Effect of Fasting on the Release of Insulin and Somatostatin from Perifused Islets of Langerhans

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SUMMARY

Release of somatostatin and insulin from perifused islets of fasted and control rats was compared. After a fasting period of 48 h glucose-induced insulin release but not somatostatin release was diminished. Islets from fasted rats released significantly more somatostatin in the presence of 3.3 mM glucose than islets from controls. Simultaneously, the somatostatin content of isolated islets from fasting rats was significantly decreased.

The results indicate that the low secretory activity of islet B cells in the fasting state is associated with a high secretory activity of islet D cells. DIABETES 28:204–207, March 1979.

Release of immunoreactive somatostatin from D cells of pancreatic islets seems to be well established,[1–11] but its possible role in islet physiology remains at present speculative.

The secretory function of islet B cells is diminished by fasting.[12–26] To learn more about the possible physiologic role of somatostatin we compared the release of insulin and somatostatin from islets of fasted and control rats.

MATERIALS AND METHODS

Experimental animals. Male Wistar rats (210–260 g) were used throughout the study. The rats had free access to food and water or were fasted for 24, 48, or 72 h.

Reagents. Bovine serum albumin was purchased from Behringwerke A. G. Marburg, Federal Republic of Germany; ^{125}I-labeled porcine insulin (sp. act. 150–200 mCi/mg) from Farbwerke Hoechst A. G., Frankfurt, FRG; crystalline rat insulin from NOVO Industri A.S. Copenhagen, Denmark. Cyclic somatostatin standard and 1-Tyr-somatostatin were kindly provided by Dr. Romandini and Mr. Harrant, Serono, Freiburg. Collagenase was purchased from Worthington Biochemical, Freehold, New Jersey.

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Isolation and perfusion of islets. Islets were isolated from rat pancreas by collagenase 2 h after intraperitoneal administration of 0.6 ml pilocarpine hydrochloride (2% wt/vol), as previously described.[27,28] Perfusions were for 60 min with Krebs-Ringer bicarbonate buffer (0.2 mg/ml BSA; 1000 KIU/ml Trasylol; pH 7.4; 37°C; flow rate, 0.8 ml/min).[29] Two batches of 250 islets were investigated in parallel, one obtained from a 48 h-fasted rat and the other from a control animal. All perifusions were preceded by a 15-min preperifusion period with 2 mM glucose to reach steady-state conditions. Extraction of insulin from isolated islets was by acid/ethanol[30]; extraction of somatostatin was by 0.2 M acetic acid.[31]

Radioimmunoassays. Insulin and somatostatin quantities released into the perifusion medium or extracted from islets were determined by radioimmunoassay with either rat insulin or synthetic cyclic somatostatin as the reference standard.[6,31,32] The somatostatin assay reads 300 fg with 95% confidence.[31]

The data are presented as means ± SEM. Statistical analysis was by Student's *t* test based on nonpaired comparisons.

RESULTS

Figure 1 shows the secretion of insulin (upper panel) and somatostatin (lower panel) from pancreatic islets isolated from 48 h-fasted or from control rats. Perifusions were with 3.3 mM glucose. Insulin release from islets of fasted rats is lower than from controls [279 ± 60(7) vs 451 ± 73(7) ng/60 min/250 islets; $P < 0.025$]. Somatostatin release from islets of fasted rats is higher than from the controls. This difference is statistically significant during the first 30 min of the perifusion [444 ± 86(7) vs 223 ± 34(7) pg/30 min/250 islets respectively; $P < 0.025$].

Figure 2 shows the secretion of insulin and somatostatin from islets of 48 h-fasted and from control rats in the presence of 10.0 mM glucose. Insulin release from islets of fasted rats is lower than from the controls [814 ± 156(8) vs 1536 ± 229(8) ng/60 min/250 islets; $P < 0.01$] (upper panel). Somatostatin release from islets of fasted and con-

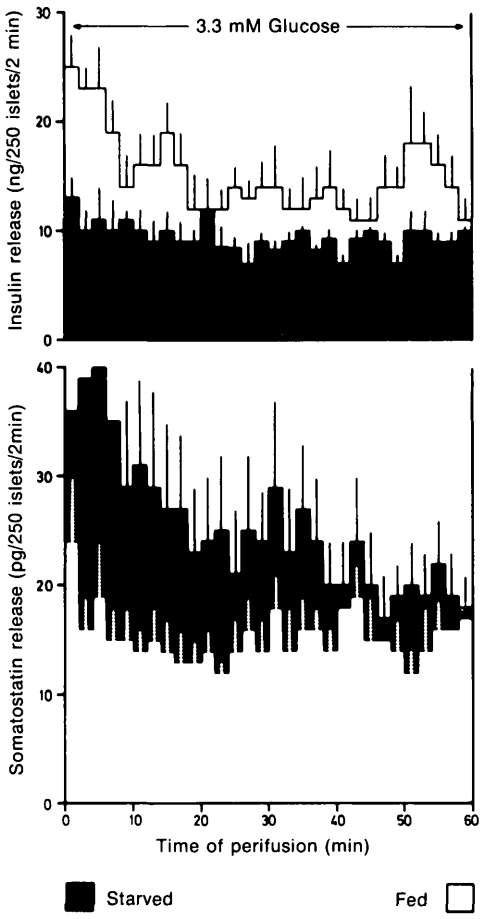


FIGURE 1. Effect of 3.3 mM glucose on insulin release (upper panel) and somatostatin release (lower panel) from perifused pancreatic islets of 48 h-fasted (shaded area) and fed rats (light area). In each experiment one batch of 250 islets from a fasted rat and one from a fed rat is perifused in parallel. Mean values ± SEM from seven perifusions are shown. Two-minute fractions are plotted. Insulin release is significantly diminished by fasting ($P < 0.025$). Somatostatin release from islets of fasted rats is increased compared with the controls. The difference is statistically significant during the first 30 min of the perifusion ($P < 0.025$).

trol rats is not significantly different [591 ± 121(8) vs 357 ± 67(8) pg/30 min/250 islets] (lower panel).

Figure 3 shows the secretion of insulin (upper panel) and somatostatin (lower panel) from islets of control or 48 h-fasted rats. Perifusions were with 25.0 mM glucose. Whereas insulin release is significantly diminished by fasting [843 ± 162(5) vs 1991 ± 369(5) ng/60 min/250 islets; $P < 0.01$], somatostatin release is not [509 ± 95(5) vs 507 ± 113(5) pg/30 min/250 islets].

The dose/response relationship between glucose concentration and release of insulin and somatostatin from islets of fasted or control rats is shown in Table 1. Islets from fasted and control rats respond to an increase of the glucose concentration with an increase in insulin release. There is also a dose/response relationship between glucose concentration and somatostatin release in islets from control rats. Such a relationship is not observed in islets from 48 h-fasted animals. They display a high somatostatin release already in the absence of 3.3 mM glucose that is not further augmented by increasing the glucose concentration to 10.0 or 25.0 mM [444 ± 86(7) vs 591 ± 121(8) vs 509 ± 95(5) pg/30 min/250 islets, respectively].

Table 2 shows the effect of fasting on the content of insulin and somatostatin from freshly isolated islets. Islet somatostatin content is significantly decreased after a fasting period of 24, 48, or 72 h [37 ± 6(20), 38 ± 5(20) and 27 ± 2(40) respectively vs 53 ± 3(30) pg/islet in the controls; $P < 0.01$]. Insulin content/islet is diminished after a 72-h fasting period only (49 ± 2(30) vs 59 ± 2(30) ng/islet; $P < 0.01$].

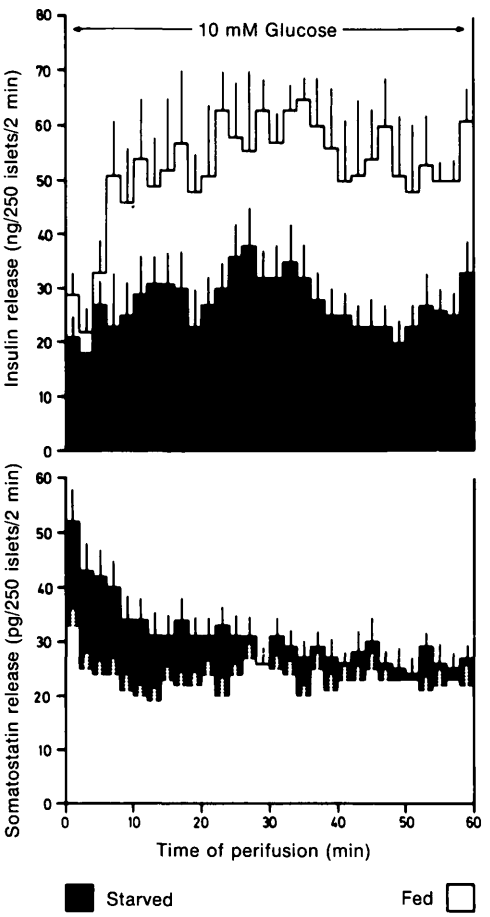
DISCUSSION

The data indicate that the release of insulin from isolated rat pancreatic islets is diminished by fasting for 48 h. Islets from fasted rats respond to an increase in the concentration of glucose with an increased insulin release; however, this response is significantly less than the response of islets from control rats (Figures 1–3, Table 1). This confirms previous observations.[12–26]

Why fasting is associated with a decreased insulin release is not known, but an abnormal (diminished) glucose metabolism[21,24,26] and/or an abnormal generation of adenosine-3′,5′-cyclic monophosphate (cAMP) in B cells[20,22,23,25] have each been considered as a possible explanation for the impaired insulin release induced by fasting.

Somatostatin release from islet D cells is not diminished

FIGURE 2. Effect of 10 mM glucose on insulin release (upper panel) and somatostatin release (lower panel) from perifused pancreatic islets of 48 h-fasted (shaded area) and fed rats (light area). Two minute fractions are plotted. Mean values ± SEM from eight perifusions are shown. Insulin release is significantly diminished by fasting ($P < 0.01$). Somatostatin release from islets of fasted and fed rats is not significantly different.



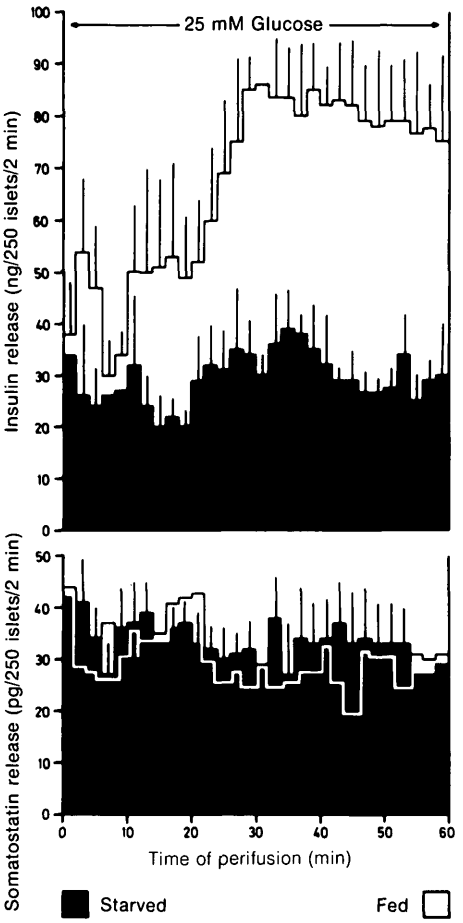


FIGURE 3. Effect of 25 mM glucose on insulin release (upper panel) and somatostatin release (lower panel) from perifused pancreatic islets of 48 h-fasted (shaded area) and fed rats (light area). Two minute fractions are plotted. Mean values ± SEM from five perifusions are shown. Insulin release is significantly diminished by fasting ($P < 0.025$). There is no significant difference in somatostatin release from islets of fasted rats compared with controls.

TABLE 1
Dose/response relationship between glucose concentration and the release of somatostatin and insulin from perifused pancreatic islets of 48 h-fasted or fed rats

	Glucose (mM)	Somatostatin release (pg/250 islets/ 30 min)	Insulin release (ng/250 islets/ 30 min)
Fasted (48 h)	3.3	444 ± 86(7)	146 ± 19(7)
Control	3.3	223 ± 34(7)*	240 ± 31(7)*
Fasted (48 h)	10.0	591 ± 121(8)	420 ± 73(8)
Control	10.0	357 ± 67(8)	715 ± 55(8)*,†
Fasted (48 h)	25.0	509 ± 95(5)	413 ± 116(5)†
Control	25.0	507 ± 113(5)†	818 ± 63(5)*,†

Mean values ± SEM are shown with the number of perifusions in parentheses. Significant differences between fasting and control rats are indicated by *; between 3.3 and 10.0 or 25.0 mM glucose by †. Somatostatin release from islets of 48 h-fasted rats is significantly higher in the presence of 3.3 mM glucose compared with controls, but is not further stimulated by increasing the glucose concentration to 10.0 or 25.0 mM.

* $P < 0.025$ or less vs fasted.

† $P < 0.025$ or less vs 3.3 mM glucose.

TABLE 2
Content of somatostatin and insulin in pancreatic islets of 48 h-fasted and fed rats. Determinations were from batches of 100 islets. Mean values ± SEM are shown with the number of individual observations obtained from 10–20 rats

Starvation (h)	Body weight (g)	IR-Somatostatin (pg/islet)	IR-Insulin (ng/islet)
—	252 ± 3(30)	53 ± 3(30)	59 ± 2(30)
24	235 ± 4(20)	37 ± 6(20)*	58 ± 3(20)
48	220 ± 4(20)	38 ± 5(20)*	54 ± 3(20)
72	202 ± 5(40)	27 ± 2(40)*	49 ± 2(30)*

* Significant difference from the respective controls ($P < 0.01$).

by fasting. Islets from fasted rats even release significantly more somatostatin in the presence of 3.3 mM glucose, and as much in the presence of 10.0 or 25.0 mM glucose as islets from control rats (Figures 1–3). This finding together with the demonstration of a decreased somatostatin content of isolated islets after fasting (Table 2) suggests a high secretory activity of islet D cells during fasting.

The low secretory activity of B cells associated with the high secretory activity of D cells induced by fasting might reflect intra-islet cell to cell interactions.

There is abundant pharmacologic evidence that somatostatin inhibits insulin release.[33] Moreover, inhibition of insulin release by endogenous somatostatin has recently been reported,[34] but there is also a negative report.[35]

If the D cell is indeed involved in inhibition of insulin release, it is conceivable that the high secretory activity of the D cell might be one of the reasons why insulin release is diminished in the fasting state.

There is, however, an alternative explanation for our observations. It was previously shown that fasting was associated with an increased glucagon release, and at low glucose concentrations an increase in glucagon secretion appears to be a constant finding.[16,36–39] Since insulin has been reported to diminish the secretory activity of the islet A cell,[38] it is conceivable that the increased glucagon release during fasting might reflect a fasting-associated lack of insulin. In analogy, somatostatin release during fasting might be high because the secretory activity of the B cell is low.

Our data thus indicate that during fasting there is a low secretory activity of the B cell but a high secretory activity of the D cell. However, in the absence of a pure cell preparation, the cause/effect relationship between the two phenomena is difficult to establish.

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