Insulin Release and Cyclic AMP Accumulation in Response to Glucose in Pancreatic Islets of Fed and Starved Rats

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ABSTRACT The dose as well as the time kinetics of insulin and adenosine-3′,5′-monophosphate (cyclic AMP) responses to glucose were compared in pancreatic islets of fed and starved rats. There was a preferential impairment of the early phase of glucose-induced insulin release in perifused islets of rats starved for 16 and 48 h. Similarly, the accumulation of ^{3}H cyclic AMP in islets prelabeled with ^{3}H-2-adenine was less in islets of 48 h starved than fed rats, during the first 10-min of stimulation with 26.7 mM glucose in the presence of 0.1 mM of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine, whereas at 30 and 60 min ^{3}H cyclic AMP responses to glucose were similar in fed and starved islets. Also, in 10-min incubations with glucose 3.3, 6.7, 10.0, 13.3, and 26.7 mM without and with 0.1 mM and 1.0 mM 3-isobutyl-1-methylxanthine, insulin release correlated strongly with the accumulation of ^{3}H cyclic AMP in the islets of fed as well as starved rats. The thresholds for glucose-induced insulin and ^{3}H cyclic AMP responses were higher and the maximal responses were lower in starved than fed islets. Preincubation of islets of 48-h starved rats with 16.7 mM glucose for 60 min corrected the impaired insulin and ^{3}H cyclic AMP responses to glucose. Starvation-induced impairment of insulin secretory responses to glucose, and their restoration by preincubation with glucose in vitro, may represent acute regulatory effects of glucose on the adenylate cyclase-cyclic AMP system in the pancreatic beta cell.

INTRODUCTION

It is well documented in all species thus far studied, in vivo (1–7) and in vitro (8–13), that starvation results in an impairment of glucose-stimulated insulin secretion. This impairment is not complete, rather there is a decreased sensitivity to glucose of the insulin secretory mechanism (10, 14, 15), and glucose refeeding in vivo (2, 4, 7, 16), or in vitro (15) has been reported to overcome this defect. It has been proposed that starvation specifically impairs a glucose-inducible enzyme system in the pancreatic beta cell (3, 7). Since the mechanisms which normally regulate insulin secretion are not well defined (17), it is hardly surprising that the nature of the impaired mechanism of insulin release observed in starvation has not been established.

Recent observations appear to link adenosine-3′,5′-monophosphate (cyclic AMP)[1] levels in islets with the decreased insulin release observed in starvation. Howell et al. (18) have shown that basal as well as glucagon-stimulated adenylate cyclase activity is decreased in islets of 48-h starved rats, and these authors have suggested that glucose, or a glucose metabolite, may exert long-term effects on this enzyme. Selawry et al. (19) found a decreased content of cyclic AMP in islets of rats starved for 48–72 h, and Capito and Hedeskov (20) observed that glucose

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Abbreviations used in this paper: Cyclic AMP, adenosine-3′,5′-monophosphate; IBMX, 3-isobutyl-1 methylxanthine; KRB, Krebs- Ringer bicarbonate.

TABLE I
Effects of Starvation on Body Weight, Plasma Glucose and Insulin Concentrations, and Islet Insulin Content

Nutrition of rats	No. of animals	Body weight	Plasma glucose	Plasma insulin	Islet insulin content
		g	*mg/100 ml*	*ng/ml*	*ng/islet*
Fed	10	237±10	116±5	2.76±0.28	34.2±2.2
Starved 16 h	12	266±9	82±4*	1.28±0.18‡	39.5±4.8
Starved 48 h	10	203±11	88±4*	1.44±0.17‡	33.8±2.4

All values are mean±SEM.
* $P < 0.02$, according to Student's t test, compared to fed rats.
‡ $P < 0.01$.

increased the cyclic AMP content in islets of fed, but not 48 h starved mice. Furthermore, insulin responses of starved islets to glucose (14), as well as to several other insulinotropic agents (21), were restored to normal by the addition of the phosphodiesterase inhibitor, caffeine.

The purpose of this study was to examine further the relationship between the impairment of insulin secretion observed in starvation, and the activity of the islet adenylate cyclase-cyclic AMP system in this condition. Accordingly, the dose as well as the time kinetics of insulin and cyclic AMP responses to glucose were compared in pancreatic islets isolated from fed and starved rats.

METHODS

Preparation of islets. Male Wistar rats weighing 200–250 g were either fed on a standard laboratory diet ad libitum, or starved for 16 or 48 h until decapitation. Pancreatic islets were isolated by the method of Lacy and Kostianovsky (22). Each pancreas was minced with scissors and incubated at 37°C for 5–6 min, with shaking, in 2 ml Hank's solution equilibrated to pH 7.4 with 95% O_2:5% CO_2, and containing 2.8 mM glucose and 8 mg collagenase (type I, Worthington Biochemical Corp., Freehold, N. J.).

Perifusion experiments. For each experiment 40 islets were transferred into each of two perifusion chambers in parallel, and the insulin content of two groups of 40 islets was also measured after acid-ethanol extraction (23). The perifusion system as applied to pieces of whole pancreas has been previously described (24). The basic medium was Krebs-Ringer bicarbonate (KRB) buffer continuously gassed with 95% O_2:5% CO_2, warmed at 37°C, and containing 5 mg/ml of bovine serum albumin and 2.8 mM glucose. The islets were perifused at a constant flow rate of 2.0–2.5 ml/min, for an initial 30 min equilibration period with 2.8 mM glucose, followed by a 30-min stimulation period with 16.7 mM glucose, then a return to 2.8 mM glucose for 10 min. The effluent from each chamber was collected in 1-min periods by using fraction collectors, and the insulin output rate was calculated as the product of the insulin concentration measured in the effluent and the perifusion flow rate.

Labeling with ^3H adenine and incubation of islets. The technique of labeling islet ATP during a period of preincubation was adapted from Kuo and DeRenzo (25) and Humes et al. (26). The total batch of islets (1,000–2,000), obtained from four to eight rats, was preincubated at 37°C for 60 min in 2.5 ml KRB buffer containing 5 mg/ml of dialyzed bovine serum albumin, 2.8 mM glucose, and 100 μCi/ml ^3H-2-adenine (25 Ci/mmol; New England Nuclear, Dreieichenhain, W. Germany). The islets were then washed four times with 10 ml of the radioactive-free incubation buffer and transferred to a Petri dish.

In the first set of experiments, groups of 25 islets were incubated at 37°C in 1.0 ml KRB buffer containing 5 mg/ml of albumin and 3.3, 6.7, 10.0, 13.3, or 26.7 mM glucose alone and with 0.1, or 1.0 mM 3-isobutyl-1-methylxanthine (IBMX, Aldrich Chemical Co. Inc., Milwaukee, Wis.). After 30 s–60 min of incubation, 0.1 ml of medium was removed for insulin assay and 0.5 ml for measuring the accumulation of ^3H cyclic AMP in the incubation medium. 100 μg of unlabeled cyclic AMP was added to the remaining 0.4 ml of medium containing the islets, and this was boiled for 5 min. The islet content of cyclic AMP was calculated as the difference between the ^3H cyclic AMP in the medium before and after boiling.

In a second set of experiments, after the 60-min preincubation with ^3H-2-adenine, one-half of the total batch of islets (1,000–2,000) was incubated at 37°C for 60 min in 2.5 ml KRB buffer containing 5 mg/ml of albumin and 3.3 mM glucose, and the other half of the islets was incubated with 16.7 mM glucose. The islets were then washed four times with the incubation buffer containing 3.3 mM glucose and transferred to separate Petri dishes. Groups of 25 islets were then randomly distributed to vials containing 1.0 ml KRB buffer with 5 mg/ml of albumin and 3.3, 6.7, 10.0, 13.3, or 26.7 mM glucose, and incubated at 37°C for 10 min. The interval between the preincubation with 3.3 or 16.7 mM glucose and the final 10-min incubations was between 15 and 40 min.

Measurements of ^3H cyclic AMP. The separation of ^3H cyclic AMP from other radioactive substances was performed by ion exchange chromatography and barium sulfate precipitation as described by Krishna et al. (27). Briefly, the incubation media and the boiled islet extracts, each containing 100 μg of unlabeled cyclic AMP (as carrier), were adjusted to 1.0 ml with water and put onto columns (4 × 0.6 cm) of Dowex 50 × 4, 200–400 mesh, H^+ form (Fluka, Buchs, Switzerland), and eluted with water. Cyclic AMP was collected in the 5th- and 6th-ml fraction, and this was further treated by $BaSO_4$ precipitation of impurities, which were removed by centrifugation. Portions (0.2 ml) of the supernate were taken for determination of optical density (at 260 nm wave length) of the carrier cyclic AMP added before purification, to correct for losses (50–60%) of ^3H cyclic AMP during this procedure. The rest of the supernate (2.2 ml) was counted in 6 ml Instagel in a Beckman liquid scintillation counter. (Beckman Instruments, Inc., Fullerton, Calif.) The efficiency of the above procedure for separation of ^3H cyclic AMP from other radioactive substances was confirmed by phosphodiesterase treatment and measurement of the resulting ^3H 5′AMP (28).

Measurements of total cyclic AMP. Groups of 100 islets were incubated at 37°C for 10 min in 50 μl KRB buffer containing 5 mg/ml of albumin plus test agents. Incubations were stopped by addition of 100 μl acetate buffer and boiling for 5 min. Boiled extracts of 50 μl (in duplicate) were taken for cyclic AMP determination by a protein binding assay employing protein kinase from muscle,

as described by Gilman (29). Standard curves were run in medium treated in the same manner as the sample extracts.

Insulin and glucose assays. Insulin was measured by using a charcoal separation method of radioimmunoassay (30). Monocomponent porcine insulin (kindly supplied by Dr. J. Schlichtkrull, Novo Research Institute, Bagsvaerd, Denmark), was iodinated with ^{125}iodine (EIDG. Institut Fur Reaktorforschung, Würenlingen, Switzerland), by using the chloramine-T method of Hunter and Greenwood (31), and purified on G50 Sephadex (Pharmacia Fine Chemicals, Uppsala, Sweden). Purified rat insulin (Novo Research Institute) was used as standard and guinea-pig antiporcine insulin antiserum (kindly provided by Dr. P. H. Wright, Indiana University, Indianapolis, Ind.) was used as antibody in the radioimmunoassay.

Plasma glucose was measured by a glucose-oxidase method (32), by using reagents kindly donated by Dr. F. Schmidt, C. F. Boehringer and Sons, Mannheim, GmbH, W. Germany.

RESULTS

Effects of starvation on plasma glucose and insulin concentrations and on islet insulin content. Rats starved for 16 h weighed about 5% less than fed controls and had significantly lower concentrations of plasma glucose and insulin (Table I). Rats starved for 48 h weighed about 14% less than fed controls, but exhibited no further decrease in the concentration of either plasma glucose or insulin. There was no significant change in the content of insulin in islets of rats starved for 16 or 48 h compared to fed controls.

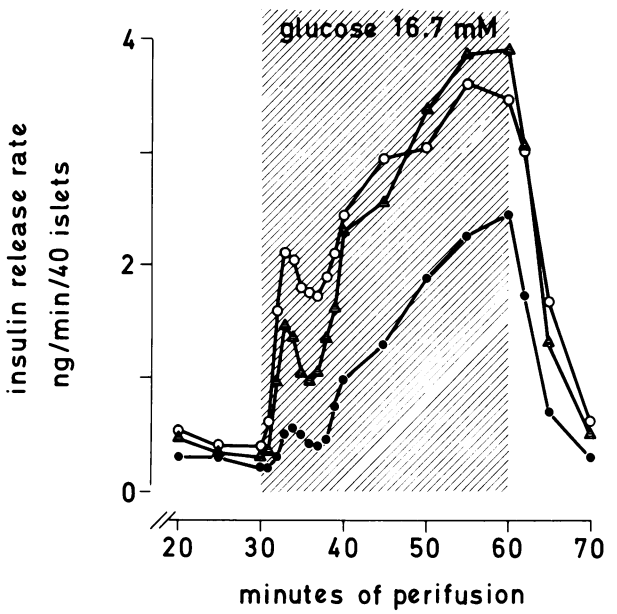

FIGURE 1 Mean rates of insulin release from islets of fed rats (○, $n = 5$ experiments) 16-h starved rats (▲, $n = 6$ experiments) and 48-h starved rats (●, $n = 5$ experiments), during perifusion with 2.8 mM glucose for 30 min (only last 10 min shown) followed by an increase in the perifusion medium glucose concentration to 16.7 mM for 30 min, then a return to 2.8 mM glucose for 10 min.

TABLE II
Effects of Starvation on the Early (I) and Late (II) Phases of Glucose-Induced Insulin Release in Perifused Islets

Nutrition of rats	No. of perifusions	Insulin release, *ng/40 islets* *			
		Phase I	Phase II	Total	Phase I/Total
					%
Fed	5	9.2±1.3	69.6±14.7	79.5±16.0	14.0±1.3
Starved 16 h	6	5.8±0.9‡	69.9±12.4	75.9±13.7	7.7±0.8§
Starved 48 h	5	2.3±0.5§	38.0±5.3‡	40.5±5.6	6.2±1.1§

* Mean values±SEM integrated over the first 6 min (phase I), and from 7 to 30 min (phase II) during perifusion with 16.7 mM glucose.
‡ $P < 0.05$, according to Student's t test, compared to fed rats.
§ $P < 0.005$.

Effects of starvation on glucose-stimulated insulin release from perifused islets. Basal insulin release rates at the end of a 30-min period of perifusion with 2.8 mM glucose were lower in islets of 48 h starved rats (0.20±0.06 ng/min/40 islets) and 16-h starved rats (0.31±0.09 ng/min/40 islets) than in islets of fed rats (0.39±0.07 ng/min/40 islets), however the differences were not statistically significant (Fig. 1). There was a significant increase in the rate of insulin release from islets of fed rats at 1 min after increasing the glucose concentration in the perifusate from 2.8 to 16.7 mM, but not until 2 min in islets of 16-h starved rats, and 3 min in islets of 48 h starved rats. Furthermore, there was a progressive decrease in the early phase of glucose-induced insulin release in islets of rats starved for 16 and 48 h, whereas the later phase was significantly less only in islets of rats starved for 48 h (Table II). Phase I insulin release represented 14.0 ±1.3% of the total insulin release in islets of fed rats, 7.7±0.8% in islets of 16-h starved rats, and 6.2 ±1.1% in islets of 48-h starved rats, thereby indicating that starvation resulted in a relatively greater decrease in the early than the late phase of glucose-induced insulin release.

Time-course of the effects of glucose on insulin release and ^{3}H cyclic AMP formation in islets of fed and starved rats. Fig. 2 shows that in incubations with 0.1 mM of the phosphodiesterase inhibitor, IBMX, 26.7 mM glucose stimulated significant increases of ^{3}H cyclic AMP in islets of fed rats (30 s) sooner than in islets of 48-h starved rats (1 min). Similarly, glucose stimulated a significant increase of insulin release at 2 min in islets of fed rats and only at 5 min in islets of 48-h starved rats. Thus, significant increases of ^{3}H cyclic AMP were detected in islets of fed as well as starved rats before insulin

release was significantly stimulated in either case. Insulin release and islet levels of ^3H cyclic AMP were less in starved than fed islets during the first 10–30 min, but not at 60 min of stimulation with 26.7 mM glucose (see legend to Fig. 2).

Glucose dose-response relationships for insulin release and ^3H cyclic AMP accumulation in islets of fed and starved rats. Fig. 3 shows that insulin and ^3H cyclic AMP responses in 10-min incubations with different concentrations of glucose were less in islets of 48-h starved than fed rats, in the absence as well as in the presence of 0.1 or 1.0 mM IBMX. Furthermore, the starved islets were less *sensitive* to glucose, in terms of both insulin release and ^3H cyclic AMP accumulation. Thus, the threshold concentration of glucose required to stimulate a significant increase of insulin release above basal (with 3.3 mM glucose alone) was 13.3 mM in fed islets and 26.7 mM in starved islets without IBMX, 10.0 mM in fed islets and 13.3 mM in starved islets with 0.1 mM IBMX, and 6.7 mM in both fed and starved islets with 1.0 mM IBMX. Since fed islets were not tested with glucose concentrations below 3.3 mM, it is not clear whether this concentration is already stimulatory in the presence of 1.0 mM IBMX. Similarly, the threshold concentrations of glucose required to stimulate a significant increase of ^3H cyclic AMP above basal was 13.3 mM in fed islets and 26.7 mM in starved islets without IBMX, 6.7 mM in fed islets and 10.0 mM in starved islets with 0.1 mM IBMX, and 6.7 mM in fed islets and 10 mM in starved islets with 1.0 mM IBMX. In these short (10 min) incubations, IBMX did not correct the impaired insulin and cyclic AMP responses to glucose; however, 0.1 mM, as well as 1.0 mM IBMX amplified the dose-dependent effects of glucose similarly in fed and starved islets.

Furthermore, there were strong correlations between insulin release and ^3H cyclic AMP accumulation in the islets of either fed or starved rats, without and with IBMX (Fig. 4). Also, the slopes of the regression lines (b, regression coefficients) were similar in fed and starved islets in the absence, as well as in the presence of 0.1 mM and 1.0 mM IBMX. This indicates that insulin release bears the same relationship to levels of cyclic AMP in

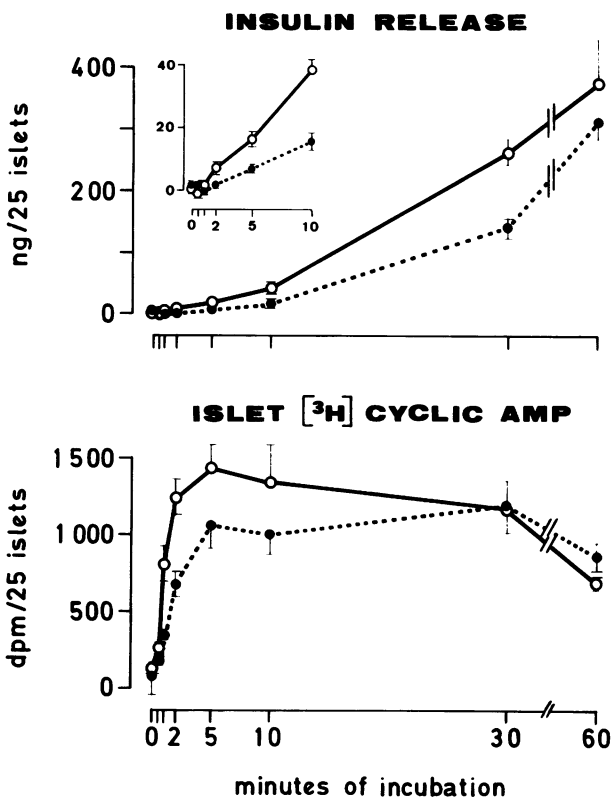

FIGURE 2 Time-course of the effects of 26.7 mM glucose on insulin release and accumulation of ^3H cyclic AMP in islets of fed (○——○) and 48 h starved (●- - -●) rats. Data are plotted as mean values±SEM for the differences between eight (fed islets) and six (starved islets) parallel incubations with 26.7 and 3.3 mM glucose. IBMX (0.1 mM) was present in all incubations. Insulin release was significantly less (Student's t test) in starved than fed islets at 2 min ($P < 0.05$), 5 min ($P < 0.05$), 10 min ($P < 0.001$), and 30 min ($P < 0.001$); and ^3H cyclic AMP accumulation was significantly less in starved islets at 1 min ($P < 0.01$) and 2 min ($P < 0.01$).

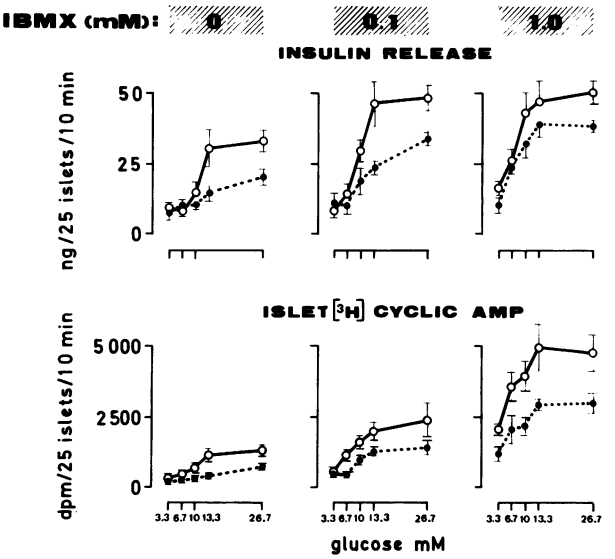

FIGURE 3 Glucose dose-response relationships for insulin release and accumulation of ^3H cyclic AMP in islets of fed (○——○) and 48-hr starved (●- - -●) rats, incubated without and with 0.1 and 1.0 mM IBMX. Data are plotted as mean values±SEM for five (fed islets) and six (starved islets) parallel incubations with and without IBMX at each glucose concentration.

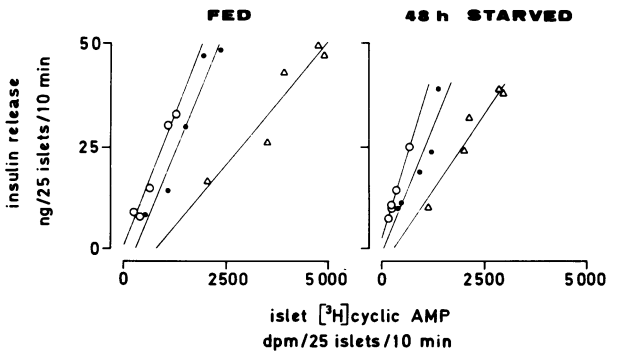

FIGURE 4 Correlations between insulin release and ^{3}H cyclic AMP accumulation in islets of fed and 48-h starved rats. Data are taken from the experiments shown in Fig. 3, in which islets were incubated for 10 min with 3.3–26.7 mM glucose alone (○), with glucose plus IBMX 0.1 mM (●), and with glucose plus IBMX 1.0 mM (△). The coefficients of correlation (r) were: in fed islets, 0.98 (○), 0.97 (●), and 0.94 (△); and in starved islets, 0.99 (○), 0.93 (●), and 0.97 (△). The regression coefficients (b, mean±SEM) were: in fed islets, 0.032±0.009 (○), 0.025 ±0.010 (●), and 0.010±0.002 (△, $P < 0.05$ *vs.* ○); and in starved islets, 0.033±0.004 (○), 0.023±0.006 (●), and 0.013±0.003 (△, $P < 0.01$ *vs.* ○).

starved as in fed islets, i.e. the sensitivity of insulin release to cyclic AMP was similar in fed and starved islets. However, insulin release in both fed and starved islets was progressively less sensitive to increases in cyclic AMP with increasing concentrations of IBMX, as indicated by the progressively smaller regression coefficients (see legend to Fig. 4). Thus, the phosphodiesterase inhibitor was not as effective as glucose alone in stimulating insulin release.

Effects of a 60-min preincubation with glucose on the subsequent insulin and ^{3}H cyclic AMP responses to glucose in islets of fed and starved rats. Fig. 5 shows that when islets of either fed or 48-h starved rats were preincubated for 60 min with 16.7 mM glucose, the subsequent glucose-induced insulin as well as ^{3}H cyclic AMP responses were greater than those observed in islets preincubated for 60 min with 3.3 mM glucose. It is also apparent that the 60-min preincubation with 16.7 mM glucose had greater effects on islets from starved than fed rats, the dose-response curves in these two nutritional states now becoming quite similar. Thus, the decreased stimulation of both insulin release and cyclic AMP formation in islets of rats starved for 48 h could be entirely reversed by a 60-min preincubation of the islets in a high glucose concentration.

Comparative effects of glucose and IBMX on ^{3}H cyclic AMP formation and total cyclic AMP content in islets of fed and starved rats. The total cyclic AMP content in islets of fed and 48-h starved rats was measured by the protein kinase binding method (29), to determine whether the effects of glucose and IBMX on the accumulation of ^{3}H cyclic AMP in islets prelabeled with ^{3}H adenine reflected changes in total islet cyclic AMP content. Table III shows that basal levels of cyclic AMP (with 3.3 mM glucose) were slightly, but not significantly lower in islets of 48 h starved than fed rats, as measured by either method. 26.7 mM glucose stimulated a greater increase in the accumulation of ^{3}H cyclic AMP in fed than starved islets, and the levels of ^{3}H cyclic AMP with 26.7 mM glucose were significantly greater in fed than starved islets ($P < 0.025$) Similarly, 26.7 mM glucose increased total cyclic AMP in fed islets more than in starved islets, and the latter increase was not significant. However, it is apparent that the glucose-stimulated increase in total cyclic AMP was considerably less than the increase in ^{3}H cyclic AMP in islets of either fed or starved rats. This may be due to the less favourable incubation condition for glucose action (100 islets in only 50 μl, necessi-

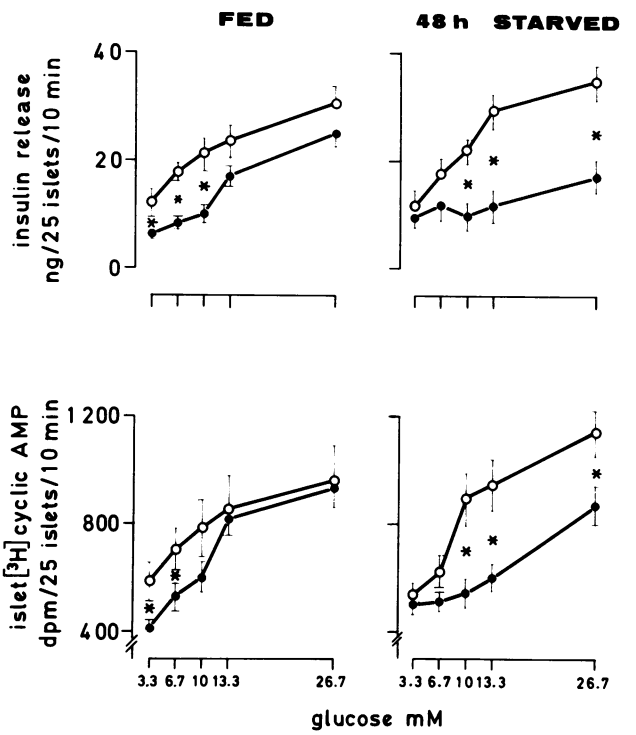

FIGURE 5 Effects of a 60-min preincubation with 3.3 mM (●) or 16.7 mM (○) glucose (followed by a 15–40-min interval at room temperature with 3.3 mM glucose) on the subsequent insulin and ^{3}H cyclic AMP responses to glucose during 10-min incubations of islets of fed and 48-h starved rats. Data are plotted as mean values±SEM for six (fed islets) and six (starved islets) parallel incubations with each preincubation glucose concentration.

* Indicate that the values for preincubations with 3.3 and 16.7 mM glucose were significantly different by Student's *t* test ($P < 0.05$ or less).

TABLE III

Effects of Glucose±IBMX on Accumulation of ^3H Cyclic AMP and Total Cyclic AMP in Islets of Fed and Starved Rats

Glucose concentration	IBMX concentration	Islet [^3H] cyclic AMP accumulation*		Total cyclic AMP content‡	
mM	*mM*	*dpm/islet/10 min*		*fmol/islet/10 min*	
		Fed	48 h starved	Fed	48 h starved
3.3	0	9.9±2.0	6.4±0.5	8.8±1.0	7.0±1.3
26.7	0	50.8±6.6	27.0±2.7	16.5±1.5	11.0±2.0
3.3	1.0	82.3±4.7	45.6±9.7	65.0±7.0	47.0±6.8
26.7	1.0	191.6±25.2	119.0±14.0	74.0±7.5	70.3±3.8

* Mean values±SEM; results of experiments shown in Fig. 3.

‡ Mean values±SEM for eight incubations of fed islets and nine incubations of starved islets.

tated by the lesser sensitivity of the protein binding assay) compared to that used (25 islets incubated in 1 ml) for the radioactive method of measuring cyclic AMP. By contrast, 1.0 mM IBMX (with 3.3 mM glucose) stimulated similar increases of ^3H cyclic AMP (+731%) and total cyclic AMP (+638%) in islets of fed rats, and slightly smaller but similar increases of ^3H cyclic AMP (+613%) and total cyclic AMP (+571%) in islets of starved rats.

DISCUSSION

Contrary to earlier reports (33, 34), the ability of glucose to stimulate an increase in the content of cyclic AMP in pancreatic islets has been demonstrated recently by several groups, by using islets isolated with collagenase from adult fed rats (35–37) or mice (20), as well as islets microdissected from obese (ob/ob) mice (38). The present study also demonstrates that glucose stimulates an increase in the content of cyclic AMP in pancreatic islets of adult fed rats, whether measured as the accumulation of ^3H cyclic AMP in islets prelabeled with ^3H-2-adenine, or the total amount of the nonradioactive cyclic nucleotide. The glucose-stimulated increase of cyclic AMP in islets of fed rats was rapid, and in static incubations, preceded a significant increase of insulin release (Fig. 2). Also, the insulin and cyclic AMP responses in islets of fed, as well as 48-h starved rats, exhibited parallel glucose dose-dependencies (Fig. 3). This was further emphasized by the strong linear correlations between the insulin and cyclic AMP responses to glucose (Fig. 4).

However, it is also apparent in Fig. 4 that the ratio of insulin release to islet cyclic AMP accumulation was lower in incubations with glucose plus the phosphodiesterase inhibitor, IBMX than with glucose alone. Similarly, Charles et al. (35), and Hellman et al. (38), by employing high concentrations of xanthine derivatives, or the adenylate cyclase stimulator, cholera toxin, have shown that islet concentrations of cyclic AMP even greater than those achieved by high concentrations of glucose are unable to induce major insulin release in the presence of low concentrations of glucose. From these observations it has been concluded that cyclic AMP cannot be the mediator of glucose-induced insulin release. Although cyclic AMP may not be the exclusive mediator of insulin release, nevertheless, the present study suggests that the *differences* between insulin release in islets of fed and starved rats may be due to differences in activity of the adenylate cyclase-cyclic AMP system in these islets.

The mechanism(s) by which starvation results in impairment of beta cell function are not clear. A role for islet cyclic AMP has been implied by recent studies (18–20). Contrary to the findings of Selawry et al. (19) and in agreement with Capito and Hedeskov (20), the *basal* content of cyclic AMP was not significantly reduced in islets of starved animals (Table III). However, in agreement with the latter authors (20), glucose stimulated a lesser increase of cyclic AMP in islets of 48 h starved than fed animals. Since starvation reduced mainly the early phase of glucose-induced insulin release (Fig. 1 and Table II), it is of particular interest that increases of cyclic AMP in islets of starved rats were less than in islets of fed rats only during the first 10-min of stimulation with glucose. Also, cyclic AMP responses were similar in fed and starved islets at 30 min, and insulin responses were similar at 60 min (Fig. 2). Thus, there was a parallel impairment in the *early* insulin and cyclic AMP responses to glucose in islets of 48-h starved rats. In addition, the dose kinetics of the insulin and cyclic AMP responses to glucose were similarly impaired in islets of 48 h starved rats. Starvation raised the threshold and decreased the capacity of the insulin as well as the cyclic AMP responses to glucose in 10-min incubations (Fig. 3). The similarity in the impairment of both time- and dose-kinetics of glucose-induced insulin and cyclic AMP responses in islets of 48-hr starved rats suggests that a major factor in the modification of glucose-induced insulin release in starvation may be a reduced responsiveness of the islet cyclic AMP system to glucose.

Inhibition of the phosphodiesterases with IBMX did not fully restore the impaired glucose-stimulated insulin response in starved islets, as reported by others (11, 39). However, IBMX amplified the dose-dependent effects of glucose similarly in fed and starved islets, and the sensitivity of insulin re-

lease to cyclic AMP was similar in fed and starved islets (Fig. 4). This finding suggests that the response of the effector secretory system to the postulated signal derived from cyclic AMP is unaltered by starvation.

Several authors (2, 4, 7, 16) have demonstrated that glucose refeeding in vivo can correct the impaired insulin release associated with starvation, and Hahn and Fiedler (15) recently reported that glucose priming in vitro was also effective. In the present study, a 60-min preincubation of islets of 48 h starved rats with 16.7 mM glucose resulted in the restoration of normal (fed) glucose dose-response relationships both for insulin release and cyclic AMP accumulation (Fig. 5). It is quite possible that the time kinetic characteristics of the cyclic AMP and insulin responses in starvation, i.e. their "normalization" at 30 and 60 min, respectively, (Fig. 2), reflect a similar restoring effect of high glucose during prolonged incubations. These findings are of considerable interest since they suggest that starvation (or glucose withdrawal) affects a glucose-sensitive beta cell mechanism which displays a high turnover rate. Having observed that the normalizing effect of glucose "priming" is blocked by actinomycin D, Grey et al. (7) concluded that the impairment of glucose-stimulated insulin secretion in starvation is associated with changes in a glucose-inducible enzyme system in the pancreatic beta cell. However, Howell et al. (18) reported that the increase in adenylate cyclase activity in rat islets incubated for 4 h with 17 mM glucose was not prevented by cycloheximide or actinomycin D. These observations, together with the short-term (1 h) action of glucose in correcting the impaired insulin and cyclic AMP responses in starvation in the present study, suggest activation of an existing enzyme(s) or "glucoreceptor" rather than their induction by the hexose.

Starvation has been reported to cause a marked reduction of glucose metabolism (14, 39) as well as a reduced activity of glucokinase and phosphofructokinase in islets (40). On the basis of these findings, the above authors have proposed that the major starvation-induced defect in the process of glucose recognition in the beta cell is located in the early steps of glucose metabolism through the glycolytic pathway. Our data do not exclude a role for altered glucose metabolism in the anomaly of islet function induced by starvation. The effect of glucose on cyclic AMP may even be linked with its metabolism (37, 38). However, other reports suggest that a defect in cyclic AMP generation, rather than in carbohydrate metabolism, might be responsible for the decreased insulin release observed in starvation. Thus, fasting ob/ob mice for 18 h reduced the initial phase of glucose-induced insulin release, but left the rise of glucose-6-phosphate unaffected (11); and the glucose-induced increase of cyclic AMP was reduced by 50% in islets of rats starved for 24 h, whereas glucose oxidation was not affected (41).

In summary, the data presented here suggest that the glucose-stimulated increase of cyclic AMP in islets may be one of the factors that control insulin release; and that the modifications induced by starvation on the metabolism of this nucleotide may be, at least in part, responsible for the impairment of insulin secretion observed in starvation.

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