PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 210-214 (1977)

Effects of Fasting on Insulin and Glucagon Secretion by Isolated Rat Islets of Langerhans (39639)

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Introduction. Fasting results in changes of hormonal balance which favor the provision of substrates necessary for the continued production of energy. An important hormone in this regard is glucagon. In pharmacological amounts, this hormone stimulates glycogenolysis and gluconeogenesis in the liver (1, 2) and the release of fatty acids from adipose tissue (3, 4). Glucagon concentrations in the blood have been shown by some investigators to increase markedly during periods of glucose deprivation (5, 6), but others have shown little or no change (7, 8, 9). Interpretation of such conflicting observations made *in vivo* is complicated by a number of factors. Thus, the antisera used for radioimmunoassay of glucagon have not always been specific for pancreatic glucagon; they can cross-react with other substances present in plasma (10, 11). In addition, it is known that fasting can cause other hormonal changes which directly affect metabolism at the tissue level and which could directly or indirectly influence glucagon secretion from the pancreas *in vivo*. In order to determine whether fasting has any direct effect upon pancreatic endocrine function, glucagon and insulin secretion were measured in isolated islets of Langerhans obtained from rats killed in the fully fed state or after fasting for periods of 24 to 72 hr.

Materials and methods. Male Sprague-Dawley rats (250-300 g) had free access to water and were fed *ad libitum* on Purina rat chow except during periods of fasting. All experiments were performed between 0900 and 1100 hr. Animals were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg body weight). After laparotomy, blood was collected by cardiac puncture into a heparinized syringe and chilled in tubes containing Trasylol (F.B.A. Pharmaceuticals, N. Y., 200 U/ml of blood). The blood was centrifuged, and plasma separated within 5 min of collection; the plasma was stored at −20° until assayed for glucagon. Glucose was determined in each sample of blood before addition of Trasylol using an Ames reflectance meter (Ames, Elkhart, Ind.).

Islets were isolated by the collagenase digestion procedure of Lacy and Kostianovsky (12). Briefly, this procedure involves cannulation of the common bile duct and injection of approximately 20 ml of buffered Hanks' solution to disrupt the acinar tissues of the pancreas. The pancreas is removed, cleaned of all connective and extraneous tissue, chopped into small pieces, and exposed to collagenase (6-10 mg/pancreas; Type IV, Worthington Biochemicals, N. J.) during rapid hand shaking at 37° for 7-10 min. Enzymatic digestion of the pancreas is terminated by 4 × 10-ml buffer washes and centrifugation steps. After a final centrifugation, the pellet of pancreas and islet tissue is poured into siliconized glass petri dishes, and the islets are removed with a small glass loop. Approximately 150 islets were transferred to a Swinnex Millipore chamber (13 mm; Millipore, Bedford, Mass.) containing a 5-μm pore size membrane. The chamber containing islets was submerged in a water bath maintained at 37° and perifused with Krebs Ringer bicarbonate buffer supplemented with bovine plasma albumin (0.2%; Fraction V, Sigma Chemical Company, St. Louis, Mo.). The perifusion medium was maintained at pH 7.4 with a 95% O_2:5% CO_2 gas mixture.

Experimental protocol. During each experiment, two chambers were perifused simultaneously at a flow rate of 1 ml/min. One chamber contained islets from a fed rat and the other chamber islets from a fasted rat. An equilibration period of 15-20 min preceded the beginning of perifusate sample collection. Effluent samples (0.1 ml), col-

ISSN 0037-9727

lected in the presence of 500 U of Trasylol, were immediately frozen and stored at −20° until assayed for insulin and glucagon. Islets were stimulated with arginine (10 m*M*; Sigma Chemical Company, St. Louis, Mo.) or with a combination of arginine (10 m*M*) and glucose (16.7 m*M*).

Radioimmunoassay procedures. Insulin was determined by the method of Makulu *et al.* (13) using alcohol precipitation for the separation of antibody bound from free hormone. Glucagon was determined using an antiserum specific for pancreatic glucagon and a double antibody technique slightly modified (14) from the method of Pagliara *et al.* (15). Porcine insulin and glucagon (Eli Lilly Company, Indianapolis, Ind.) were used as standards and for labeling with iodine-125.

Data analysis. Students *t*-test was used for statistical analysis of data. A *P* value of 0.05 or less was considered to be significant.

Results. Blood glucose and plasma glucagon. Table I shows blood glucose and plasma glucagon levels for fed animals and animals fasted for periods of 24, 48, and 72 hr. Glucose concentrations were significantly lower for all fasting animals when compared with fed controls ($P < 0.001$). Glucagon concentrations were not altered by fasting.

Perifusion studies. As shown in Fig. 1–3, islets, perifused in low glucose media (1.67 m*M*) and stimulated with arginine (10 m*M*) after 10 min, exhibited biphasic secretion of glucagon. Fasting for 24, 48, or 72 hr had little or no effect on either the first or second phase of secretion. Increasing the concentration of glucose in the perifusion media to 16.7 m*M* after 30 min resulted in a decrease of glucagon secretion by islets from both fed and fasted animals. Addition of arginine (10 m*M*) to islets perifused in low glucose (1.67 m*M*) media did not affect basal insulin secretion rates. Increasing the glucose concentration to 16.7 m*M* then stimulated biphasic insulin secretion by is-

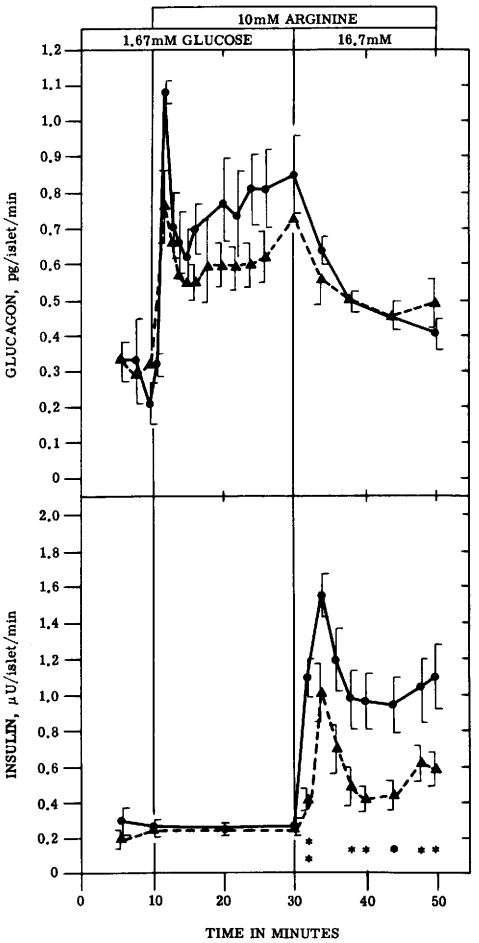

FIG. 1. Upper panel shows glucagon secretion rates by islets prepared from fed (●——●) and 24-hr fasted (▲-----▲) rats. Lower panel shows insulin secretion rates for the same islets. Each point represents the mean ± SEM for six observatons. (*) Represents $P < 0.05$, and (**) represents $P < 0.005$.

TABLE I. BLOOD GLUCOSE AND PLASMA GLUCAGON CONCENTRATIONS FOR FED CONTROL AND FASTED ANIMALS[a]

Period of fast (hr)	Glucose (mg/100 ml)		Glucagon (pg/ml)	
	Fed control	Fasted	Fed control	Fasted
24	166 ± 5_a (6)	95 ± 4_b (6)	188 ± 24_g (6)	204 ± 28_h (6)
48	145 ± 4_c (22)	84 ± 4_d (20)	167 ± 13_i (22)	146 ± 12_j (19)
72	156 ± 4_e (16)	105 ± 5_f (14)	142 ± 10_k (14)	137 ± 11_l (13)

[a] Statistical analysis: $P < 0.001$ for a versus b, c, versus d, and e versus f. There were no significant differences between g and h, i and j, or k and l. Numbers of observations are shown in parentheses.

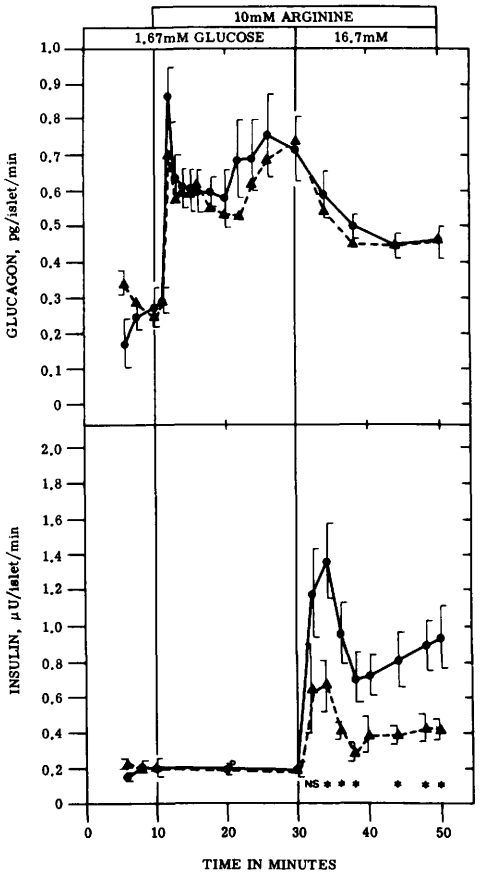

FIG. 2. Upper panel shows glucagon secretion rates by islets prepared from fed (●——●) and 48-hr fasted (▲-----▲) rats. Lower panel shows insulin secretion rates for the same islets. Each point represents the mean ± SEM for six observations. (*) Represents $P < 0.05$.

lets from both fed and fasted animals. Fasting was seen to cause significant reductions in both phases of insulin secretion. The onset of insulin secretion in response to glucose was not altered.

Discussion. Results from these studies indicate that, while fasting (24–72 hr) caused a significant decrease in blood glucose concentration, plasma glucagon concentration was not significantly affected. Furthermore, when stimulated with arginine islets, from fed and fasting animals demonstrated similar glucagon secretion rates. Buchanan *et al.* (8), using 3-day fasted rats, obtained similar results. However, in their studies, flask incubated islets were used, a system which does not permit either detection of subtle changes in secretion rate or analysis of the dynamics of glucagon secretion. In experiments using fed and 5-day fasted dogs, Buckman *et al.* (9) were unable to show any differences between plasma glucagon concentrations either in the basal state or during an alanine tolerance test.

Contrary to our findings are those of Aguilar-Parada *et al.* (7) who showed slightly elevated plasma glucagon concentrations in 3- to 4-day fasted human subjects and marked elevations in glucagon secretion in response to arginine infusion. Other investigators (5, 6) have reported increases in plasma glucagon concentrations after fasting, in man. Also, experiments carried out in the rat (16) demonstrated elevated

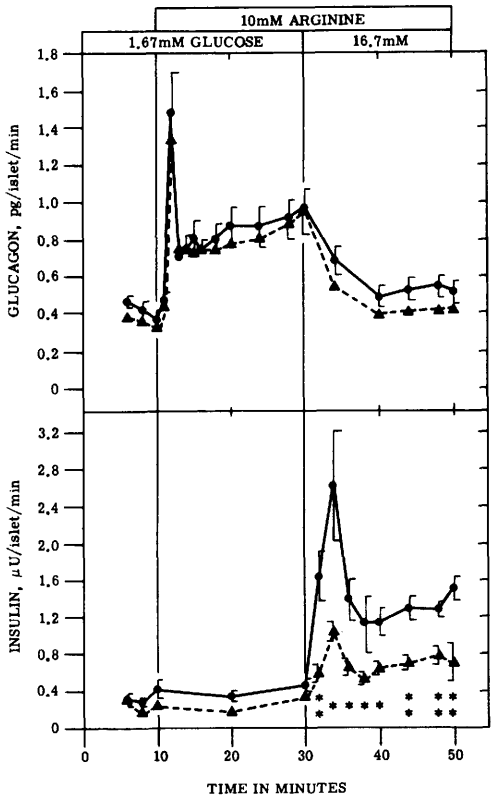

FIG. 3. Upper panel shows glucagon secretion rates by islets prepared from fed (●——●) and 72-hr fasted (▲-----▲) rats. Lower panel shows insulin secretion rates for the same islets. Each point represents the mean ± SEM for seven observations. (*) Represents $P < 0.05$, and (**) represents $P < 0.005$.

plasma glucagon concentrations, and, in the hamster (17), increased glucagon secretion rates by islets from starved animals. The reasons for differences between our findings and those of others is not readily apparent, but they may be due to differences in experimental design or animal species selected for study.

In regard to insulin secretion, it is readily apparent from our results that after 24 hr and up to 72 hr of fasting, insulin secretion rates were markedly reduced. Similar findings of reduced insulin secretion after fasting have been reported by many investigators (17–19). However, our results show, for the first time, that fasting will suppress both phases of insulin secretion, indicating a possible loss of sensitivity by the islets. Malaisse *et al.* (17) and Coddling *et al.* (18) have suggested such a mechanism on the basis of an altered threshold concentration at which glucose stimulates insulin secretion. Another possible explanation for reduced insulin secretion is decreased concentration of cyclic AMP in islet tissue during fasting (20). Islet content of insulin does not appear to be an important factor since Malaisse *et al.* (17) and Buchanan *et al.* (8) have shown little or no change in insulin content of islets prepared from fed and fasted (48–72 hr) rats.

The significance of our results in explaining the role of glucagon on glucose homeostasis is difficult to assess. However, the results are consistent with the thesis of Unger (21) that, during periods of acute glucose deprivation or prolonged fasting, it is the balance between glucagon and insulin which controls blood glucose concentration. Studies *in vitro* have clearly shown the counter regulatory roles of these two hormones in carbohydrate (22, 23) and fat metabolism (24). Thus, increases in plasma glucagon concentration may not be required during fasting, since the inhibitory effects of insulin on the glucogenic actions of glucagon will be reduced at the lowered plasma insulin concentration associated with this state. However, because of the many hormonal changes occurring during periods of glucose deprivation, the exact mechanism for maintenance of blood glucose still remains to be elucidated.

Summary. Rats fasted for periods of 24, 48, or 72 hr showed no changes in plasma glucagon concentration despite lowered blood glucose concentration. Islets isolated from both fed and fasted animals elicited biphasic glucagon and insulin secretion when stimulated with arginine and glucose, respectively. There were no differences in glucagon secretion rates between islets prepared from fed and fasted animals. Significant reductions in both the first and second phases of insulin secretion were observed in islets prepared from fasting animals.

The authors wish to thank Ms. Carol Grimme and Ms. D. Barr for typing the manuscript, and Dr. A. Pagliara for anti-glucagon serum. This work was made possible by Public Health Service Grant AM-16534.

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Received June 4, 1976. P.S.E.B.M. 1977, Vol. 154.