REVISTA ESPAÑOLA DE FISIOLOGIA, 34, 291-294. 1978

# Effect of Prolonged Fasting Upon Insulin and Glucagon Secretion From Isolated Rat Pancreatic Islets

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(Received on November 14, 1977)

J. C. PRIETO, S. ORTIZ, F. SOBRINO, M. T. HERRERA, F. BEDOYA and R. GOBERNA. *Effect of Prolonged Fasting Upon Insulin and Glucagon Secretion From Isolated Rat Pancreatic Islets.* Rev. esp. Fisiol., 34, 291-294. 1978.

To study insuling-glucagon interrelationships in the regulation of pancreatic islet functions, glucose-mediated insulin and glucagon secretion have been studied in isolated pancreatic islets from fed and from 4 and 8-day fasted rats. At low glucose levels (50 mg %) a continuous decrease of insulin and increase of glucagon secretion were observed during prolonged fasting. High glucose concentrations (300 mg %) stimulated insulin and inhibited glucagon secretion until 4 days, but did not cause any effect after 8 days fasting. These results suggest that the secretory mechanisms of the two hormones may have a common basis.

Functional interrelationships between the pancreatic hormones insulin and glucagon are generally accepted (4, 11). Furthermore, insulin secretion is inhibited by insulin itself (2) and somatostatin, the third pancreatic hormone, possesses an inhibitory effect on both insulin and glucagon secretion (12).

The possible inductive effects of glucose on pancreatic islet function are pertinent to changes in insulin and glucagon secretion observed in undernutrition. An evident rise of basal glucagon levels and a concomitantly reduced basal insulin concentration are observed after relatively short food deprivation periods of 24-48 hours in the rat (5, 9), but it is not well established if the glucose induced insulin secretion is responsible of the simultaneous inhibition of glucagon secretion or if this last effect is directly due to the sugar.

The purpose of this paper is to study the secretory capacity of pancreatic rat islets at low and high glucose levels and the modifications induced by increasing fasting periods in order to elucidate the relationships between insulin, glucagon and glucose during long periods of food deprivation.

## Materials and Methods

Three groups of male Wistar rats weighing between 300-350 g were used: *a*) control, with free access to a standard diet; *b*) 4 days fasting; *c*) 8 days fasting. Water *ad libitum* was supplied to the three groups of animals. Isolated pancreatic islets from each rat were prepared with a collagenase (Worthington Biochemical Co., Freehold) method (8).

Groups of 5 isolated islets were removed with a Pasteur pipette and preincubated for 10 min at 37° C in plastic tubes containing Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with 1 % bovine serum albumin (Behringwerke, Marburg-Lahn) and 1,000 U of kallikrein-trypsin inhibitor (Trasylol, Bayer Leverkusen) to prevent any damage of the glucagon released. Glucosa was added at the end of this period so that the final concentration was 50 or 300 mg % for a total volume of 1 ml; incubation with the sugar lasted 30 min. The tubes were subjected to a gas phase consisting of 95 % $O_2$: 5 % $CO_2$ and to constant shaking. Samples of the medium were frozen immediately after the incubation and stored at —25° C until assay of insulin and glucagon.

Immunoreactive insulin (IRI) was measured by a modified charcoal radioimmunoassay mehtod (10) using crystalline pork insulin (Lilly Co., Indianapolis) and our own antibody. Immunoreactive glucagon (IRG) was also measured by a modified charcoal radioimmunoassay method (1) using crystalline pork glucagon (Lilly Co.) and 30-K antibody (Dr. R. H. Unger, Dallas, Texas). Results were expressed as $\mu$U IRI or ng IRG released per 5 islets per 30 minutes.

## Results

The effects of glucose at 50 and 300 mg % on insulin release from rat islets at 0, 4 and 8 days fasting are shown in figure 1. Glucose 50 mg % evoked a basal insulin secretion of 70.7 ± 2.8 $\mu$U/5 islets/30 min in fed rats. This value was significantly decreased at 4 (55.4 ± 4.0, $p < 0.002$) and 8 (41.5±3.3, $p < 0.0005$) days fasting. Differences between the last two groups also were significative ($p <$

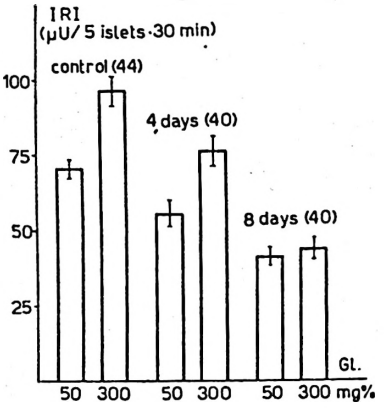


Fig. 1. *Glucose-induced insulin release at 0, 4 and 8 days fasting.*
Five islets were incubated for 30 min in the presence of 50 or 300 mg % glucose. Insulin concentrations after incubation are given as mean values (± S.E.M.) of the number of experiments indicated in parenthesis.

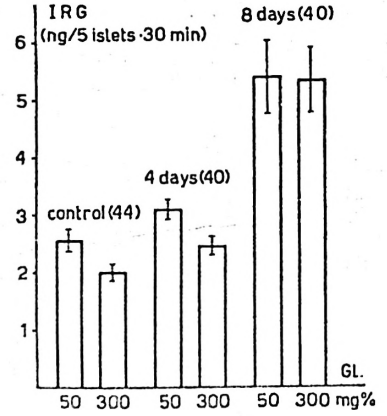


Fig. 2. *Glucose action on glucagon release at 0, 4 and 8 days fasting.*
Five islets were incubated for 30 min in the presence of 50 or 300 mg % glucose. Glucagon concentrations after incubation are given as mean values (± S.E.M.) of the number of experiments indicated in parenthesis.

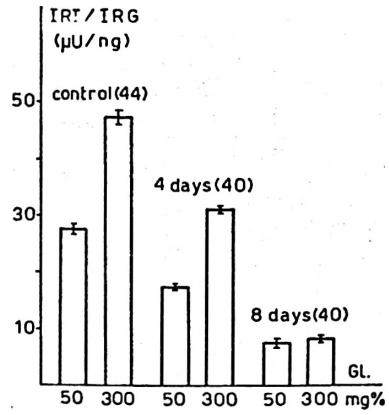


Fig. 3. *Insulin/glucagon ratios at 0, 4 and 8 days fasting for glucose 50 and 300 mg %.* Mean values (± S.E.M.) are shown.

0.005). Glucose 300 mg % significantly increased insulin secretion in fed (95.8 ± 5.0, 35.5 % above the basal, $p < 0.0005$) and in 4-day fasted rats (76.4 ± 4.7, 37.9 %, $p < 0.002$), but not after 8 days fasting (44.2 ± 3.5, 6.3 %, N.S.).

Figure 3 shows the opposite effect with glucagon. Basal glucagon secretion at glucose 50 mg % was 2.56 ± 0.21 ng/5 islets/30 min in fed rats; it was significantly increased at 4 (3.10 ± 0.18, $p < 0.05$) and 8 (5.39 ± 0.71, $p < 0.0005$) days fasting. Differences between the last two groups also were significative ($p < 0.0005$). A significantly decreased glucagon secretion was observed with glucose 300 mg % in fed (2.02 ± 0.15, 21.1 % under the basal, $p < 0.02$) and in 4-day fasted rats (2.44 ± 0.16, 21.3 %, $p < 0.005$), but not after 8 days fasting (5.33 ± 0.63, 1.1 %, N.S.).

Figure 3 shows the ratio between insulin and glucagon levels (μU/ng) at each glucose concentration. It can be seen that this ratio decreases during fasting at the two glucose concentrations studied. The ratio increases by 71 and 75 % above the basal at 0 and 4 days fasting, respectively, when glucose in the medium reaches a concentration of 300 mg %. However, it remains practically unchanged after 8 days of food deprivation (7.9 %).

## Discussion

It is a well known fact that glucose-induced insulin release leads to a decrease of glucagon secretion (4). Nearly complete inhibition of glucagon release can be obtained, both *in vivo* and *in vitro*, by appropriately high glucose concentrations. There is evidence (7) that the diminished glucose-induced insulin release from isolated islets from rats fasted for a period of 24-48 hours is similar to that obtained following maintenance of the islets *in vitro* with low glucose concentrations.

The results with isolated pancreatic islets from fed rats presented in this study are in agreement with previous literature: glucose 300 mg % evokes stimulation of insulin and inhibition of glucagon release with respect to the values obtained with glucose 50 mg %. Whether this response is due to the high glucose level or to the concomitantly released endogenous insulin or both, remains to be elucidated. It has been suggested that endogenous insulin is important to control glucagon secretion (13). Various states of insulin deficiency and hyperglycemia result in an inappropriate suppression of glucagon (3) and it has been shown (6, 14) that the level of adenylate cyclase activity in islets from fasted rats is significantly lower than that in islets from fed rats.

It can be seen from present results that 4 days fasting causes a diminished basal insulin secretion, but it does not alter the proportional response to high glucose levels; the reverse is true for glucagon. Then, 4 days fasting is not able to modify the whole response of pancreatic islets to the glucose surcharge. On the contrary, 8 days fasting suppresses this sensitivity to high levels of the sugar, despite the diminished insulin and augmented glucagon secretion at low glucose concentration. It is interesting to note that 8 days fasting is a situation nearly terminal for normal rats weighing around 300-350 g, as are the animals here studied (These

rats begin to die after 8 days fasting). This is not the case in 4-day fasted rats.

These results sugget that prolonged fasting, besides a continuous decrease of basal insulin and increase of basal glucacon secretion, blocks the response of A and B pancreatic cells to high glucose levels after 8 days of food deprivation, but not after 4 days. These simultaneous loss of sensitivity observed after 8 days fasting suggest that insulin and glucagon are secreted by mechanisms with a common basis.

*Acknowledgements*

We are also indebted to the Foundation Alexander von Humboldt for their material support in performing these studies.

## Resumen

Se estudia la secreción de insulina y glucagón en islotes pancreáticos aislados de ratas alimentadas o sometidas a ayuno de 4 a 8 días con el objeto de establecer las interrelaciones entre estas dos hormonas en la regulación de las funciones del páncreas endocrino. A bajos niveles de glucosa (50 mg %) se observa un continuo descenso de la secreción de insulina y un aumento de la de glucagón a medida que el ayuno progresa. Elevadas concentraciones de glucosa (300 mg %) estimulan la secreción de insulina e inhiben la de glucagón hasta el cuarto dia de ayuno, pero no a los 8 dias, sugiriendo que los mecanismos de secreción de ambas hormonas pueden tener una base común.

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