Pancreatic Compartmentalization of Insulin

Evidence for Pituitary Control

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SUMMARY

African pygmies who appear to be insensitive to the metabolic effects of human growth hormone (HGH) are insulinopenic after administration of either glucose or arginine. Contrary to our expectation, insulin responses in this group were normal after tolbutamide and glucagon. To clarify this observation, insulin secretion was studied in twenty-nine normal controls and six dwarfs with growth hormone deficiency.

After arginine and glucose, peak insulin responses in controls were, respectively, 90.4 ± 8.6 μU./ml. and 108.0 ± 12.0 μU./ml. In HGH deficient dwarfs, responses were 38.6 ± 6.0 μU./ml. and 40.0 ± 6.4 μU./ml. ($p < .01$). In controls as well as HGH deficient dwarfs, the mean maximal insulin responses to arginine and glucose were increased two- to fourfold by HGH treatment (5 mg. twice a day for five days). Similar results occurred after ingestion of mixed meals.

Both tolbutamide and glucagon caused normal insulin responses in the HGH deficient group. Mean maximal responses to tolbutamide were 69.4 ± 15.0 μU./ml. in controls and 61.6 ± 7.7 μU./ml. in HGH deficient dwarfs. Mean maximal responses to glucagon were 101.6 ± 19.4 μU./ml. and 102.0 ± 9.4 μU./ml., respectively. Unlike arginine and glucose, HGH had no effect upon insulin responses to either agent in controls.

The data support the division of pancreatic pools of insulin into two major groups: those dependent and those not dependent upon pituitary secretion of HGH. DIABETES 22:25-29, January, 1973.

Over the past several years a variety of models have been proposed to explain insulin storage in the pancreas and patterns of insulin release.[1-3] Although many points remain to be clarified, most investigators would accept the fact that insulin release involves multiple receptors, which vary in their sensitivity to different stimuli and in degree of suppressibility by such agents as norepinephrine.[3] Whether there are, in addition, multiple compartments of dischargeable insulin remains to be established, but the factors controlling basal insulin secretion certainly appear to vary from those modulating the acute discharge of insulin.

The present investigation stems from our earlier work which showed that human growth hormone (HGH) increased insulin secretion in dwarfs with a monotropic deficiency of HGH.[4,5] Because of a chance finding, which will be discussed in greater detail, we reinvestigated insulin secretion in this group after the subjects were exposed to various stimuli causing acute release of insulin. Our findings indicate that pancreatic beta cell receptors, pancreatic insulin pools, or both vary in their dependency upon pituitary secretion of HGH.

METHODS

Six dwarfs with a monotropic deficiency of HGH were admitted to the General Clinical Research Center of Boston University at Boston City Hospital. Each had been studied extensively on previous admissions and had been shown to have a deficiency of growth hormone, whereas other pituitary functions were normal.[6] Although each dwarf had been examined previously after both an infusion of arginine and a glucose tolerance test, these studies were repeated in conjunction with additional investigations. Studies carried out were as follows: (A) Arginine infusion: On the morning of the study, an antecubital vein was cannulated and its patency was then maintained by a slow infusion of saline. At least three basal blood samples were collected prior to infusion of arginine, which was given at a dose of .25 gm. per pound of body weight. Arginine was infused from time zero to thirty minutes; samples were collected every fifteen to thirty minutes for two hours. (B) Glucose tolerance: Each subject received 100 gm. of glucose orally at time 0. Samples were taken every thirty minutes for three hours via a needle previously placed in an antecubital vein. (C) Glucose/beef meal: In a manner similar to the glucose tolerance test, subjects ingested a combination meal consisting of 100 gm. of glucose and 350 gm. of beef tenderloin. The total

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time allotted for ingesting the meal was fifteen minutes. Samples were then collected every thirty minutes for three hours. (D) Glucagon infusion: Over a fifteen-minute period, 1 mg. of glucagon was infused. To facilitate early sampling, two intravenous cannulas were used, one for administering glucagon and the second for sample collection. Blood was collected every ten minutes for forty minutes, and then every thirty minutes for an additional hour. (E) Tolbutamide infusion: As with glucagon, two veins were utilized. Tolbutamide, 1 gm., was injected over a two-minute period, and blood was sampled from a contralateral vein at periods identical with the glucagon infusions.

Glucose was measured in all samples in duplicate by a glucose oxidase method with the maximum variation between duplicates of a sample being 1.5 mg. per 100 ml. Insulin was measured by a charcoal modification of the initial immunoassay technic of Berson and Yalow.[7] Samples were assayed in duplicate for insulin with the maximum variation between duplicates being no greater than 10 per cent. For purposes of comparison, data from glucose tolerance tests and glucose/protein meals in twenty-two normal subjects were utilized. Tolbutamide and glucagon infusions were carried out in eight normal controls in a manner similar to that used in the dwarf group. Seven of the control subjects were treated with HGH (5 mg. twice a day for five days) and restudied after each stimulus.

RESULTS

Insulin responses to glucose, arginine, and mixed glucose/beef meals. Figure 1 compares the mean insulin concentrations in plasma of control subjects and growth hormone deficient dwarfs after the infusion of l-arginine. Figures 2 and 3 compare similar responses after glucose and after glucose/protein meals. The mean maximal insulin responses to all stimuli are indicated in table 1.

Following the infusion of l-arginine, insulin reached a peak concentration of 35.0 ± 8.9 μU./ml. in HGH deficient dwarfs at thirty minutes, which contrasted with a concentration in normal subjects of 90.4 ± 8.6 μU./ml. ($p < .01$). A similar significant difference was noted fifteen minutes after the start of the infusion of arginine. Following ingestion of glucose, the greatest plasma insulin concentration in the dwarfs occurred sixty minutes after the ingestion (40.0 ± 6.3 μU./ml.), which was likewise significantly less than in the control group (77.3 ± 2.9 μU./ml.). A similar degree of insulinopenia occurred after mixed glucose/protein meals at all time intervals from thirty minutes to three hours.

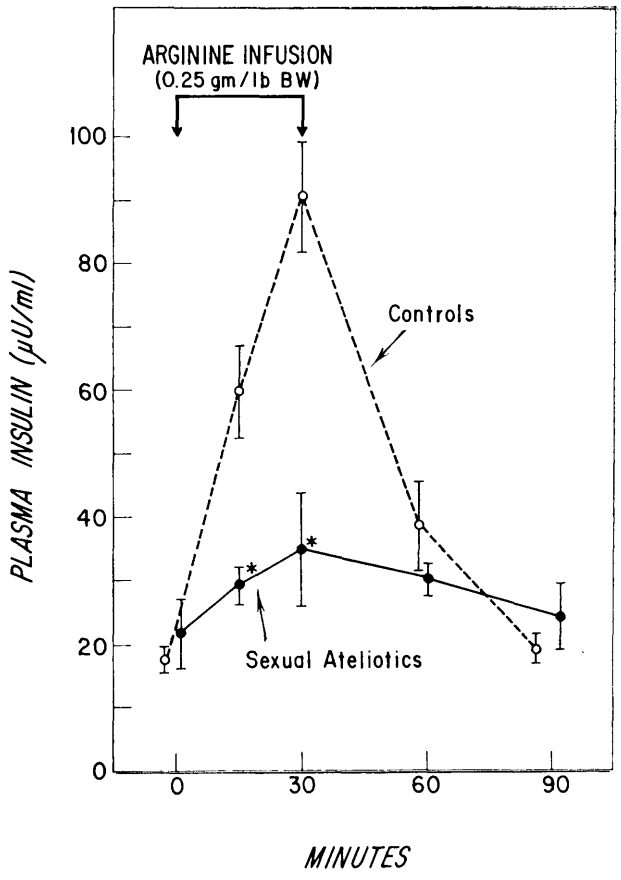

FIG. 1. Plasma insulin concentrations are shown after the infusion of l-arginine. Each point is a mean ± SEM. An * indicates that values at a given sampling period differ with $p < .01$.

In the glucose tolerance test the insulinopenia was actually more pronounced than suggested by the absolute figures, since the glycemic stimulus was greater in the dwarf group than in controls (see figure 2). On a body weight basis, dwarfs actually received an average of 1.4 gm. of glucose per kilogram of body weight, whereas controls received .75 gm. per kilogram of body weight.

Insulin responses to tolbutamide. Maximal insulin responses to tolbutamide in the growth hormone deficient dwarfs ranged from 46 μU./ml. to 90 μU./ml. The maximal response was 61.6 ± 7.7 μU./ml. In normal controls the mean maximal response to tolbutamide was 69.4 ± 15.5 μU./ml., which was statistically similar to that of the dwarf group. The insulin concentrations in both groups at selected time intervals following tolbutamide are compared in figure 4. The values in the dwarfs were similar statistically at all sam-

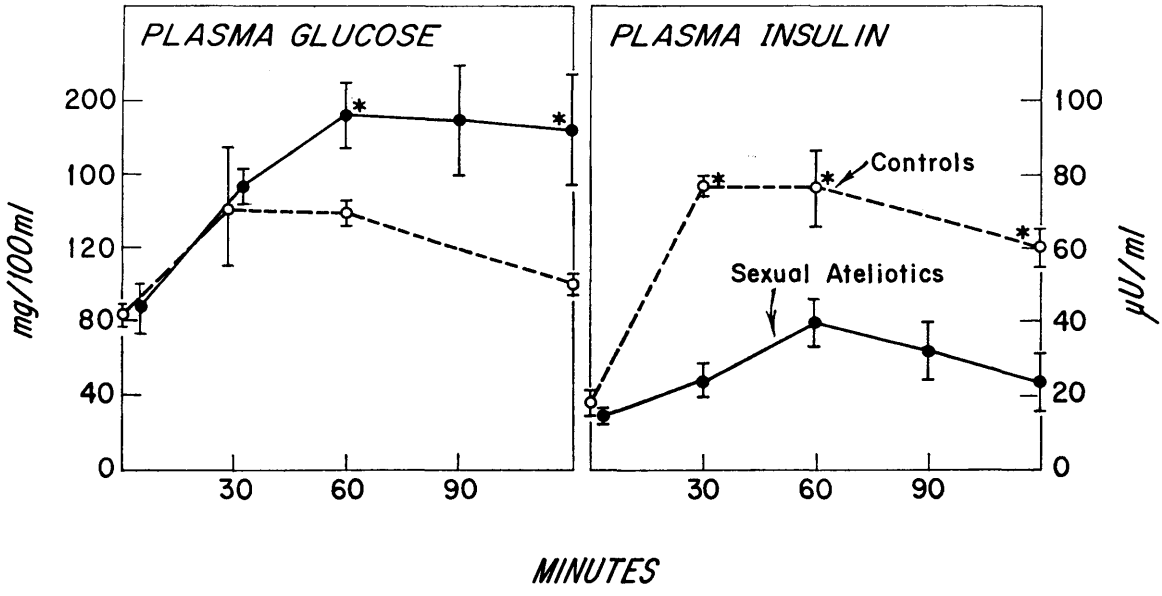

FIG. 2. Plasma glucose and insulin concentrations are shown after the ingestion of 100 gm. of glucose. Each point is a mean ± SEM. An * indicates that values at a given sampling period differ with $p < .01$.

pling periods to those observed in the control population.

Glucagon. As with tolbutamide, the mean maximal insulin responses to glucagon were similar in each group: the control response was 102.0 ± 9.4 μU./ml.; the mean maximal response in the dwarfs was 101.6 ± 18.3 μU./ml. Since glucagon also causes an increase in the plasma concentrations of glucose, the hyperglycemic response to glucose must also be taken into consideration. If one compares the insulin and glucose concentrations in plasma ten minutes following the injection of glucagon, these are similar in both groups. The mean insulin response in the dwarfs at the latter time interval was 99.2 ± 11.5 μU./ml., while glucose measured only 109.4 ± 3.2 mg./100 ml. In controls, plasma insulin concentration at this time was 102.6 ± 19.0 μU./ml., and the plasma glucose concentration was 105.5 ± 5.0 mg./100 ml. Hyperglycemia followed the peak insulin response in each individual.

HGH treatment and insulin responses. We showed previously the ability of HGH to increase insulin responses to glucose and arginine in sexual ateliotics.[4] It was not possible to perform a similar study after tolbutamide and glucagon in this group. These studies were done in the control subjects. Insulin responses to glucose and arginine were significantly augmented as in the dwarfs, while those occurring after tolbutamide and glucagon were unaffected (see table 2).

DISCUSSION

Our reason for investigating the effect of HGH on several stimuli for insulin release stems from observations made initially in the African pygmy. We had shown previously that African pygmies, in common with the majority of growth hormone deficient dwarfs, had insulinopenia after the administration of either arginine or glucose.[8] We wished to further substantiate this finding in the pygmy and chose glucagon and tolbutamide as insulinogenic stimuli. The results were completely the opposite of those expected—insulin responses were not decreased but normal.[9] By a variety of other criteria, such as a failure of HGH to raise plasma free fatty acids and decrease serum urea nitrogen, the pygmy was not responsive to HGH. We therefore sought some reasonable explanation for these data and postulated that insulin release mediated by tolbutamide and glucagon was not growth-hormone dependent, whereas other stimuli of insulin secretion were modulated by pituitary control.[8,9]

To test this hypothesis, we chose six dwarfs known to be deficient only in HGH who had been studied previously after at least two arginine infusions and two glucose tolerance tests. Each had been consistently insulinopenic at all time intervals during the latter tests. The arginine infusion, glucose tolerance test, and glucose/protein meals were repeated and, on separate days, tolbutamide and glucagon were administered.

The results were unequivocal. In each case, HGH de-

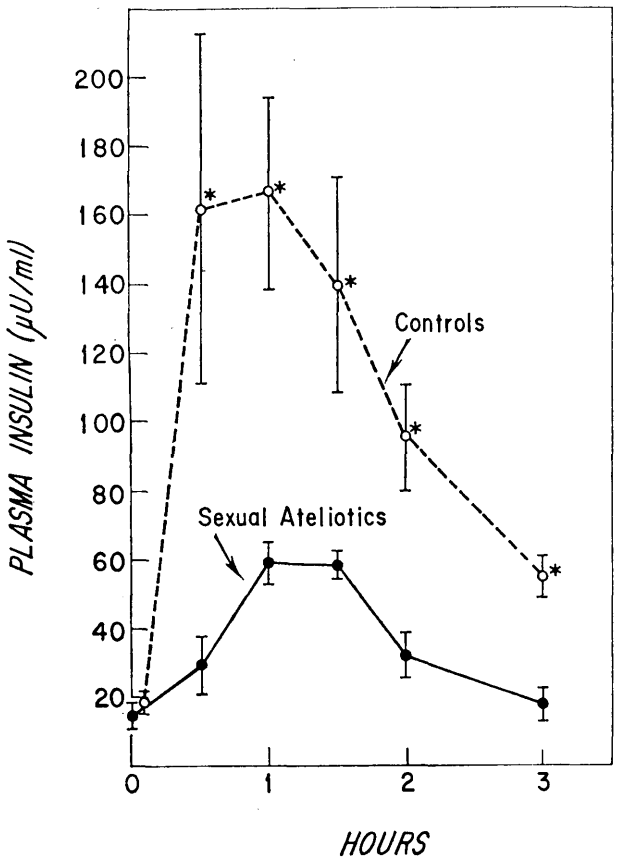

FIG. 3. Plasma insulin responses are compared after the ingestion of 100 gm. of glucose and 350 gm. of beef tenderloin. Each point is a mean ± SEM. An * indicates that values at a given sampling period differ with $p < .01$.

ficient dwarfs, showed a normal increase of plasma insulin concentration after tolbutamide and glucagon, whereas insulin responses continued to be significantly less than normal after the other stimuli. The comparison of insulin responses in controls after HGH treatment also supported these data. After HGH treatment, insulin responses to arginine and glucose were strikingly augmented, whereas those following tolbutamide and glucagon remained unaltered.

TABLE 1

Mean maximal insulin concentration in plasma after a variety of stimuli*

Stimulus	HGH deficient subjects	Control subjects
Glucose tolerance test	40.0 ± 6.3†(6)	90.4 ± 8.6(29)
Glucose/protein meal	67.0 ± 5.8†(6)	160.6 ± 27.9(12)
Arginine	38.6 ± 6.0†(6)	108.0 ± 12.0(27)
Tolbutamide	61.6 ± 7.7(6)	69.4 ± 15.5(7)
Glucagon	102.0 ± 9.4(6)	101.6 ± 19.3(7)

* Insulin is given in μU./ml. of plasma. Values are mean ± SEM. Numbers in parentheses indicate number of subjects.
† Value differs from the control value with a $p < .01$.

To attempt a specific localization of this effect of HGH on insulin release is tempting but would be hazardous considering the present state of knowledge. The mechanism of insulin biosynthesis and secretion is only partially understood. Biochemical evidence indicates that insulin release involves a complex system with numerous components such as calcium, beta and alpha autonomic receptors and cyclic 3′5′ AMP. In addition, insulin in the pancreas appears to be stored in a variety of compartments.

The concept has gradually evolved that insulin in the pancreas is distributed in muliple compartments dischargeable by different stimuli such as secretin, tolbutamide, and arginine.[1-3,10] In addition, these compartments are subserved by a variety of beta cell receptors each of which probably vary in their sensitivity, excitability, and latent period.

The studies reported in this paper indicate that such compartments, or the receptors subserving them, must differ in their dependency upon pituitary control—a control mediated by HGH. We cannot make any broad statement regarding the site of this action, but if the present data in the literature are valid, then it would seem clear that there must be more than one "acute phase" compartment of insulin release.

It has been noted that tolbutamide discharges insulin primarily if not solely from a single compartment.[1] Such insulin release is rapid (acute phase) and not sustained. More complex stimuli cause a rapid (acute phase) and more sustained release, supposedly from a separate storage compartment of insulin.[1,2,10] The acute phase or rapid release compartment involved with complex stimuli such as glucose has been considered as identical with that stimulated by tolbutamide.[1] This would seem unlikely from our data, unless the differences we have noted are secondary to differences in the release mechanism rather than to differences in the compartments of insulin.

Considering all circumstances, we deem it best to record the observations as noted and make only the broadest generalizations regarding this action. Accordingly, we conclude simply that there is a difference in the effect of HGH upon either pancreatic beta cell receptors or upon pancreatic release of insulin. This is manifested by the fact that the acute release of insulin following tolbutamide and glucagon is normal in the HGH deficient state but is consistently subnormal after the administration of glucose and amino acids.

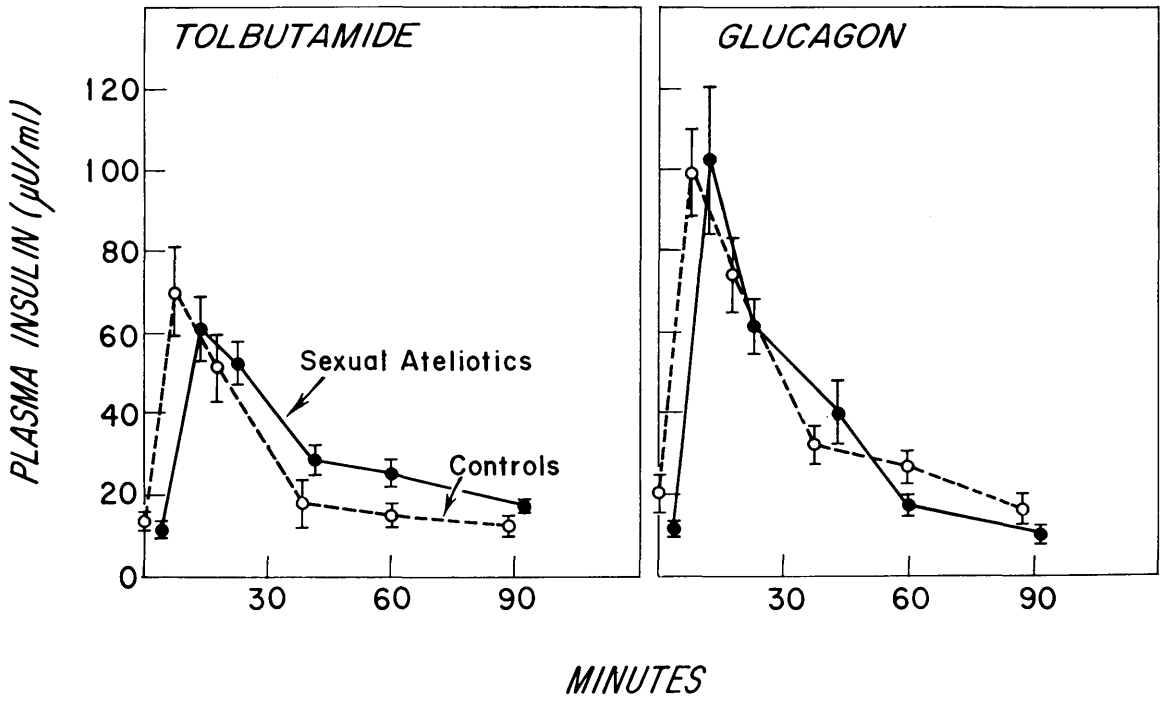

FIG. 4. Plasma insulin concentrations are compared after intravenous tolbutamide and glucagon (see text). All points are mean ± SEM.

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TABLE 2

Effect of human growth hormone upon maximum insulin responses of seven normal subjects*

Stimulus	Before treatment	After treatment
Arginine	81.5 ± 19.0 μU./ml.	290.0 ± 65.0†
Glucose	68.5 ± 14.4 μU./ml.	156.0 ± 9.8†
Tolbutamide	69.4 ± 15.5 μU./ml.	77.2 ± 14.0
Glucagon	71.1 ± 9.0 μU./ml.	81.8 ± 10.0

* The amount of HGH was 5 mg. twice a day for five days. Values are mean ± SEM.

† Increases are significant with $p < .01$

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