Evidence for an Insulin-Induced Inhibition of Insulin Release by Isolated Islets of Langerhans* (33608)

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The existence of a direct feedback inhibition of insulin secretion has been an object of controversy. Logothetopoulos and his collaborators (1) have shown that degranulation of the B cells and pancreatic insulin depletion were more marked in rats injected with guinea pig anti-insulin serum (GPAIS) than in rats maintained at a comparable level of hyperglycemia by a glucose infusion. They suggested that a possible explanation for this difference could be the establishment of a gradient of insulin concentration between the B cells and the blood, by the GPAIS-binding of circulating free insulin. Later, other investigators [cited in (2)], reached the conclusion that there is an equilibrium between the insulin stored in the ribosomes and the insulin present in the incubation medium, equilibrium which is ruled by the law of mass action. Frerichs and collaborators (3, 4), using glucose-stimulated pancreatic slices, found evidence for an inhibition of insulin secretion by endogenous insulin allowed to accumulate in the medium or by exogenous insulin added to the medium at the concentration of 10 mU/ml. Loubatières and collaborators (5) demonstrated an inhibition of insulin release by perfused rat pancreas, when the glucose-containing medium was enriched with exogenous insulin at a concentration of 1.5–2 mU/ml. It should be pointed out that the concentrations of insulin used in the above mentioned experiments are very high when compared to those found in normal serum. On the other hand, using the perfused rat pancreas, Grodsky (6) was unable to detect any inhibition of insulin release by high concentrations of fish insulin. Similarly, in a few experiments of Malaisse and collaborators (7) neither insulin nor GPAIS modified the rate at which insulin was secreted by isolated islets in response to glucose.

During the course of other studies we uncovered evidence suggesting that the secretion of insulin by the pancreas of hamsters with a transplantable islet-cell tumor, was suppressed and that insulin itself was an important factor in determining the suppression (8). This observation provided the stimulus to undertake the experiments described in this paper.

Materials and Methods. Adult female golden hamsters, weighing 85–135 g and fasted for 24 hr, were killed by cervical dislocation. The islets were prepared according to a modification of the method of Lacy and Kostianovski (9), without previous staining with neutral red or disruption of the pancreas with Hanks–Wallace (H-W) solution (10). The pancreas was dissected and trimmed in an ice-cold bath of H-W solution containing no glucose, transferred into a weighing bottle, cut into small pieces and digested with collagenase (50 mg/pancreas) for 20 min at 37°. The suspension was poured into a centrifuge tube, diluted to 30 ml with ice-cold H-W solution and spun at 10*g* for 2 min. Twenty-five ml of supernatant fluid were discarded and the islets were washed again in the same manner, selected for general appearance and diameter using a dissecting microscope and a micrometric eyepiece and picked out with a small wire loop. Thirty islets from each animal were obtained and distributed into six 25-ml rubber-stoppered Erlenmeyer flasks containing 10 ml of Krebs–Ringer bicarbonate buffer, enriched with bovine serum albumin (1%) and glucose (100, 200, or 300 mg/100 ml). Three flasks were used as controls, the other three contained bovine insulin (250, 500, or

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1000 μU/ml).[2] To insure that each flask contained the same amount of islet tissue, the islets were distributed so that their mean diameter was the same in each vial. After equilibration with a mixture of 95% O_2 and 5% CO_2 for 5 min, the flasks were incubated for 2 hr at 37° in a Dubnoff metabolic shaker. The time elapsed from the death of the animal to the start of incubation was 40–60 min. At the end of the incubation, the islets were removed by centrifugation at 280*g* for 5 min, 0.5 ml of medium was reincubated for 1 hr at 37° with 0.5 ml of a solution containing an excess of GPAIS[3] in phosphate buffer, pH 7.0, and 1% bovine serum albumin. Following this second incubation, the residual unbound GPAIS was back-titrated according to the method of Wright and Malaisse (11, 12). All insulin measurements were performed in triplicate, using beef insulin as standard and as labeled material; therefore, the results must be considered beef insulin equivalents.

Results. As shown in Table I, insulin release by the islets was not affected by the presence of added insulin when the glucose concentration in the medium was 100 mg/100 ml. On the other hand, when the concentration of glucose in the medium was 200 mg/100 ml, exogenous insulin, at a concentration of 250 μU/ml, significantly inhibited IRI output. Islets stimulated maximally by glucose at the concentration of 300 mg/100 ml were insensitive to exogenous insulin at the concentration of 250 μU/ml, barely inhibited by exogenous insulin at a concentration of 500 μU/ml ($p = 0.06$) but strongly inhibited when the concentration of exogenous insulin was 1000 μU/ml.

Discussion. Plasma IRI levels of 250, 500, and 1000 μU/ml are not seen in normal individuals, although levels of 250 or 500 μU/ml may occur in cases of pathologic hyperinsulinism accompanying islet cell tumors, maturity onset diabetes and obesity or following the administration of insulin or sul-

TABLE I. Effect of Exogenous Insulin on the IRI Output of Isolated Pancreatic Islets Stimulated with Glucose, Golden Hamster.

Glucose (mg/100 ml)	Av body wt. (g)	Added insulin (μU/ml)	Islet diameter (μ)	Islet volume ($\mu^3 \times 10^6$; av ± SE)	IRI output (μU/islet/2 hr; av ± SE)	IRI output ($\mu U/\mu^3 \times 10^6/2$ hr; av ± SE)	Significance (no. of animals)[a]
100	120	—	287.5 ± 7.2	12.61 ± 0.90	177.4 ± 34.51	13.70 ± 2.54	NS (8)
		250			179.6 ± 35.3	14.32 ± 2.60	
200	117	—	281.7 ± 7.3	11.96 ± 0.88	394.3 ± 65.7	33.5 ± 4.78	$p < 0.0025$ (12)
		250			270. ± 54.8	22.96 ± 4.35	
300	115	—	255 ±13.8	9.20 ± 1.35	498.5 ±112.5	51.35 ± 6.76	NS (8)
		250			484.2 ±117.3	48.23 ± 6.49	
300	122	—	288.5 ± 7.5	12.88 ± 0.99	647.8 ± 84.11	49.36 ± 5.07	$p = 0.06$ (13)
		500			571.2 ± 81.4	43.48 ± 6.05	
300	94	—	253.1 ± 8.1	8.67 ± 0.82	804.9 ±115.6	95.73 ±12.97	$p < 0.0005$ (8)
		1000			409.9 ±132.5	44.36 ±12.75	

[a] Number of animals in parentheses.

[2] Gift of Dr. Mary Root, Lilly Research Laboratories.

[3] Lot 400, a gift of Dr. P. H. Wright, University of Indiana.

fonylurea. In these conditions, persistent hyperinsulinism could lead to pancreatic suppression or atrophy and for this reason, we chose these concentrations of insulin for our *in vitro* experiments. Interpretation of our results requires the assumption that significant amounts of either added or released insulin were not lost by proteolytic destruction or adsorption to the glass during incubation. We believe this assumption to be reasonable for the following reasons: (i) the incubated islets were carefully freed of all exocrine tissue; (ii) the amount of tissue was very small in relation to the volume of the incubation mixture (approximately 50 μg in 10 ml of medium); (iii) albumin provides a degree of protection from both the proteolytic destruction of insulin and its adsorption to the glass; (iv) the amounts of insulin recovered in the experimental flasks were very close to theoretical. We have expressed the results both as insulin output per islet and as insulin output per unit volume of islet tissue;[4] the latter expression, which more accurately reflects the amount of tissue, was used for statistical calculations. The results indicate that the addition of insulin to the medium, at a concentration of 250 μU/ml, inhibited insulin release only when the islets were moderately stimulated by glucose, but not under basal conditions or when the islets were stimulated more strongly. Under conditions of "strong" stimulation, the insulin concentration had to be raised to 1000 μU/ml to obtain a significant inhibitory effect. We believe that these results provide evidence that the concentration of insulin required for the inhibition of insulin secretion increases in proportion to the metabolic stimulus. This variable adjustment would permit a continuous minimal output of insulin under basal conditions, while allowing it to reach successively higher cutoff levels, as the glucose concentration in the serum rises. Further experiments are necessary to confirm this hypothesis and to establish the physiologic significance of this mechanism *in vivo*. It should be pointed out that 3′, 5′-cyclic AMP in the B cell is believed to mediate the stimulatory effect of glucose, glucagon, and theophylline on insulin secretion (13) and that insulin lowers the level of this nucleotide in adipose tissue (14) and in the liver (15). It is possible that insulin may suppress the formation of cyclic AMP in the B cell, thus decreasing further insulin synthesis and release. Experiments designed to investigate this possibility are in progress. Meanwhile, we would like to suggest that the hypothesis of a feedback inhibition of insulin secretion by insulin itself, may explain, at least in part, the inhibition of B-cell activity in patients (16) and in hamsters (8) with insulin producing tumors or following prolonged insulin hypoglycemia (17, 18). The reason why our results do not agree with those of Grodsky (6) and of Malaisse and collaborators (7) are not clear: they could be attributed to differences in animal species, to the relative strength of the positive (glucose concentration) and negative (insulin concentration) insulinogenic stimuli, or to differences in technique, but these explanations are unsatisfactory even though temptingly obvious. The results presented in this paper justify the use of the method of Malaisse and collaborators (11) for the measurement of insulin release by isolated islets or pancreatic slices *in vitro*, because GPAIS binds the released insulin and, thus, prevents it from interfering with the secretory activity of the B cells.

Summary. Isolated islets of Langerhans were incubated in a medium containing different concentrations of glucose with or without different concentrations of insulin. The results suggest that insulin inhibits further insulin release and that the insulin level at which this feedback mechanism operates is determined by the intensity of the insulinogenic stimulus.

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[4] Assuming that no significant changes in the specific gravity of the islet tissue occurred during incubation, this is equivalent to expressing the results in terms of insulin output per unit of islet weight.

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Plateauing and Intermittent Treatment with Growth Hormone Extract (33609)

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Treatment of adult Norway rats with anterior pituitary growth hormone (GH) or GH-containing extracts initially produces a major renewal of growth, followed by reduced growth and eventual growth stasis (plateauing) while on treatment at the same GH dosage per rat or per unit weight (1–3). Plateauing on GH treatment is not due to aging (2), diabetes (1), altered steroid metabolism (4, 5), increased diameter of skeletal muscle fibers or decreased nuclear density (6, 7), or change in cardiac index (8). Attempted detection of GH-inactivating plasma antibodies in plateaued rats (9) was unsuccessful by the method used. Plateaued GH-treated rats show a major weight loss and cachexia when taken off treatment (3, 10), demonstrating continued effect of the hormone. This investigation attempts to establish the response to re-institution of GH treatment in rats showing the above weight loss when taken off treatment after plateauing at nearly twice normal adult size.

Methods. Normal intact 7-month-old female Long-Evans rats, averaging 260 gm in weight, were divided into 3 groups by split-litter technique. The GH extract, containing all anterior pituitary extractives soluble in 0.15 *M* NaCl at pH 10.5, was prepared in a single batch by a method previously described (1), designed to produce minimal denaturation. The extract, stored frozen in injection vials, and thawed just prior to injection, was assayed before and after the present investigation by the 10-day normal rat assay (11) and found not to have changed in potency. The daily dose per rat contained the pH 10.5 extractives of 100 mg (wet weight) bovine anterior lobe tissue and was equivalent by the above assay to 3.2 mg crystalline GH. Injections were given once daily s.c., under aseptic conditions, at systematically