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Research Article

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Adaptations of α_2- and β-Cells of Rat and Mouse Pancreatic Islets to Starvation, to Refeeding after Starvation, and to Obesity

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ABSTRACT The effects of starvation and refeeding and of obesity on pancreatic α_2- and β-cell responses to glucose or tolbutamide were studied with the isolated rat or mouse pancreas perfused with an amino acid mixture in the presence and absence of glucose. It was observed that the physiological adaptation to a regimen of fasting and realimentation and to obesity differed greatly in the two types of endocrine cells. Whereas β-cells of rats showed a dramatic reduction of glucose- and tolbutamide-stimulated insulin release during starvation that was reversed by refeeding, α_2-cells preserved their response to stimulators and inhibitors during this experimental manipulation. Amino acid stimulation of glucagon release occurred equally well with the pancreas from fed and starved rats and was suppressed efficiently by glucose and tolbutamide in both nutritional states. Surprisingly, the rate of onset of glucose suppression of α_2-cells was significantly higher in the fasted than in the fed state. This glucose hypersensitivity was apparent 2 d after food deprivation and had disappeared again on the 2nd d of refeeding. In the pancreas from animals starved for 3 d, glucose and tolbutamide suppression of α_2-cells took place in the absence of demonstrable changes of insulin release.

In the isolated perfused pancreas taken from the hyperphagic obese hyperglycemic mouse (C57 Black/6J; ob/ob), the observed rate of insulin secretion as a result of a combined stimulus of amino acids and glucose and of glucagon release stimulated by amino acids was about four times higher than achieved by the pancreas of lean controls. However, glucose was unable to suppress the α_2-cells in the pancreas of obese animals, in spite of the hypersecretion of the β-cells, again in contrast to the α_2-cells of controls that were readily inhibited by glucose.

These data imply that the acute suppression of α_2-cells by glucose is largely independent of a concomitant surge of extracellular insulin levels and that the adaptation of the islet organ to starvation leads to decreased glucose sensitivity of β-cells, which contrasts with an improved glucose responsiveness of α_2-cells. However, hyperphagia, which is assumed to be the primary abnormality in the ob/ob mouse, leads to overproduction of insulin and glucagon by the pancreas while greatly reducing the α_2-cell sensitivity to glucose.

An attempt is made to incorporate these data on starvation, refeeding, and obesity, as well as previous results with experimental diabetes, in a comprehensive picture describing a regulative principle underlying the glucose responsiveness of α_2-cells.

INTRODUCTION

The pancreatic islet is considered a biological unit, and it is hypothesized that the close anatomical association of α_2- and β-cells and of other endocrine and neuronal elements in this microscopic organ is essential for normal function of these cells individually (1). In particular, it is a widely held view that the functions of the glucagon-producing α_2-cells and of the insulin-producing β-cells of the islets of Langerhans are interdependent in the sense that the two cell types require continuous local paracrine communication between each other (2, 3). This concept is commonly

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illustrated by reports showing stimulation of β-cells by glucagon and inhibition of α_2-cells by insulin. The hyperglucagonemia typical for diabetics is another example consistent with this notion. Even though this concept is attractive and seems plausible physiologically, it does not jibe with all experimental findings (4). For example, it seems predictable from this concept of islet tissue physiology that in vitro the diabetic pancreas would hypersecrete glucagon and would be nonsuppressible by glucose (1–3). However, the pancreas of streptozotocin and alloxan diabetic animals perfused extracorporally and stimulated with amino acids showed α_2-cell secretory activity that seemed paradoxically low in view of the pronounced hyperglucagonemia that characterized the diabetic pancreas donors (4). Even more surprising was the finding that α_2-cells of diabetics were efficiently suppressed by 5 mM glucose in vitro, albeit less swiftly than α_2-cells of normals. In further pursuits of these results, it was demonstrated that the diabetic α_2-cell can be modified by insulin treatment of the donor animals (5). The rate and degree of glucose suppression of glucagon secretion in vitro was improved by treatment of the diabetic donor animals, but the response of the α_2-cell to amino acid stimulation in vitro remained abnormally low. In vitro treatment with as much as 1 μg/ml of insulin for up to 1 h did not help (5). It is noteworthy, however, that arginine-induced glucagon secretion is normal or increased in the isolated perfused rat pancreas when studied 1 h after in vivo streptozotocin treatment, which indicates that suppressed α_2-cell function is not an absolute resultant of the use of this agent (6). Corresponding studies with genetically diabetic Chinese hamsters revealed that the α_2-cell response in vitro mirrored the α_2-cell hyperactivity inferred from the elevated serum glucagon levels in vivo (7). Suppression of stimulated α_2-cells with glucose was shown to be blocked in an in vitro perfusion system in another animal model of diabetes mellitus, the obese hyperglycemic mouse (C57 Black/6J) both of the ob/ob or db/db genotype (8, 9). Therefore, questions arise about the significance of the results obtained with tissues taken from animals made diabetic by chemical agents like alloxan or streptozotocin. One possible explanation for the discrepancies might be that these agents damage the α_2-cells as well. There are indeed data consistent with an impairment of α_2-cell function by alloxan in vitro (10).

To expand our knowledge on α_2-cell function in diabetes-like states, we now studied the effect of "starvation-diabetes" and of diabetes in obesity on α_2-cell responsiveness to stimulation by a physiological amino acid mixture and to suppression of stimulated glucagon release by glucose or in selected conditions by tolbutamide with the isolated perfused pancreas. The actual design of the experiment was based on the following considerations: (*a*) Excellent preservation of the α_2- and β-cells in the in vitro setting needs to be achieved and the temporal resolution power of the approach should be of the order of 30–15 s to allow reliable kinetic release measurements. The isolated perfused pancreas has been shown to be excellent in both regards (7–13). (*b*) It is assumed that the hormone levels measured in the perfusion fluid are indicative of the hormone levels achieved in the extracellular fluid of the islet tissue bathing α_2- and β-cells. Because of dilution of perfusate by tissues other than islets (exocrine, pancreas, stomach, spleen, and part of the duodenum) the perfusate level is probably at least two orders of magnitude lower than the actual level bathing the islet cells. Nevertheless, it can be reasonably postulated that this β-cell response observed in the perfusate should precede or at least coincide with α_2-cell response for insulin to qualify as the mediator of glucose suppression of the α_2-cell. (*c*) In starvation, basal insulin levels are maintained that might be needed for supporting α_2-cell function in vivo, thus avoiding possible secondary impairment developing in those cells in severe chronic diabetes. (*d*) It was reasoned that the normal organization of the islet tissue complex might be little affected by starvation and that starvation might cause hyperglucagonemia in vivo and glucose resistance of the α_2-cells in vitro if glucose suppression of the α_2-cells were to be de-

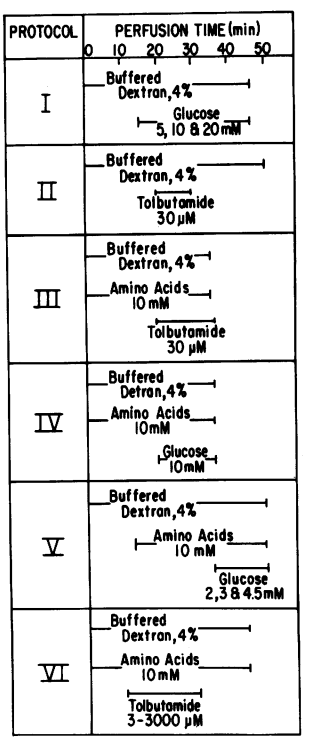

FIGURE 1 Outline of perfusion protocols.

TABLE I

Levels of Glucose, β-hydroxybutyrate, Glucagon, and Insulin in Plasma and the Contents of Glucagon and Insulin in the Pancreas of Normal, Starved, and Refed Rats

Experimental conditions	Plasma glucose*	Plasma β-OHB‡	Plasma glucagon	Plasma insulin	Total hormone content per pancreas	
					Glucagon	Insulin
	mM	*μM*	*pg/ml*	*ng/ml*	*μg*	*μg*
(*a*) Fed (group 1)	10.8±0.4	245±28	239±11	3.7±0.6	—	—
(*b*) Fed (group 2)§	9.9±0.4	245±21	207±8	3.6±0.4	6.5±0.2	49.0±2.5
(*c*) 1-d fasted	9.1±0.2	909±107	216±10	1.6±0.2	—	—
(*d*) 2-d fasted	8.0±0.2	1,523±115	209±7	0.7±0.2	—	—
(*e*) 3-d fasted (group 1)	7.8±0.3	1,530±222	225±5	0.3±0.1	—	—
(*f*) 3-d fasted (group 2)§	7.2±0.6	2,183±282	220±5	0.3±0.2	5.3±0.2	42.7±1.7
(*g*) 3-d fasted, 1-d refed	18.1±0.8	271±40	310±5	1.0±0.1	4.8±0.3	44.0±3.9
Statistical treatment						
a vs. *c*	<0.01	<0.001	NS	<0.01		
a vs. *d*	<0.001	<0.001	NS	<0.001		
a vs. *e*	<0.001	<0.001	NS	<0.001		
c vs. *d*	<0.001	<0.001	NS	<0.01		
d vs. *e*	NS‖	NS	NS	NS		
b vs. *f*	<0.01	<0.001	NS	<0.001	NS	NS
b vs. *g*	<0.001	NS	<0.001	NS	NS	NS
f vs. *g*	<0.001	<0.001	<0.001	<0.001	NS	NS

The values represent the means±SEM (n = 6 for each group).

* The plasma glucose levels as seen here are atypically high, which is probably a result of the stressful situation of the animal during surgery. On repetition of the blood sugar assay with samples taken from the tip of the tail of unanesthetized animals from a different group, the results were as follows (n = 6): fed, 5.0±0.2 mM; 1-d fasted, 3.87±0.2 mM; 2-d fasted, 3.87±0.2 mM; 3-d fasted 4.17±0.1 mM; 3-d fasted and 1-d refed, 7.03±0.2 mM.

‡ β-OHB, β-hydroxybutyrate.

§ Experiments *b* and *f* were carried out in addition to those shown in groups *a* and *e*, respectively, to assess the hormone stores of the pancreas, which was not possible with the perfused pancreas preparations of these particular experiments.

‖ $P \geq 0.05$.

pendent upon the secretory response of the β-cells. (*e*) It has recently been shown that tolbutamide is able to suppress glucagon secretion and it has been proposed that this effect was indirect, because of the concomitant stimulation of insulin secretion (3). If this were true, α_2-cells should not be blocked by tolbutamide, when the β-cells are nonresponsive as a result of starvation. The use of tolbutamide was also attractive because as a drug it contrasted well with the physiological inhibitor glucose. (*f*) It is assumed that the islet alterations that develop in the obese hyperglycemic state in the mouse are a consequence of hyperphagia and it is reasoned that mouse and rat behave similarly (14). (*g*) Finally, it was felt that these experiments might help delineate the overall role of the glucagon system in fuel homeostasis during starvation, an important, still unsettled problem of physiology.

METHODS

Animals. Male Holtzman rats with original weight of 250–300 g were used in all experiments. All animals had free access to water. The animals were either fed (Rat Chow, Ralston Purina Co., St. Louis, Mo.); fasted for 1, 2, or 3 d; or were fasted for 3 d and then refed for 1, 2, or 3 d. Obese hyperglycemic mice (C57 Black/6J, ob/ob) from the Jackson Laboratories, Bar Harbor, Me., were used. C57 Black mice from a local supplier served as controls. The mice were all in the fed state. The average weight of the obese animals was 65.4±1.9 g and that of the lean animals 22.1±2.5 g.

Perfusion system. The pancreas was isolated and perfused by the procedure described by Grodsky et al. (11), with minor modifications (12). The animals were anesthetized with pentobarbital (3 mg/100 g body wt). The surgical preparation of the animals and the composition of the perfusion medium have been described (4, 11, 12). All experiments were performed between 8:00 a.m. and 3:00 p.m. Animals were sacrificed at times in accordance with a schedule for fasting or refeeding (within ±30 min). Six different perfusion conditions were employed (Fig. 1). A total of 154 experiments were performed.

Samples of perfusate (0.5 ml) were obtained at suitable intervals from the cannula in the portal vein, cooled on ice, and, after completing the experiment, frozen for storage at −20°C until assayed. Samples from protocols II through VI were collected in tubes that contained 500 kallikrein inactivating units/ml of Trasylol (FBA Pharmaceuticals, Inc., New York). Because glucagon, in contrast to insulin,

TABLE II

Effect of Starvation on Concentration and Time Dependency of Glucose-stimulated Insulin Release

Experimental conditions	Insulin release: First phase t_{15}–t_{20}	Second phase t_{20}–t_{45}	Total release t_{15}–t_{45}
	ng/5 min	*ng/25 min*	*ng/30 min*
Glucose, 5 mM			
(*a*) Fed	118±32	287±61	406±93
(*b*) 1-d fasted	25±7	92±29	118±35
(*c*) 2-d fasted	16±6	78±30	94±35
(*d*) 3-d fasted	7.5±1.5	30±10	38±12
Statistical treatment			
a vs. *b*	<0.05	<0.05	<0.05
a vs. *c*	<0.05	<0.05	<0.05
a vs. *d*	<0.05	<0.01	<0.05
Glucose, 10 mM			
(*a*) Fed	386±86	1,589±291	1,975±369
(*b*) 1-d fasted	159±44	895±231	1,054±269
(*c*) 2-d fasted	142±26	930±192	1,072±214
(*d*) 3-d fasted	40±10	314±31	354±38
Statistical treatment			
a vs. *b*	NS*	NS	NS
a vs. *c*	<0.05	NS	NS
a vs. *d*	<0.01	<0.01	<0.01
Glucose, 20 mM			
(*a*) Fed	509±114	2,444±90	2,954±472
(*b*) 1-d fasted	307±30	2,065±318	2,371±207
(*c*) 2-d fasted	233±28	1,898±318	2,122±345
(*d*) 3-d fasted	175±41	1,093±67	1,169±72
Statistical treatment			
a vs. *b*	NS	NS	NS
a vs. *c*	NS	NS	NS
a vs. *d*	<0.05	<0.05	<0.05

The values represent the means ±SEM of the integrated secretion rates of insulin as obtained by calculating the areas under the curves extending over the indicated time periods ($n = 4$ for each condition).

* $P \geq 0.05$.

is degraded in the perfusate (unpublished data), it has been found necessary to add Trasylol. This measure stabilized the glucagon values. This precaution of adding Trasylol was omitted in protocol I. The rates of insulin and glucagon release were calculated by multiplying the concentration of the respective samples by the flow rates, which were measured at frequent intervals (3 ml/min in protocol I and 6 ml/min in protocols II through VI). For performing the studies with the isolated perfused pancreas of the mouse, the perfusion apparatus was miniaturized appropriately and the perfusion flow rate was reduced to 1 ml/min. As the flow rate is lowered, the temporal resolution of the system is reduced. Therefore, measurements of initial rates of stimulation or suppression seem to be less accurate than observed in the rat pancreas. This was of little consequence in our study. We studied 10 ob/ob mice and eight lean controls.

In vivo experiments. Blood samples were obtained from all rats undergoing the perfusion experiment just before removal of the pancreas from the heparinized animals (4). The samples (2 ml) were drawn by puncturing the inferior vena cava. The blood was injected into tubes that contained 500 kallikrein inactivating units/ml Trasylol and 1.25 mg EDTA/ml and was kept on ice for no longer than 30 min. The plasma sample was used to determine glucose, β-hydroxybutyrate, glucagon, and insulin, and all values were corrected for dilution by Trasylol.

Analytical methods. Immunoreactive glucagon (4, 15) and insulin (16) were measured by double antibody systems as previously described. Glucose and β-hydroxybutyrate in plasma were measured by enzymatic assays, either fluorometrically (17) or spectrophotometrically (18).

RESULTS

In vivo symptoms of reversible "starvation diabetes." The fasting-refeeding regimen used here led to 20% weight loss within 3 d of food deprivation. During the period of realimentation, animals consumed ~40 g of food daily. The weight loss was regained within 2 d of refeeding, and after 3 d the body

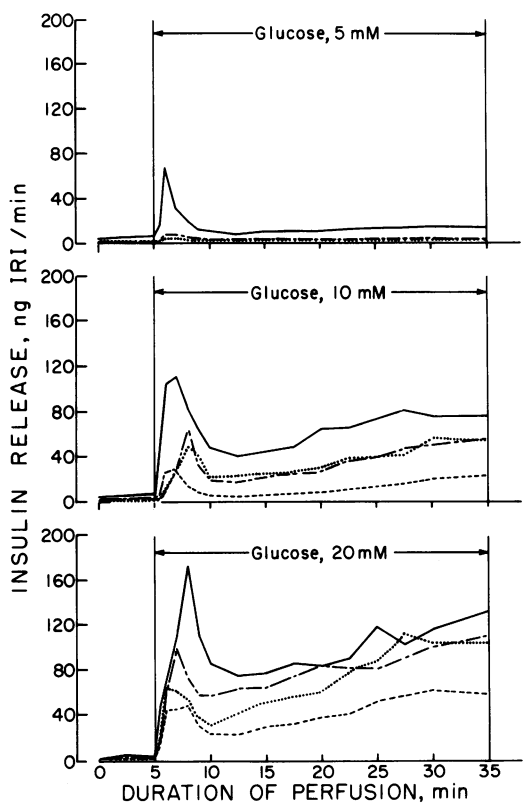

FIGURE 2 Effect of starvation on kinetics of insulin release by the perfused rat pancreas stimulated with low, medium, and high glucose. The means of four perfusion experiments are recorded in each group of animals. Protocol I (Fig. 1) was used. SEM are similar to those shown in other figures (e.g., Fig. 3). Including the SEM would have obscured the graph. The results of studies with low glucose after 3 d of fasting are not shown because insulin was virtually undetectable. A statistical treatment of the data can be found in Table II. Fed rats, ——; fasted 1 d, –·–; fasted 2 d, ·····; fasted 3 d, – – –. IRI, immunoreactive insulin.

TABLE III

Effect of Starvation and Refeeding on β-Cell Stimulation by Amino Acids and Glucose Combined

Experimental conditions	Insulin release		
	First phase t_{20}–t_{25}	Second phase t_{25}–t_{35}	Total release t_{20}–t_{35}
	ng/5 min	*ng/10 min*	*ng/15 min*
(*a*) Fed			
1 −glucose	5.0±1.1	10.0±3.1	15.0±3.2
2 +glucose	780±29	1,241±70	2,022±43
(*b*) 1-d fasted			
1 −glucose	2.2±1.2	7.3±2.8	9.5±4.0
2 +glucose	341±71	518±115	859±183
(*c*) 2-d fasted			
1 −glucose	3.4±1.0	8.6±1.9	9.7±2.0
2 +glucose	110±22	233±53	342±72
(*d*) 3-d fasted			
1 −glucose	2.0±0.5	5.9±0.3	8.0±1.5
2 +glucose	69±14	125±19	193±29
(*e*) 3-d fasted, 1-d refed, plus glucose	260±38	478±94	741±122
(*f*) 3-d fasted, 2-d refed, plus glucose	595±86	970±157	1,565±236
(*g*) 3-d fasted, 3-d refed, plus glucose	442±65	917±164	1,359±229
Statistical treatment			
a_2 vs. b_2	<0.001	<0.001	<0.001
a_2 vs. c_2	<0.001	<0.001	<0.001
a_2 vs. d_2	<0.001	<0.001	<0.001
d_2 vs. f	<0.001	<0.001	<0.001
d_2 vs. g	<0.001	<0.001	<0.001
a_2 vs. e	<0.001	<0.001	<0.001
a_2 vs. f	NS*	NS	NS
a_2 vs. g	<0.01	NS	<0.05

The concentration of the amino acid mixture was 10 mM. The values represent the means±SEM of the integrated secretion rates of insulin as obtained by calculating the areas under the curves extending over the indicated time periods. There are four experiments in each group.

* $P \geq 0.05$.

weight exceeded the starting values of the animal's weight by ~10%. The alterations of plasma glucose, β-hydroxybutyrate, glucagon, and insulin levels and the maintenance of the pancreatic glucagon and insulin stores during the starvation-refeeding schedule were for the most part as expected (Table I): blood sugar and insulin fell, the ketone bodies increased almost 10-fold, and there were no changes of the pancreatic insulin and glucagon stores. It is particularly noteworthy that 3 d of food withdrawal had little effect on plasma levels of immunoreactive glucagon in contrast to the pronounced drop of plasma insulin to <10% of the levels found in the fed state.

The changes of plasma glucose, β-hydroxybutyrate, and insulin values observed here during starvation and refeeding agree with the results of other studies (19–22). The relative constancy of pancreatic glucagon and insulin stores has also been noted before (20, 23–25). The apparent lack of effect of starvation on plasma glucagon levels as observed in our investigation is consistent with certain data published by others (26). But they are in contrast with the results of Unger et al. (27) and of Lawrence (28), who found marked elevations of plasma glucagon during periods of glucose deprivation. These discrepancies are not easily reconciled but could be related to the analytical difficulties inherent in measuring plasma levels of immunoreactive glucagon. It is emphasized that, for the purpose of this discussion, it matters little whether starvation caused an absolute hyperglucagonemia or not. The glucagon:insulin ratio was certainly greatly increased in accordance with the basic thesis of the "double trouble" hypothesis (1).

At the end of 1 d of refeeding after starvation, the animals exhibited signs of starvation-diabetes: they were hyperglycemic, had a relative insulin deficiency in the plasma, and showed high plasma glucagon levels. The insulinogenic index of animals fasted 3 d and refed for 1 d was 0.10 ng in contrast to a value of 0.36 ng of insulin/μmol of glucose in plasma found in fed animals. The glucagon levels rose by 50% (much less impressively than the threefold increase of insulin) when the rats were refed for 1 d.

The mild hyperglycemia seen in refed animals is compatible with data by Grey et al. (23), who studied insulin secretion of fed and 48-h fasted rats after oral glucose: In fed rats, the elevated plasma glucose returned to base line promptly, whereas hyperglycemia persisted in fasted animals.

In vitro studies of altered β-cell responsiveness during starvation-refeeding in the rat. Decreased responsiveness of the β-cell to stimulation by glucose itself or to a combination of glucose and amino acids was readily demonstrated with the isolated perfused pancreas. The studies performed here with glucose as the sole stimulus serve to reassess data in the literature on that subject (Table II, Fig. 2). The β-cell impairment was most pronounced with levels of glucose (5 mM) close to threshold for stimulation. It is noteworthy that glucose deprivation for the 15 min of the preperfusion period did not preclude a brisk first peak response to a glucose stimulus just above threshold in the pancreas of the fed animal. At an intermediate glucose level (10 mM) the defect was also seen as early as 1 d after fasting, but statistical significance was achieved only after 2 d of food withdrawal. Both phases of the typical release profile, observed with the isolated perfused pancreas exposed to square wave of high glucose were affected. With 20 mM glucose as stimulus, it

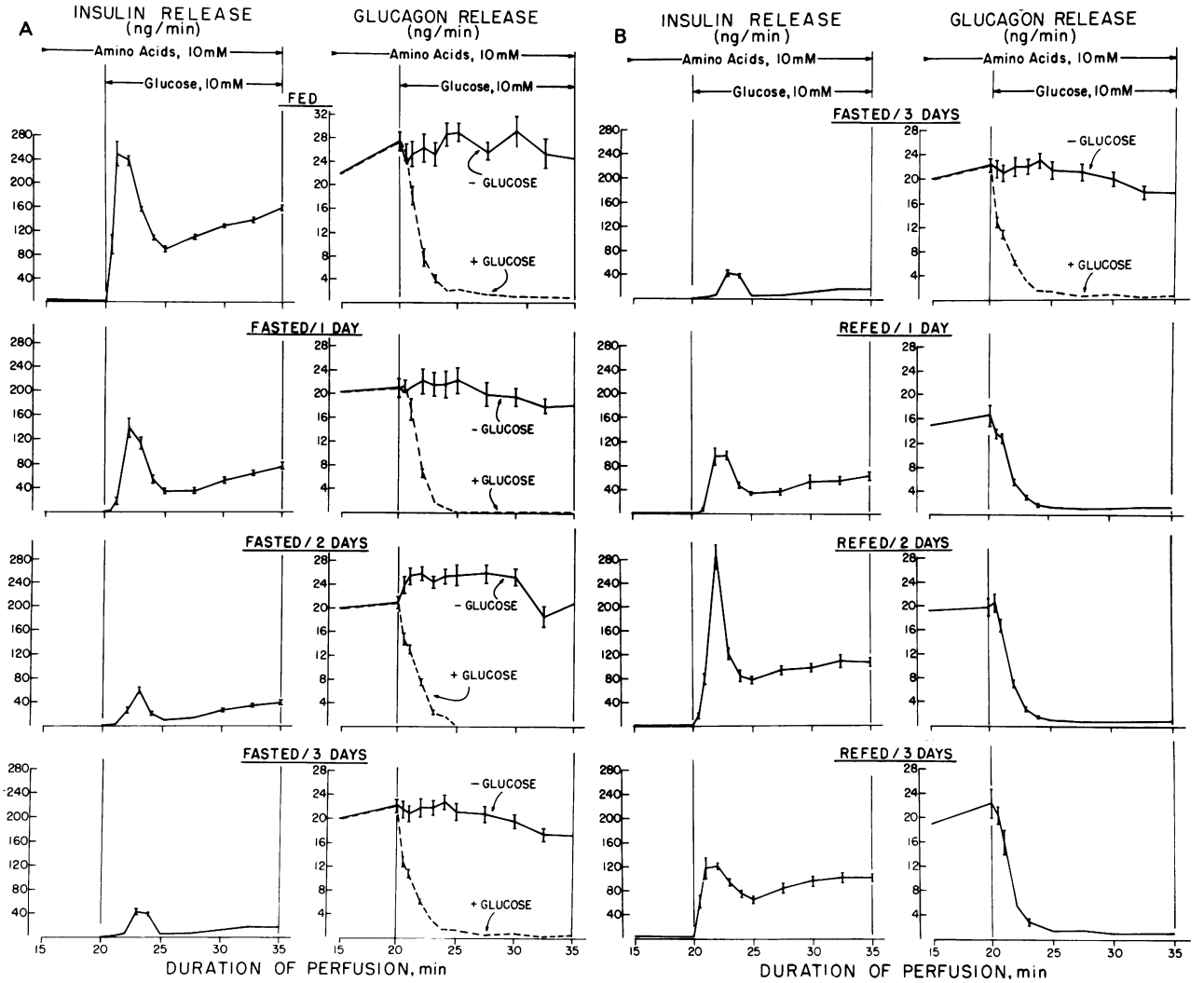

FIGURE 3 The effect of starvation (A) and refeeding (B) on α_2- and β-cell responses. The means ±SEM of experiments are given in each group. Protocol IV (Fig. 1) was used. In the panels that show the insulin release profiles, only the data with glucose plus amino acids present are given ($n = 4$). Insulin levels were negligible with amino acids alone and were not recorded. In the panels that show glucagon release profiles, the data of eight perfusions are pooled for the first 20 min of the experiment, whereas, during the ensuing 15 min, means±SEM of four experiments each in the absence and presence of 10 mM glucose are given. In the refeeding experiment, there were only four perfusions and all had glucose present during the last 15 min. For a statistical treatment of all the results see Table III.

took 3 d of starvation to alter the entire profile of release sufficient to achieve statistical significance. Considering the kinetics of the profile (Fig. 2), there was a decrease of first phase release with high glucose observable even after 24 h of starvation, but steady-state release during the second phase was altered only after 3 d of starvation. These apparent changes did not reach statistical significance.

This portion of the in vitro study confirms numerous findings of others that fasting reduces the sensitivity of the β-cells to its main physiological stimulus glucose (19, 20, 23–25, 29–36). It also documents the startling feature that the β-cell responsiveness to the physiological threshold dose of glucose (i.e., 5 mM) is well maintained after a brief extracorporal perfusion with a solution free of glucose.

The results obtained with an amino acid mixture and glucose combined agreed in general with the results obtained with glucose as the sole stimulus but differed in some important details (Table III, Figs. 3–5). As shown previously, amino acids require glucose for β-cell stimulation. With the pancreas from fed animals,

there was no apparent delay of the β-cell response. The effect of starvation on β-cell function became apparent after 24 h and affected both phases of the profile. Starvation reduced and delayed the first phase of the combined stimulus of 10 mM amino acids and 10 mM glucose. In the fed state, the maximum of the first phase was reached within 1 min after the switch. With starvation, the delay in the onset of the secretory response increased from 1 min after 1 d to 3 min after 3 d of starvation. The magnitude of the second phase response fell gradually with fasting. The β-cell response was nearly normalized after 2 d of refeeding as to onset, magnitude, and kinetics of the response.

Because it was expected that the effect of starvation on β-cell might manifest itself more readily when low glucose levels are used together with the glucose-dependent amino acid stimulus, the concentration dependency of glucose induced insulin release was assessed in fed and 3-d fasted rats (Fig. 5). The glucose threshold with the amino acid mixture present was ~3 mM, and a substantial monophasic β-cell response was achieved with 4.5 mM glucose. Starvation completely blocked insulin release as a result of these low sugar levels combined with the amino acid mixture, similar to the result when low sugar alone was used (cf. Fig. 2).

An exploratory dose-response study with tolbutamide concentrations between 3 μM and 3 mM showed that the β-cells of the pancreas perfused with an amino acid mixture was extremely sensitive to tolbutamide. At 30 μM the response was approximately one-half maximal and was clearly monophasic, but at higher concentrations (≥90 μM) biphasic release patterns were observed (Fig. 6). The effect of starvation was assessed with 30 μM tolbutamide, and it was found that after 3 d of food deprivation β-cells did not respond at all to this dose of the drug (Table IV).

Maintenance of α_2-cell in vitro responses to amino acids and glucose during starvation and refeeding in the rat. Glucagon release as a result of a maximal stimulus with amino acids was maintained during the starvation-refeeding regimen applied here (Table V, Figs. 3–5). The data indicate that both phases are fully preserved. Glucose suppression was also maintained during starvation. There was even an indication that α_2-cells might become supersensitive to glucose during fasting: in fed and realimented animals there was a definite 30- 60-s delay before glucose inhibition of glucagon secretion became apparent, but in animals fasted 2 or 3 d the release rate dropped precipitously when glucose was introduced and inhibition was clearly demonstrable in the first sample taken 30 s after the switch.

Suppression of amino acid-stimulated glucagon release by low glucose levels was very efficient (Fig. 5). As little as 2 mM glucose reduced the rate by ~40% and 4.5 mM reduced it >80%. Starvation for 3 d had only little, if any, effect of α_2-cell suppression by low glucose (2, 3, or 4.5 mM).

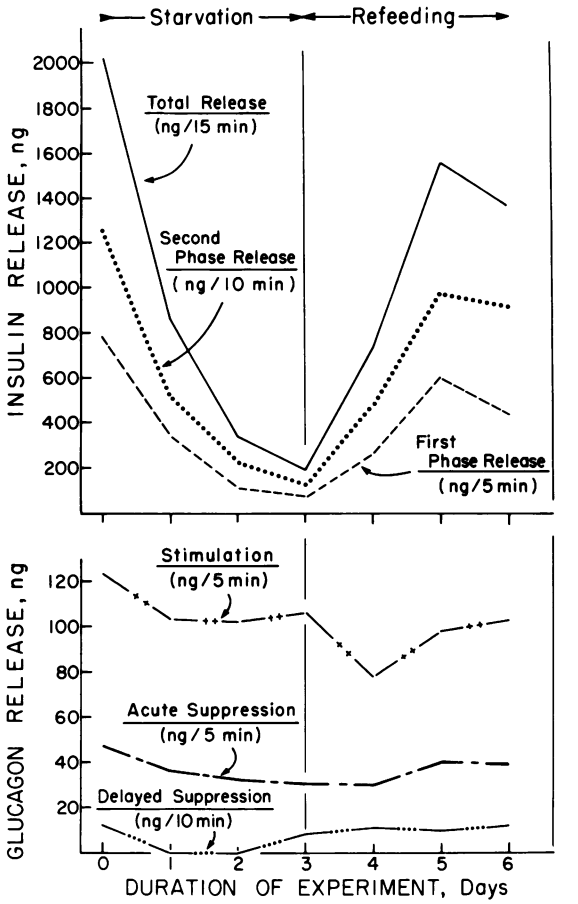

FIGURE 4 Altered β-cell and preservation of α_2-cell function during starvation. The figure is a graphic representation of data recorded in Tables III and V. Total insulin release was measured over the entire period of glucose exposure (t_{20}–t_{35}), first-phase release was obtained from t_{20} to t_{25} and second phase release from t_{25} to t_{35} of glucose exposure. The glucagon release observed during amino acid exposure from t_{15} to t_{20} was taken as a measure of stimulated glucagon release, that from t_{20} to t_{25} was used as measure of acute suppression, and delayed glucose suppression represents glucagon release from t_{25} to t_{35}.

These results generally agree with the finding of Oliver et al. (26) obtained with isolated perifused islets taken from starved rats. However, because of the limited time resolution of the perifusion system employed in that study and because these investigators used only high glucose levels (16.7 mM), presumably because the glucose responsiveness of isolated islets was not optimal, there remained some uncertainty whether subtle alterations of α_2-cell function as a result of starvation might have remained undiscovered. Our data settle this question.

It was confirmed here that tolbutamide is a powerful

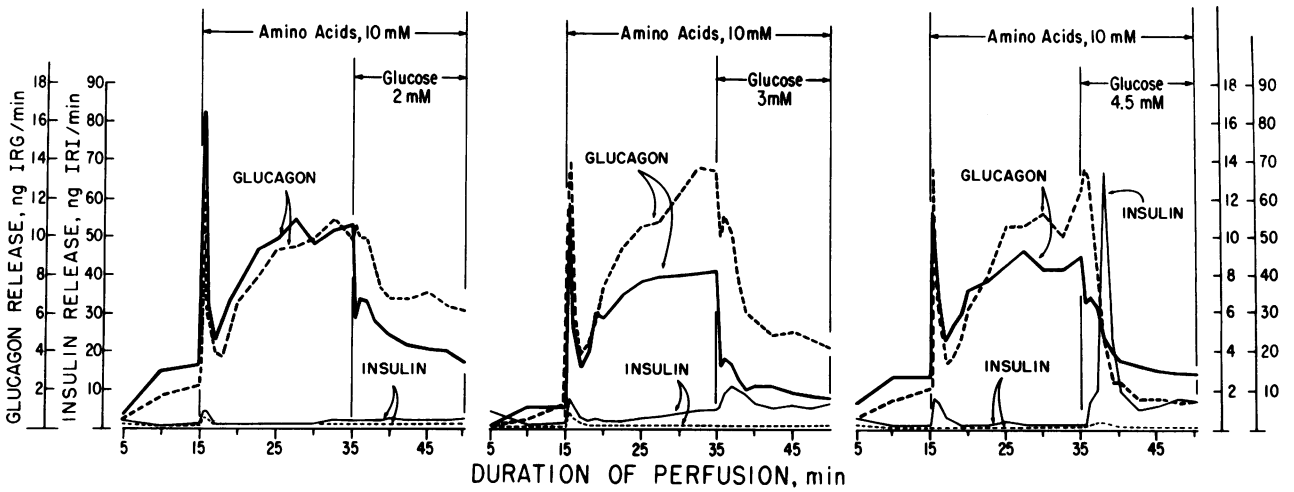

FIGURE 5 Effect of starvation of α_2- and β-cell responsiveness to low glucose levels. Protocol V as sketched in Fig. 1 was used. There were four experiments in each group, i.e., a total of 24 perfusions. Means of the results are recorded. A statistical treatment of the data seemed superfluous. For insulin (IRI) profiles: fed, ——; starved, -----. For glucagon (IRG) profiles: fed, -----; starved, ——.

inhibitor of amino acid-stimulated glucagon release (Fig. 6) (3, 37). Concentrations as low as 10 μM were effective. It must be recalled, however, that the response of the α_2-cell to tolbutamide is complex, because stimulation can be observed in the absence of amino acids (3, 37). For example, with protocol II (Fig. 1), we found that 30 μM tolbutamide infused alone stimulated α_2- and β-cells concomitantly (not shown). Starvation for 3 d had little, if any, effect on the α_2-cell suppression by a submaximal dose of tolbutamide (Table IV).

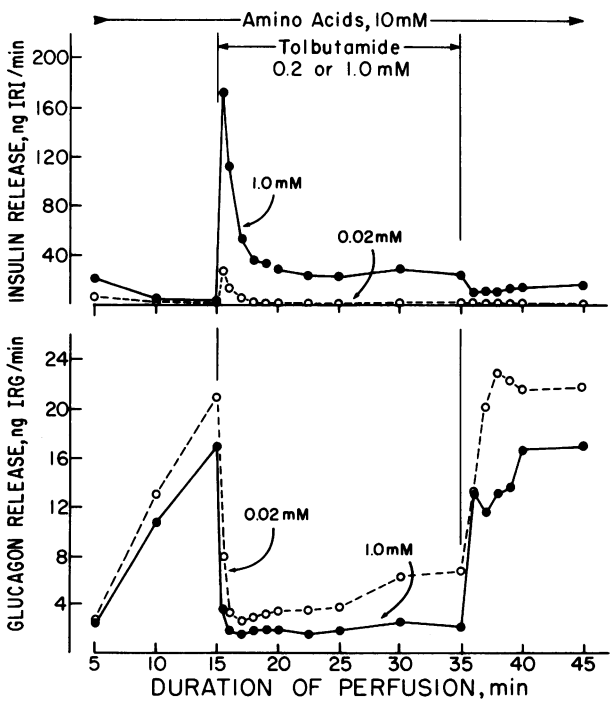

FIGURE 6 Effect of tolbutamide on α_2- and β-cells of the isolated perfused rat pancreas. The means of two perfusion experiments at two drug levels each are shown. Protocol VI (Fig. 1) was used. The results are representative of a more complete dose-response study with 0.003–3.0 mM tolbutamide (not shown). The threshold for α_2- and β-cell action was 0.01 mM, and maximal rates were obtained at 1.0 mM. IRG, immunoreactive glucagon; IRI, immunoreactive insulin.

Glucose suppression of amino acid-stimulated glucagon release in obese mice of the C57 Black/6J strain (Fig. 7). To assess the possible impact of obesity on the glucose responsiveness of α_2-cells, perfusion experiments were performed with the pancreas of

TABLE IV
Differential Effect of Starvation on α_2- and β-Cell Responsiveness to Tolbutamide

Time of perfusate sampling	Insulin release		Glucagon release	
	Fed rats	Starved rats	Fed rats	Starved rats
min	*ng/min*		*ng/min*	
15	2.1±0.1	ND*	25.9±2.7	26±3
20	1.6±0.4	ND	31.9±3.0	28±2
20.5	105±12	ND	7.5±1.4	6.8±1.7
21	61±11	ND	3.8±0.5	6.4±1.3
22	21±4	ND	3.6±0.7	6.4±0.4
35	4.3±2.2	ND	6.2±0.6	9.8±1.1

Protocol III was used. The tolbutamide concentration was 30 μM and was introduced at 20 min. The tolbutamide exposure was extended to 35 min and the experiment was terminated at that point. The means±SEM of four experiments are shown. The data of strategic time points of the release profile are given. Compare these data with corresponding results shown in Fig. 6.

* ND = not detectable.

genetically obese hyperglycemic mice [C57 Black/6J (ob/ob)] and of lean C57 Black mice. The data obtained with the pancreas from fed lean mice were compatible with results obtained with rats. However, with the pancreas from fed obese hyperglycemic mice, several striking observations were made: First, the β-cells showed high basal activity in the absence of known stimuli in the perfusate; second, the β-cells responded to an amino acid mixture with a burst of increased insulin release, even in the absence of glucose; third, the β-cell response to the combined amino acid-glucose stimulus was 3–4 times as big as that seen in the lean controls; fourth, the α_2-cell response to the amino acid mixture was 2–3 times as big as the response in the lean controls; and fifth, glucose, even at 20 mM, did not suppress amino acid-stimulated glucagon release, in contrast to the results obtained in controls.

TABLE V
Preservation of α_2-Cell Function in Starvation

Experimental conditions	Glucagon release			
	t_{15}–t_{20}	t_{20}–t_{25}	t_{25}–t_{35}	t_{20}–t_{35}
	ng/5 min	*ng/5 min*	*ng/10 min*	*ng/15 min*
(*a*) Fed				
1 −glucose	122±12	131±20	267±42	398±60
2 +glucose		42±9	12±6	59±14
(*b*) 1-d fasted				
1 −glucose	103±14	108±21	194±38	302±58
2 +glucose		36±9	0	36±9
(*c*) 2-d fasted				
1 −glucose	102±10	127±12	244±24	371±36
2 +glucose		32±3	0	33±3
(*d*) 3-d fasted				
1 −glucose	106±10	111±14	194±27	305±40
2 +glucose		31±3	8±3	40±5
(*e*) 3-d fasted, 1-d refed, plus glucose	79±17	30±4	11±1	42±4
(*f*) 3-d fasted, 2-d refed, plus glucose	98±20	40±4	10±1	51±4
(*g*) 3-d fasted, 2-d refed, plus glucose	103±22	39±7	12±1	51±8
Statistical treatment				
a_2 vs. b_2	NS*	NS		NS
a_2 vs. c_2	NS	NS		NS
a_2 vs. d_2	NS	NS		NS
d_2 vs. e	NS	NS		NS
d_2 vs. f	NS	NS		NS
d_2 vs. g	NS	NS		NS
a_2 vs. e	<0.05	NS		NS
a_2 vs. f	NS	NS		NS
a_2 vs. g	NS	NS		NS

Protocol IV was used. Amino acids (10 mM) were present throughout. Results obtained in the presence and absence of 10 mM glucose are shown. Glucose was not included until t_{20}. The values represent the means±SEM of the integrated secretion rates of glucagon as obtained by calculating the areas under the curves extending over the indicated time periods. There were four experiments in each of the two groups. From t_{15}–t_{20} the pooled results of eight perfusions are shown, since during this phase glucose was absent in all experiments.
* $P \geq 0.05$.

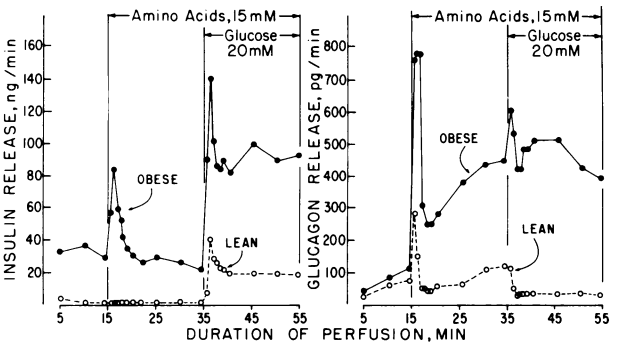

FIGURE 7 Islet cell responsiveness of the isolated perfused pancreas of the obese hyperglycemic mouse. Means of 10 and 8 experiments are given in the groups of obese (solid line) and control animals (broken line), respectively. Standard errors were similar to those observed in other experiments (e.g., Fig. 3).

These in vitro data confirm and extend findings of others who investigated β- and α_2-cell function of obese hyperglycemic mice with the isolated perfused pancreas (8, 9). The results are also consistent with the amply documented fact that C57 Black/6J mice with the ob/ob gene are characterized by high plasma levels of glucose, insulin, and glucagon (38, 39).

DISCUSSION

Our results pertain to two aspects of islet physiology and endocrine fuel homeostasis: (*a*) the possible functional role of postulated paracrine hormonal interactions between α_2- and β-cells during starvation, and (*b*) the nature of the physiological adaptive processes in α_2- and β-cells during starvation, refeeding after starvation, and in one particular form of obesity.

Insulin and glucagon as local hormones. Samols et al. (2, 3) have postulated the existence of an intra-islet feedback system now described by the term paracrine (1) with insulin functioning as the transmitter of glucose and drug-induced α_2-cell inhibition and glucagon serving as mediator of amino acid-induced insulin release. This concept of paracrine regulation of islet cell function has important implications for the characteristics of the postulated peptide hor-

mone receptors of islet cells. With ambient islet hormone levels approaching the micromolar range, the apparent K_m values of the postulated receptors can be expected to be much higher than those found in other peripheral target tissues, e.g., adipose tissue (40). Support for the existence of such a paracrine system was derived from data that showed inhibition of glucagon secretion by exogenous insulin in vitro. However, in a recent study by Weir et al. (41) it was found that as much as 50 mU of insulin/ml of perfusate suppressed glucagon secretion only slightly, and did so only in 3 of 7 perfusion experiments with the isolated dog pancreas. We have adopted a simpler working hypothesis, which proposes that the action of nutrients on α_2- and β-cells might be direct, without excluding short-term and long-term modulation of secretory responsiveness by endocrine and neural factors (4, 5, 10). For example, α_2-cells may require the continuous presence of basal insulin for maintenance of normal responsiveness to glucose, but may be relatively insensitive to the wide fluctuations of insulin levels that acutely result from varied nutritional loads. Our data seem to support this latter concept, because glucose and tolbutamide suppression of stimulated glucagon release occurred in the absence of a detectable increase in insulin secretion. Similar conclusions were drawn from the results of previous in vitro studies with the pancreas taken from acutely or chronically diabetic animals. In these studies, glucose was capable of suppressing glucagon release in the virtual absence of insulin secretion (4, 5). Additional evidence comes from extensive yet-to-be-published in vitro studies with β-hydroxybutyrate. β-Hydroxybutyrate, which is a powerful insulin-releasing agent in the perfused pancreas, provided basal glucose (2.5–5.0 mM) is present, does not enhance the α_2-cell suppressive effect of glucose in that range. It was also found that alloxan, when added to the perfusate, blocked glucose-induced insulin release of the isolated perfused rat pancreas without preventing glucose suppression of the α_2-cells (10). Finally, it has been shown here that tolbutamide inhibits stimulated glucagon release in the isolated perfused pancreas taken from starved animals, again in the absence of concomitant changes of insulin release.

Even though it is tempting to argue that the assemblage of α_1-, α_2-, and β-cells in the islet organ might be essential for the physiological regulation of islet cell functions through local paracrine interactions, the direct evidence for such a concept is meager as examplified by this discussion. An experimental design more sophisticated than used in our study may be needed to find functional evidence for the postulated cell-cell interaction among different pancreatic islet cells. One should also not discount the possibility that the association of different cell types in one endocrine organ (the "islet organ") may serve other purposes in addition to, or instead of, providing an opportunity for intercellular communication. For example, the peculiar requirement for vascularization and innervation for the different endocrine cell types may be similar, and it would seem to be biologically economical to consolidate such cells in one endocrine organ.

Physiological adaptation of α_2-cell responsiveness to glucose. Most investigators share the view that glucose suppressibility of glucagon-producing α_2-cells is directly proportional to β-cell activity. This concept was developed because hyperglucagonemia is commonly observed in diabetes mellitus and because of the beneficial effect of insulin therapy of diabetics, which lowers plasma glucagon levels (42). Our in vitro data suggest that pathophysiological phenomena, which characterize the diabetic state, tend to obscure certain physiological adaptive processes possibly operative in α_2-cells. It seems that the α_2-cell is most responsive to glucose in the state of subacute fuel deprivation and hypoinsulinemia and least responsive in the obese, most likely hyperalimented state accompanied by hyperinsulinemia. Our interpretation rests on the assumption that it is the hyperphagia and hyperalimentation that leads to islet cell hyperplasia and glucose resistance of α_2-cells in the ob/ob mouse. There is good evidence for this (14). Our hypothesis predicts that mice or rats made hyperphagic and fattened by lesions in the ventromedial hypothalamus produced electrolytically or by goldthioglucose should show β-cell hyperplasia and glucose blindness of α_2-cells as seen in the ob/ob mouse. It also follows from the above assumption that food restriction of the ob/ob mouse should remedy the defect. Those predictions are testable with our approach. In the normally fed state, α_2-cell suppression by glucose occupies an intermediate position. The dysregulation of α_2-cell function, as observed in insulin-deficient diabetes mellitus, and the remedial affect, seen after insulin treatment in this situation, can be incorporated in this concept, if it is assumed that optimal glucose sensitivity of α_2-cells can only be maintained if a minimal requirement for insulin is fulfilled. This concept provides a framework that incorporates all presently available data.

Our data and data in the literature dealing with the question of glucose responsiveness of α_2-cells can be best explained if one assumes that these cells need the maintenance of an optimal insulin level for maximal glucose sensitivity and that deviations from this optimum to a situation with relative insulin deficiency or insulin excess leads to glucose resistance. Given the maintenance of basal insulin levels, it might be of little consequence for the glucose responsiveness of α_2-cells whether glucose elicits an acute surge of insulin from the β-cell or not. Glucose resistance of α_2-cells may be

a result of alteration of a glucoreceptor speculated to exist on the α_2-cell membrane (4, 13). In such a case it needs to be assumed that too little or too much insulin causes a glucoreceptor defect or deficiency. Another equally attractive way of explaining the results can be based on the hypothesis that proposes that an event related to transmembranous glucose transport and/or to glucose metabolism is responsible for α_2-cell suppression. In such a case it needs to be assumed that too little or too much insulin might interfere with glucose entry in the α_2-cell or glucose degradation (40). Because research dealing with the nature of glucose metabolism of pancreatic α_2-cells has just been initiated, it is not possible to decide which explanation applies (5, 43). Our analysis must neglect the possible role of somatostatin in glucose and tolbutamide inhibition of glucagon. It is conceivable that the glucose and tolbutamide effects on α_2-cells are indirect, mediated through somatostatin. To our knowledge, there are no data in support of such a supposition.

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