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Restoring Effects of Refeeding and Dibutyril Cyclic AMP on the Increased Glucagon Secretion of Rat Pancreatic Islets During Starvation

S. Ortiz, J. C. Prieto, F. Bedoya, E. Arilla and R. Goberna

Cátedra de Bioquímica
Facultad de Medicina
Universidad de Sevilla
Sevilla (Spain)

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The glucagon release in the presence of glucose and the interaction of dibutyril cyclic AMP was studied in isolated pancreatic islets from fed and 96 h-fasted rats incubated for 30 min. In both states the increase of glucose concentration produced a similar inhibition of glucagon release and stimulation of cyclic AMP content. Higher glucagon secretion and lower cyclic AMP contents were observed in islets from fasted than in those from fed animals at both 2.75 and 16.7 mM glucose. Islets from rats starved for 96 h and refed for 48 h with normal diet or oral 20 % glucose showed glucagon release patterns similar to those of controls. Addition of 2 mM dibutyril cyclic AMP to the incubation medium inhibited the fasting-induced increase of glucagon secretion at both glucose levels. These results show an inverse correlation between glucagon secretion and islet cyclic AMP content. However, the sensitivity of the islet to glucose-induced inhibition of glucagon secretion appears to be independent of the islet cyclic AMP levels.

It is widely accepted that intracellular levels of adenosine 3′:5′-monophosphate (cyclic AMP) in endocrine cells may play an important role in the regulation of hormone secretion (19). The involvement of cyclic AMP in glucagon release by the pancreatic A cell is not completely understood. Dibutyril cyclic AMP (dbcAMP) has been reported to stimulate glucagon secretion in isolated rat pancreas (21), splenic lobes of rat pancreas (14) and guinea pig islets (11); however, cyclic AMP was shown to inhibit glucagon release in monolayer pancreatic cell cultures (26). Theophylline, which acts through an increase in intracellular concentration of cyclic AMP by inhibiting phosphodiesterase, stimulates glucagon release from mouse (5), rat (17) and guinea pig (11) islets, splenic lobes of rat pancreas (14) and isolated rat pancreas (21); however, the simultaneous administration of amino-

phylline and arginine decreases the arginine-induced hyperglucagonemia *in vivo* (18).

Several studies (2, 9, 27), with some exceptions (6), indicate that glucose does elevate total islet cyclic AMP levels concomitantly with an inhibition of glucagon secretion. Starvation, a dietary change of the most extreme type, has been recognized to stimulate glucagon secretion, both *in vivo* (24) and *in vitro* (20).

This report presents an analysis of the relationship between glucose-dependent glucagon secretion and islet cyclic AMP in the fed and in the fasted state in order to stablish additional evidence on the role of the cyclic nucleotide in the regulation of the secretory mechanisms of the pancreatic A cell.

Materials and Methods

Male Wistar rats weighing between 300-350 g were selected and either fed on a standard laboratory diet *ad libitum* or starved for 96 h with free access to drinking water. One additional group consisted of animals starved for 96 h and then refed for 48 h.

The pancreas was taken from each rat under sodium pentobarbital anesthesia. Pancreatic islets were isolated using a collagenase (Worthington Biochemical Corp., Freehold) technique (16).

Groups of 5 isolated islets were preincubated for 10 min at 37° C in tubes containing Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with 1 % (w/v) bovine serum albumin (Behringwerke, Marburg Lahn) and 1,000 U of kallikrein-trypsin inhibitor (Trasylol, Bayer Leverkusen) to prevent any damage of the glucagon released. Glucose was added at the end of the preincubation period at a final concentration of 2.75 or 16.7 mM (total volume 1 ml). In a set of experiments dbcAMP was added at a final concentration of 2 mM. After being gassed with 95 % O_2 and 5 % CO_2, the tubes were stoppered and incubated for 30 min at 37° C under constant shaking. Aliquots of the medium were immediately frozen after the incubation and stored at —25° C until assay of glucagon.

Immunoreactive glucagon (IRG) was measured by a modified charcoal radioimmunoassay method (1) using crystalline pork glucagon (Lilly Co., Indianapolis) and 30K antibody (a gift from Dr. R. H. Unger, Dallas, Texas). Results were expressed as ng IRG released per 5 islets per 30 minutes.

For the experiments where cyclic AMP was measured, batches of 25 islets were incubated in the presence of 2.75 or 16.7 mM glucose as indicated above. An islet homogenate was then prepared by sonication (10 s at half-maximal amplitude, MSE sonifier) of the incubation mixture. One ml of 30 % trichloroacetic acid (TCA) was added and the tubes were centrifuged for 10 min at 3,000 rpm (Beckman TJ-6). The TCA was removed from each supernatant by shaking with 3 volumes of ether saturated with water and the remaining solution was lyophylized and stored at —25° C until cyclic AMP determination. Cyclic AMP was measured by a modification (23) of the method of GILMAN (8) using cyclic AMP binding protein purified from fresh rabbit skeletal muscle through the DEAE-cellulose column step. Results were expressed as total concentration (islets plus medium) in pmol cyclic AMP per 25 islets per 30 min.

The TCA precipitates of the islet homogenates were assayed for DNA by the method of Kissane and Robins (12) using calf thymus DNA (Sigma) as standard.

For each parameter, the mean ± S.E.M. was calculated. Statistical significances between groups were analyzed by the Student's t-test.

Results

There were no differences in islet DNA content between fed (219 ± 20 ng/islet,

n = 13) and fasted (217 ± 18, n = 13) rats, thus making possible the reference of glucagon secretion or cAMP concentration to the number of islets employed.

The accumulation of glucagon in the incubation medium during 30 min at glucose 2.75 or 16.7 mM is shown in figure 1. In islets from both fed and fasted rats glucagon secretion was inhibited at the high glucose concentration with respect to basal values (at 2.75 mM glucose). Islets from fasted rats secreted more glucagon than those from fed rats at both glucose levels.

Due to this fasting-induced onset of pancreatic A cell hyperfunction, it was thought that the elevated glucagon secretion might revert to normal upon resumption of refeeding. For this purpose, the A cell function was evaluated in islets from rats that had been starved for 96 h and then refed for 48 h with a standard laboratory diet (fig. 1). These islets showed a pattern of glucagon secretion at both carbohydrate levels that closely approached the pattern in the fed state, rendering the differences between these groups statistically non-significant. Thus, refeeding virtually abolished the fasting-induced stimulation of glucagon secretion. A similar effect was obtained after selective refeeding with 20 % glucose administered orally (data not shown).

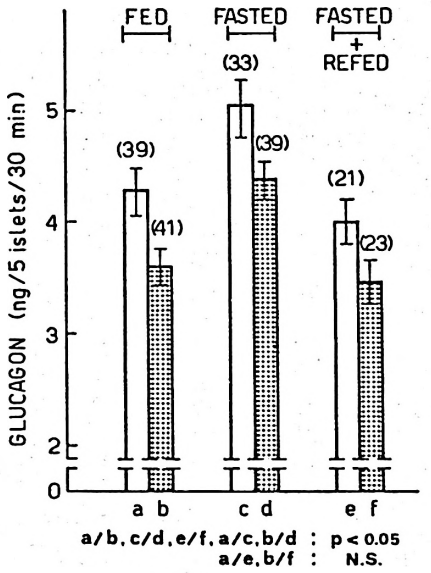

Fig. 1. *Glucagon release from islets incubated with glucose.*
Islets from fed, 96 hours-fasted or 96 hours-fasted and 48 hours-refed rats were incubated for 30 min in the presence of 2.75 (open bars) or 16.7 (shaded bars) mM glucose. Glucagon concentrations in the medium after incubation are expressed as mean values ± S.E.M. of the number of experiments indicated in parenthesis.

Table I shows the cyclic AMP contents in islets from fed and 96 h-fasted rats. A glucose-induced increase in cyclic AMP content was observed in both fed and fasted states. In islets from fed rats, cyclic AMP at 30 min of incubation in the presence of 16.7 mM glucose showed a 60 % increase over the values observed at 2.75 mM glucose and the same was true in islets from fasted rats. Starvation resulted in a 60 % decrease in islet cyclic AMP content with respect to control values, both at 2.75 and 16.7 mM glucose (table I).

The fact that the fasting-induced decrease of islet cyclic AMP correlated closely the fasting-stimulated glucagon secretion at both glucose levels led the authors to study the effect of the cyclic nucleotide in the mechanisms of hormone secretion

Table I. *Cyclic AMP contents in islets from fed and 96 hours-fasted rats after incubation with 2.75 or 16.7 mM glucose for 30 min at 37° C.*
Means of 8-10 rats per category ± S.E.M.

Addition mM	Cyclic AMP content (pmol/25 islets/30 min)		
	Fed	96 hours-fasted	
Glucose 2.75	1.30±0.26	0.72±0.13	p<0.05
Glucose 16.7	1.99±0.33	1.23±0.22	p<0.05
	p<0.05	p<0.05	

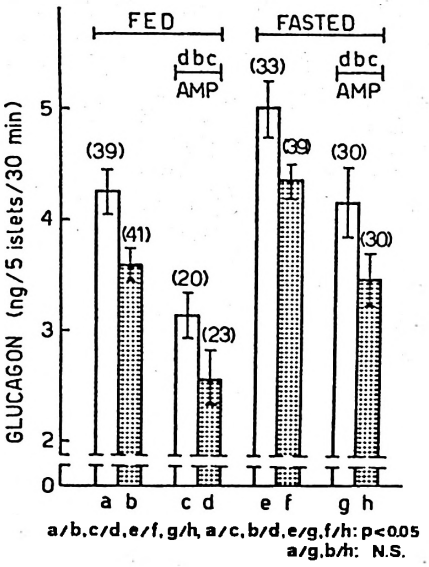

Fig. 2. *Effect of dbcAMP on glucagon release from islets incubated with glucose.* Islets from fed and 96 hours-fasted rats were incubated for 30 min in the presence of 2.75 (open bars) or 16.7 (shaded bars) mM glucose with or without 2 mM dbcAMP. Glucagon concentrations in the medium after incubation are expressed as mean values ± S.E.M. of the number of experiments indicated in parenthesis.

in the A cell. In this context, batches of islets from fed and fasted rats were incubated in the presence of dbcAMP. This nucleotide was chosen, rather than cyclic AMP because it passes through the cell membrane more easily.

The effect of 2 mM dbcAMP on glucagon secretion in isolated islets is shown in figure 2. The increased sensitivity of the secretory mechanism in islets from starved rats returned to normal fed control values in the presence of the nucleotide either at 2.75 or 16.7 mM glucose. This dbcAMP inhibition of glucagon secretion was also observed in islets from fed animals at the two concentrations of glucose studied (figure 2) since hormone concentrations measured were about 50-60 % of those obtained in the absence of the nucleotide.

Discussion

It is assumed in this work that the number of cells in islets from fed and fasted rats is the same, as it is shown by the maintenance of the DNA content, in agreement with previous reports (10). Whether the proportion of A cells changes with the nutritional state is not known.

The present demonstration of glucose-induced elevation of islet cyclic AMP (table I) in the absence of phosphodiesterase inhibitors such as theophylline or 3-isobutyl-1-methylxantine is in agreement with previous reports (2, 22, 27, 29), thus indicating that islet cyclic AMP is related to a glucose-sensitive mechanism. This dependence of islet cyclic AMP on glucose concentration was true both in the fed and in the fasted state, although starvation caused a marked reduction of the cyclic nucleotide content at the two glucose concentrations studied. This agrees with studies on islets from 24 to 72 h fasted rats (2, 27), although the cyclic AMP response to glucose reported there was lower in the fasted than in the fed state. In contrast, CAPITO and HEDESKOV (3) reported that 16.7 mM glucose increased cyclic AMP content in islets from fed mice but had no effect in islets from 48 h-fasted mice

Present results confirm the stimulatory effect of fasting on glucagon secretion in the presence of glucose (20). Complete restoration of the secretory response to glucose was observed after 48 h refeeding, both with a standard diet or a selective diet of just 20 % glucose. It is worth noting the inverse correlation that exists between glucagon secretion and islet cyclic AMP at both low and high glucose levels in the fed as well as in the fasted state. It was also observed in experiments in which islets were incubated with glucose and dbcAMP. The presence of the cyclic nucleotide diminished glucagon secretion in islets from both fed and fasted animals. The latter group exhibited a secretory pat-

tern similar to that of islets from fed rats in the absence of dbcAMP (fig. 2). Contradictory results have been reported in the literature: *a*) dbcAMP stimulated glucagon release (11, 14, 21) but cyclic AMP produced the opposite effect (26) and cyclic GMP was inactive (15); *b*) methylxantines stimulated glucagon secretion at low glucose concentration (5, 11) or regardless of glucose level (13), had no effect at high glucose concentration (25) and lowered the glucagon response induced by arginine (18).

From present and previous results (2, 22, 27, 29) it appears that fasting modifies the mechanism by which glucose stimulates cyclic AMP production in islets of Langerhans. Although this mechanism is largely unknown, membrane-bound calcium is apparently implied. In this connection, islet cyclic AMP has been reported to be dependent on calcium (4, 7, 29) and it is interesting that fasting causes a partial depletion of islet calcium content (28).

In conclussion, the sensitivity of islets to inhibition of glucagon secretion by glucose does not appear to be modified after a 4-day fasting period. During starvation, the enhancing of the secretory response of pancreatic A cells correlates well with the lower cyclic AMP levels present in the islets. That the effectiveness of glucose for inhibition of glucagon secretion is not regulated by the prevailing cyclic AMP content of the islet, may be subjected to speculation.

Resumen

Se estudia la secreción de glucagón en presencia de glucosa y la interacción del dibutiril AMPc en islotes pancreáticos aislados de ratas alimentadas o sometidas a 96 h de ayuno, incubados durante 30 min. En ambas situaciones, el aumento de la concentración de glucosa da lugar a una inhibición similar de la secreción de glucagón y a una estimulación del contenido de AMPc. Los islotes de ratas sometidas a ayuno presentan mayor secreción de glucagón y menor contenido de AMPc que los islotes de ratas alimentadas. La alimentación con dieta completa o con glucosa 20 % oral en ratas sometidas previamente a ayuno normaliza la secreción de glucagón. La adición de dibutiril AMPc 2 mM al medio de incubación de los islotes de ratas sometidas a ayuno normaliza también el patrón de secreción de glucagón a ambos niveles de glucosa Estos resultados muestran una correlación inversa entre la secreción de glucagón y el contenido de AMPc de los islotes. Sin embargo, la sensibilidad del islote a la inhibición de la secreción de glucagón provocada por la glucosa no parece depender de los niveles existentes de AMPc.

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