrease in basal levels. The mechanism responsible for the decrease in basal somatostatin and the increase in stimulated somatostatin secretion by fasting, although not clear at present, deserves consideration.

TABLE 1
Basal somatostatin, insulin, and glucagon secretion in the presence of 4.4 mM glucose from the isolated perfused pancreas of fed, 16-h fasted, and 48-h fasted rats

		Body weight (g)	Somatostatin secretion (pg/ml)	Insulin secretion (μU/ml)	Glucagon secretion (pg/ml)
Fed	(6)	280 ± 16	33 ± 6	6 ± 1	56 ± 3
16-h fasted	(7)	261 ± 5	23 ± 6	8 ± 4	69 ± 4
48-h fasted	(5)	252 ± 9	15 ± 1*	< 3	81 ± 9†

Results are shown as mean ± SEM with numbers of rats in parenthesis.
* $P < 0.005$ versus fed group.
† $P < 0.05$ versus fed group.

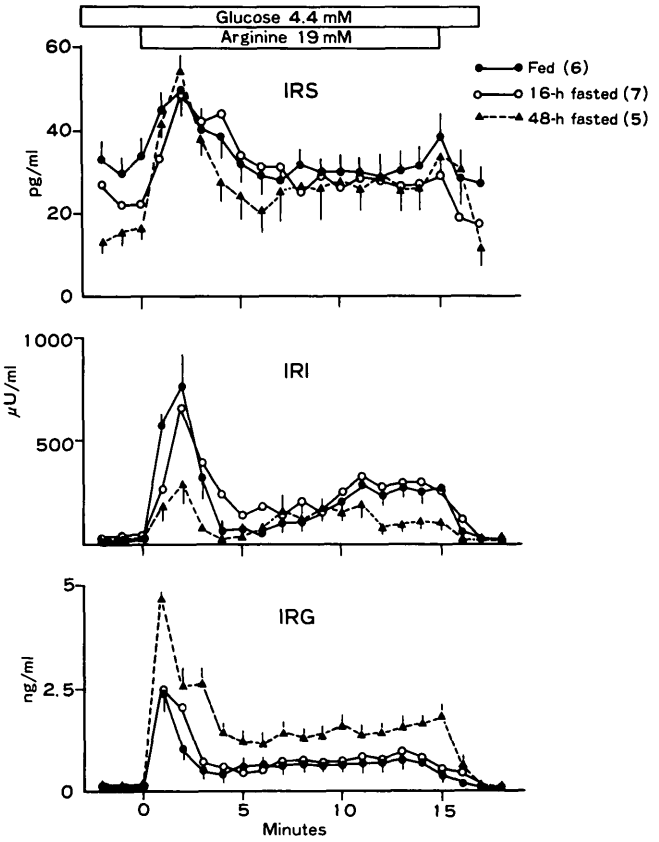

FIGURE 1. Somatostatin, insulin, and glucagon responses to 19 mM arginine in the presence of 4.4 mM glucose from the isolated perfused pancreas of fed, 16-h fasted, and 48-h fasted rats. Means ± SEM are shown in fed and 48-h fasted rats.

Recent studies have suggested that somatostatin may locally interact with regulation of insulin and glucagon secretion within the pancreatic islets as a "paracrine" mechanism.[1-3,20,21,23-26] Glucagon stimulates somatostatin release,[20,21] while there is evidence that insulin may inhibit somatostatin secretion.[24-26,29] Therefore, somatostatin secretion may be affected by the changes of endogenous glucagon or insulin secretion induced by fasting. However, as to the basal somatostatin levels, this seems unlikely because the present data showed that basal somatostatin levels were actually reduced despite the increased basal glucagon and the decreased basal insulin secretion by the starvation. On the contrary, the increase in arginine-stimulated somatostatin secretion by fasting may be due to endogenously enhanced glucagon or reduced insulin secretion, resulting from the interaction of somatostatin, glucagon, and insulin within the islets.

The present study confirms previous observations that arginine stimulates pancreatic somatostatin release[20-22] and further demonstrates that the 48-h fasting enhances arginine-induced somatostatin release, when decreased basal levels were taken into consideration. Recently, Shapiro et al.[28] reported that somatostatin-like immunoreactivity (SLI) in portal blood was decreased in 72-h fasted rats compared with that in the fed rats. Moreover, they also demonstrated that the decrease in basal SLI was associated with an increased tissue SLI, thus indicating the possibility of re-

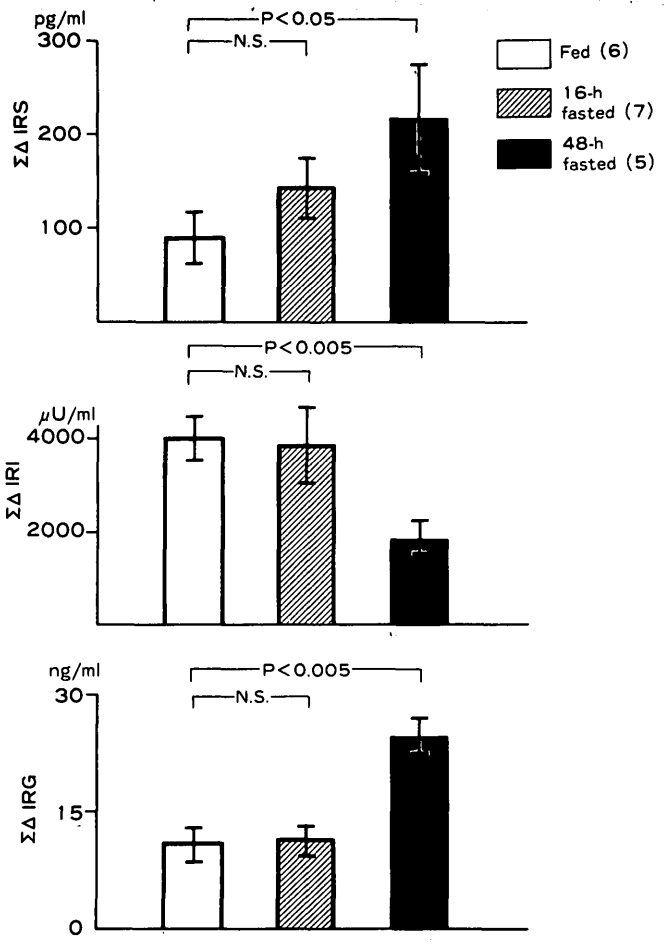

FIGURE 2. Somatostatin, insulin, and glucagon responses to 19 mM arginine in the presence of 4.4 mM glucose from isolated perfused pancreas, expressed as the sum of the increments above basal levels during arginine infusion. Abbreviations are shown as ΣΔIRS, ΣΔIRI, and ΣΔIRG.

duced secretion. On the other hand, Schauder et al.[29] observed an increase in stimulated somatostatin secretion from perifused isolated islets of 48-h fasted rats, although they used glucose as the stimulus. Our present data with low baseline and high stimulated somatostatin secretion seem to agree with the results of Shapiro et al. and Schauder et al., respectively. Further investigation should clarify the exact mechanism by which somatostatin secretion changes during fasting.

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