The Inexhaustible Beta Cell

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SUMMARY

Repeated intensive pancreatic beta-cell stimulation was carried out in 42 subjects, comprising 22 normal controls, 10 mild to "severe" maturity-onset diabetics, and 10 chronic pancreatitis patients. Each subject received 75 gm. oral glucose twice and 1 mg. glucagon plus 0.5 gm. tolbutamide intravenously three times at short intervals. Each of the three combined stimuli caused almost equivalent marked spikes of insulin release in all experimental groups. The total calculated output of insulin was equivalent to the total daily insulin output in normal subjects. Pancreatitics and those with severe diabetes (fasting blood sugar greater than 120 mg./100 ml.) had qualitatively similar but a quantitatively smaller response. Those with mild diabetes were similar to the normal subjects but had an exaggerated response to the second oral glucose dose, suggesting overactivity of the enteroinsular axis. Despite the inordinate insulin levels, hypoglycemia did not occur. DIABETES *25*:11-15, January, 1976.

The pancreatic beta cell responds to a variety of insulin secretagogues, including glucose, amino acids, pancreatic glucagon, and other hormones of gastrointestinal origin, and the sulfonylureas. It seems that most agents stimulate the release of insulin from a small finite physiologic pool, as yet anatomically undefined and containing only 2-3 per cent of the total pancreatic insulin.[1-4] Glucose has the ability to stimulate insulin output from this acute-release pool and also to activate storage and release from a larger pool.[5] These secretagogues appear to act by different mechanisms at the membrane surface or within the beta cell.[6,7]

Earlier studies showed that elderly people,[8] genetic diabetics, and diabetics with chronic pancreatitis[9] had reduced insulin output when three of these agents (oral glucose, intravenous tolbutamide, and glucagon) were used in combination to provoke intense beta-cell stimulation.[10]

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Accepted for publication October 30, 1975.

We report here on the effects of repeated intensive stimulation of insulin secretion in normal control subjects, in persons with "mild" and "severe" maturity-onset diabetes, and in patients with chronic pancreatitis. The results we obtained do not readily fit a definable two-pool system.

MATERIALS AND METHODS

Forty-two subjects were studied. Twenty-two were normal, healthy, nonobese blood donors. Their ages were 23-53 years, and none were overweight. None had a family history of diabetes, none were taking drugs known to affect insulin secretion, and all were on a diet estimated to provide at least 200 gm. carbohydrate daily.

Ten patients attending the Groote Schuur Hospital Diabetes Clinic volunteered for the study. All had been taking either a sulfonylurea, a biguanide, or a combination of these, and they were instructed to discontinue medication for four days before being tested. Ages ranged from 33-60 years. None were more than 15 per cent above desirable body weight and none had ever received insulin. These diabetics divided themselves into two groups: four had fasting blood glucose levels above 120 mg./100 ml. (mean = 225) and six under 120 mg./100 ml. (mean = 98). For present purposes these two groups are referred to in the text as "severe" and "mild."

Ten patients with proved chronic pancreatitis[11] who were 22-62 years old, nonobese, and on no medical treatment, were also studied. One turned out to behave in a diabetic fashion (fasting blood glucose level at 110 mg./100 ml.), but all others had blood glucose levels little above that of the normal subjects with a mean fasting level of 65 mg./100 ml. Informed consent was obtained from all subjects.

Test Procedure

After an overnight fast each patient rested for half an hour in the laboratory in a comfortable armchair or a bed, and a polyethylene cannula was then placed in

an antecubital vein. Venous blood samples were drawn from and injections given into this cannula. Three basal samples (−30, −15, 0 minutes) were taken and 75 gm. glucose then ingested; further samples were taken at 1, 3, 5, 10, 20, and 30 minutes. Glucagon (Lilly) 1 mg. and 0.5 gm. tolbutamide (Rastinon, Hoechst) were injected rapidly and further samples taken at 31, 33, 35, 40, 50, and 60 minutes. A second injection of glucagon and tolbutamide ("combined injection") was then given and further samples drawn at 61, 63, 65, 70, 80, and 90 minutes. A further 75 gm. of glucose was rapidly ingested and blood samples taken at 91, 93, 95, 100, 110, and 120 minutes, after which the third combined injection was given and samples taken at 121, 123, 125, 130, 140, 150, and 160 minutes. Untoward reactions included severe nausea, which occurred regularly after the second glucose dose and was partly, but not entirely, eliminated by flavoring with citric acid, and thrombophlebitis in some instances. (We do not recommend the procedure for general use.)

An aliquot of each venous blood sample was taken for estimation of glucose by the Hoffman method on the Technicon AutoAnalyzer and the remaining blood allowed to clot. The serum was separated by centrifugation and deep-frozen for insulin estimation by radioimmunoassay with Amersham kits.

Statistical Analysis

Absolute means in each group and following each stimulus were compared by Student's *t*-test. The areas (μU./min.) of insulin responses were computed for each "spike" above the projected rising baseline and compared similarly.

Calculation of Insulin Secretion (Delivery) Rates

The equation used to calculate the pancreatic insulin-secretion rate was that derived by Manougian et al.,[12] where insulin delivery rate, $R(t) = Vdx(t)/dt + 220\ x(t) - 4{,}900$ (μU./min.). V, the insulin distribution space in milliliters, has been shown to be 20 per cent of the body weight and relatively constant. For the purpose of this paper, it was assumed to be 1.4×10^4 ml. The value x(t) (μU./ml.) is the plasma insulin concentration, and 220 x(t) − 4,900 (μU./min.) the irreversible loss rate of insulin.

Thus R(t) can be calculated at any time t from the plasma insulin curve. A correction factor has to be applied for the hepatic uptake of insulin, which is approximately 35 per cent, and the total amount of insulin secreted by the pancreas from time t_1 to t_2 minutes is:

$$\int_{t_1}^{t_2} \frac{R(t)\,dt}{0.65} = \frac{1}{0.65}\left(V \int_{t_1}^{t_2} \frac{dx(t)}{dt}\,dt + 220 \int_{t_1}^{t_2} x(t)\,dt - 4{,}900 \int_{t_1}^{t_2} dt \right) \quad \ldots (1)$$

A program calculating the total amount of insulin released was written for use with a Hewlett Packard 9100B Calculator and Digitizer.

An initial plot of plasma insulin concentration x(t) (μU./ml.) against time was done, and the area under selected parts of the concentration curve was found, i.e. ${}^{t_2}\int_{t_1} x(t)\,dt$. At the same time as the coordinates of the curve were transformed into Cartesian coordinates, the first differential of insulin concentration with respect to time was generated, and the area under this differential curve was determined for the same time limits t_1 and t_2 thus yielding ${}^{t_2}\int_{t_1} dx(t)\,dt$. The final term, $4{,}900\ {}^{t_2}\int_{t_1} dt$, reduces on integration to $4{,}900\ [t_2 - t_1]$.

The final calculation was then performed, as expressed in equation 1, giving the total pancreatic insulin release between times t_1 and t_2 minutes.

RESULTS

The response to the intensive stimulus in control subjects is illustrated in figure 1, which shows that the insulin response to repeated intense provocation within the space of 180 minutes was not exhausted. In fact the responses to the three combined glucagon and tolbutamide injections were very similar to each other (table 1). The mean total insulin release amounted to 46.3 ± 3.8 units. Oral glucose stimulation accounted for only a small fraction (0.87 ± 0.15 units) of the insulin output compared to each combined glucagon and tolbutamide stimulus (12.7 ± 1.8-16.0 ± 1.4 units); the second oral glucose dose elicited a significantly smaller response than the first (table 1). These extremely high insulin levels did not cause clinical hypoglycemia, and the blood glucose concentration fell below the fasting level only after the second and third combined injection. The rise in blood glucose after the second oral glucose load was smaller than after the first.

Figure 2 compares the response in controls, diabetics, and patients with pancreatitis. "Severe" diabetics showed no insulin response to either oral glucose load—i.e., before or after glucagon/tolbutamide. The insulin secretion following each combined injection was far less than that of the other groups but was of similar magnitude after each injection. Total insulin release was only 8.3 ± 2.9 units. "Mild" diabetics on the other hand secreted insulin almost as well as con-

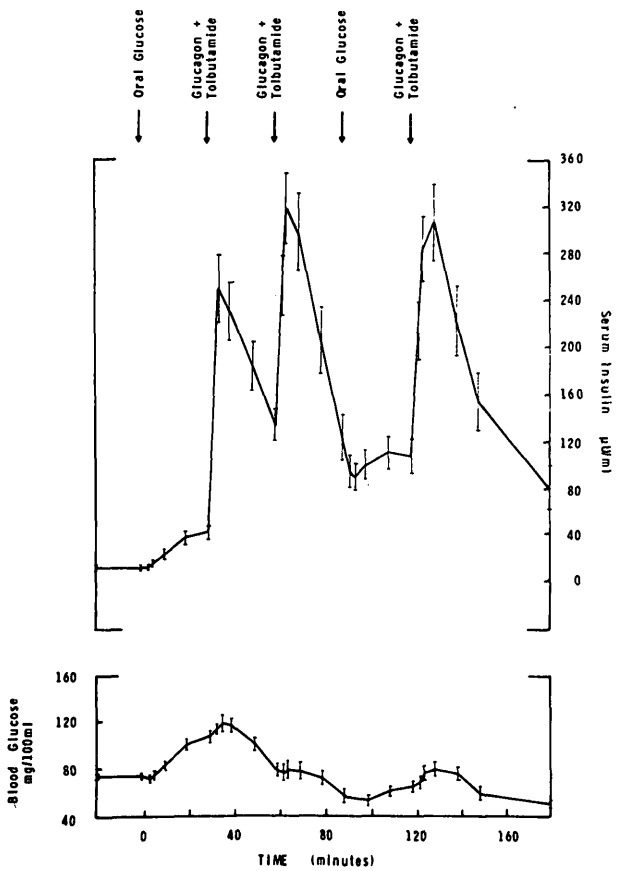

FIG. 1. Serum insulin and blood glucose responses to repeated stimulation in normal controls.

trols in response to all stimuli and indeed appeared to overrespond to the second dose of oral glucose (table 1, figure 2). Their total insulin release was 39.4 ± 9.5 units, which was similar to that of controls. Mean responses of patients with chronic pancreatitis were intermediate, midway between those of controls and "severe" diabetics but, again, equivalent amounts of insulin were released with each repeated combined injection. Their total insulin release was 19.1 ± 6.1 units. The circulating glucose concentration tended to fall after the rise in IRI following each combined injection.

DISCUSSION

Earlier workers who attempted to estimate pancreatic insulin reserve by using intensive beta-cell stimulation[9,10] did not discuss the fact that the total amount of insulin released was greater than could have been derived from a small finite pool[4,13] that comprises only 2-3 per cent of the total pancreatic insulin content.[2] The present studies corroborate the general findings of the earlier work and further show that repeated intensive stimulations yield almost identical peaks so that any single one can be taken to reflect the size of the "pool" or the reserve involved. By this argument the diabetics here clearly show reduction in the size of this "pool" but nevertheless are able to refill it very rapidly to its initial holding after each depletion.

The ability of the beta cells to release large quantities of insulin at intervals of 30 minutes without "tiring" suggests a rapid "refilling" process from a larger "provisional" source, the time required for this process being less than that suggested by some authors.[2] Even in the severely compromised diabetic

TABLE 1

Insulin responses (μU./ml. min.) to repeated intensive pancreatic beta-cell stimulation

Period	I Oral glucose	II Glucagon + tolbutamide	III Glucagon + tolbutamide	IV Oral glucose	V Glucagon + tolbutamide
Group					
Normals (n = 22)	514 ± 87	4,242 ± 457	3,742 ± 1,003	−514 ± 331	4,011 ± 790
Mild diabetics (n = 6)	306 ± 72	2,933 ± 890	1,917 ± 318	1,176 ± 745*	1,486 ± 1,538
Severe diabetics (n = 4)	41 ± 18*	413 ± 150†	397 ± 168†	172 ± 129	452 ± 287*
Pancreatitics (n = 10)	164 ± 47‡	1,953 ± 661†	930 ± 337‡	61 ± 123	2,444 ± 1,330

Differences significant from normal * = < 0.05; † = < 0.01; ‡ = < 0.02.

The mild diabetics (fasting glucose < 120 mg./100 ml.) have significantly ($p < 0.05$) greater insulin responses than the severe diabetics (fasting glucose > 120 mg./100 ml.) in periods I, II, and III. The insulin responses to the repeated doses of glucagon and tolbutamide (II, III, V) do not differ statistically in any group, but the response to the second oral glucose dose is significantly ($p < 0.01$) reduced in normals and enhanced in mild diabetics.

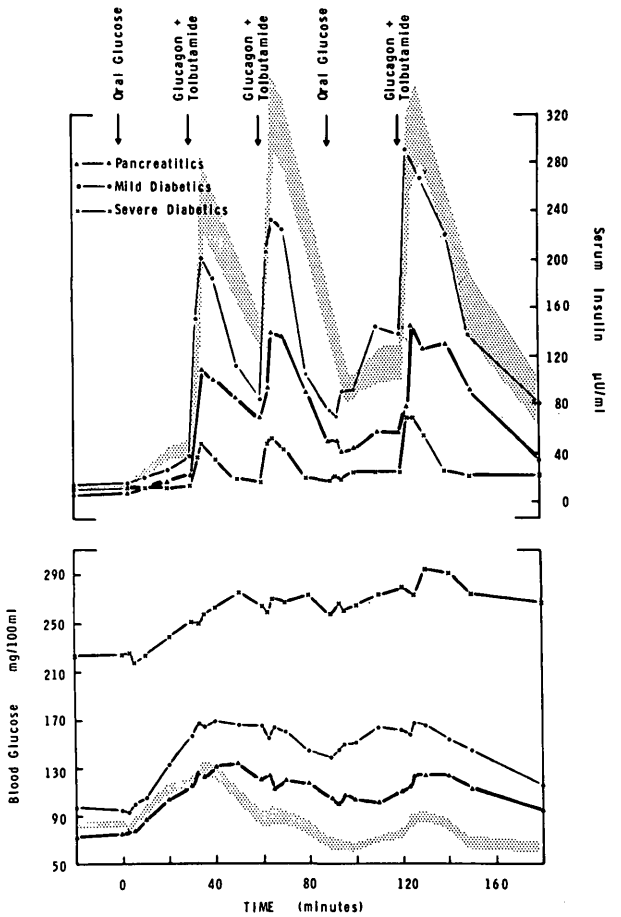

FIG. 2. Serum insulin and blood glucose responses to repeated stimulation in normal controls (shaded area), pancreatitics, and mild and severe diabetics.

pancreas, no refractoriness to the repeated pulses was seen. In contrast, repeated doses of glucose given intravenously in "staircase,"[14] or increasing pulse amounts,[15] result in attenuated acute insulin responses, suggesting depletion of the finite glucose-releasable insulin pool, which nonetheless may depend on various factors such as feedback inhibition or saturation of glucose receptors.[14] It is clear therefore that the equivalent increments in peak insulin responses found by us were not mediated by glucose alone but possibly by glucose (and tolbutamide plus glucagon) interaction with intestinal factors released by oral glucose.

The large amounts of insulin rapidly released with each combined stimulus considerably exceed those occurring after acute intravenous pulses of glucose,[16] arginine,[17,18] secretin,[19] tolbutamide,[20] or glucagon[21] of whatever magnitude. This seems incompatible with the generally held belief that insulin is released from a small pool comprising 2-3 per cent of the total pancreatic insulin content and refilling only slowly after depletion. It is possible that the acute-release pool is really considerably larger, but has been previously underestimated because submaximal stimuli have been used. Alternatively the greater stimulus in the present work may have elicited additional responses from more than one pool,[16] or possibly the whole "pool" concept may be an oversimplification.

The complete lack of insulin response to oral glucose in the "severe" diabetics, with retention of some response to the combined injection, suggests independent and separate mechanisms for the stimulation of insulin release, though the same "pool" may be present in each case. It is possible that the continuous supply of glucose from the large (75 gm.) oral dose and/or priming by intestinal factors facilitated rapid refilling of the small insulin pool stimulated by glucagon and tolbutamide.[16]

Of further interest are the virtually normal insulin responses in the "mild" diabetics, which would seem to indicate little or no reduction in their "pool" sizes. Nevertheless these normal insulin levels were ineffective in maintaining normal blood glucose levels, thus confirming the existence of some degree of insulin resistance[22,23] rather than insulin sensitivity[24] at this stage of the disease. An unexpected observation is the exaggerated rise in insulin in the "mild" diabetics after the second oral glucose load, consistent with our earlier suggestion that there is overactivity of the enteroinsular axis in mild maturity-onset diabetes.[25]

In conclusion we can state that intensive stimuli for insulin release did not lose their effect when repeated at short intervals in subjects with normal or compromised beta cells, while in all but "severe" diabetics the output of insulin *from each stimulus* was greater than the total amount currently believed to exist in the so-called acute-release pool. Very "mild" diabetics showed no evidence of any loss of this postulated "pool"; in fact to one oral glucose stimulus they secreted significantly more insulin than any other group. We are driven to suggest that the "pool" hypothesis, as currently formulated, is not entirely valid.

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