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A Possible Role of Adenylate Cyclase in the Long-Term Dietary Regulation of Insulin Secretion from Rat Islets of Langerhans

By SIMON L. HOWELL, IRENE C. GREEN and WILLIAM MONTAGUE
Biochemistry Group, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K.

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1. Adenylate cyclase activity and patterns of insulin release in response to various concentrations of glucose were determined in islets of Langerhans isolated from starving, fed, or glucose-loaded rats. 2. Basal and glucagon-stimulated activities of adenylate cyclase were lower in islets from starved than from fed rats. The minimum glucose concentration required for stimulation of insulin secretion was higher, whereas the maximum secretory response to glucose was lower, in islets from starved than from fed rats. 3. Adenylate cyclase activity in islets of Langerhans obtained from fed rats loaded with glucose by intermittent intravenous or intraperitoneal injections over 5h was significantly higher than that seen in islets from normal fed rats. Islets obtained from glucose-loaded rats required a lower glucose concentration for stimulation of insulin secretion and attained a higher maximal response to glucose stimulation than those derived from fed rats. 4. Incubation *in vitro* of islets isolated from normal fed rats, for periods of 1 to 24h in the presence of high concentrations of glucose resulted in an activation of adenylate cyclase that occurred progressively from 2 to 7h and which was maintained during 24h of incubation. The increase of adenylate cyclase activity in isolated islets incubated for 4h in the presence of glucose was not prevented by addition of cycloheximide or actinomycin D. Galactose or 2-deoxyglucose was ineffective in increasing adenylate cyclase activity, and pyruvate (20mM) was less effective than glucose. 5. It is suggested that glucose or a glucose metabolite may exert long-term effects on islet cell adenylate cyclase.

There is much evidence to suggest the existence of a long-term regulation of the insulin secretory response of the pancreatic β-cell by the carbohydrate content of the diet (for review see Malaisse, 1972). In addition, starvation appears to inhibit insulin secretory responses to glucose in man (Cahill *et al.*, 1966), dog (Vance, *et al.*, 1968) and rat (Malaisse *et al.*, 1967*a*; Grey *et al.*, 1970*a*), whereas the secretory response to theophylline is unaffected (Grey *et al.*, 1970*a*). This effect of starvation can be prevented by intermittent administration of glucose during the period of starvation, and can be overcome by the intraperitoneal or intravenous injection of glucose, or by re-feeding on a high-carbohydrate diet (Grey *et al.*, 1970*a*). An ability to respond to theophylline but not to glucose similar to that seen in starvation, is also seen in foetal pancreatic islet tissue. Nevertheless foetal pancreas maintained in tissue culture in the presence of 3mg of glucose/ml shows, after some days of culture, an adult pattern of response to glucose stimulation, together with a massive secretory response to theophylline (Asplund, 1970; Erlandsen *et al.*, 1968; Lambert *et al.*, 1969). These findings suggest that the initial inability of foetal β-cells to respond to glucose may be related to the presence of a low adenylate cyclase activity or high phosphodiesterase activity. In addition, Cerasi & Luft (1969) have suggested that the altered secretory response to glucose in prediabetes in man may be related to a defect in β-cell adenylate cyclase and they have postulated an effect of glucose in the activation of this enzyme as a mechanism for the acute regulation of insulin secretion by glucose (Cerasi & Luft, 1970, 1972).

These findings have together focused attention on the relationship between glucose and adenylate cyclase activity in the β-cells. In an attempt to explore this relationship, we have investigated the possibility that the long-term effects of starvation, re-feeding and glucose loading on insulin secretory responses might be mediated by changes of adenylate cyclase activity in isolated rat islets of Langerhans.

Experimental

Sources of reagents

The following reagents were obtained from Sigma (London) Chemical Co. Ltd., Kingston-on-Thames, Surrey, U.K.: phosphocreatine, creatine phosphokinase, Trizma base, crystalline albumin, glucagon, imidazole, D-galactose, 2-deoxy-D-glucose and sodium pyruvate. Actinomycin D was obtained from

Servac Ltd., Heidelberg, Germany; actidione (cycloheximide) was obtained from Koch-Light Ltd., Colnbrook, Bucks., U.K.; 3 - isobutyl - 1 - methylxanthine was a gift from G. D. Searle, Chicago, Ill., U.S.A.; glibenclamide was a gift from Hoechst Pharmaceuticals, Hounslow, Middx., U.K.

Methods

Animals. Female rats of Sprague–Dawley strain weighing 260–350g were used, and were maintained on a diet containing 53% carbohydrate (cereal starch), 20% protein and 4% fat. Those animals that were subjected to a glucose load received 2g of glucose/kg body weight intraperitoneally, or intravenously via a tail vein under ether anaesthesia, every hour for 4h and were killed for isolation of the islets 1h after the final injection; control animals received equal volumes of 0.9% saline. In other experiments animals were starved but allowed free access to water, during the 48h before death.

Isolation of islets. Islets of Langerhans were isolated by methods previously described (Howell & Taylor, 1966) and were homogenized directly for determination of adenylate cyclase activity. In further experiments, islets from fed rats were incubated *in vitro* for periods of up to 24h in a bicarbonate-buffered medium (Gey & Gey, 1936) supplemented by the addition of amino acids (Eagle, 1959) and either 1.7mM-, 5.5mM- or 17mM-glucose. Hellerström *et al.* (1972) have demonstrated the continuing viability of islets incubated in these conditions. After incubation the islets were homogenized as before for measurement of their adenylate cyclase activity.

Adenylate cyclase assay. Adenylate cyclase activity was measured by a procedure described in detail elsewhere (Howell & Montague, 1973). Homogenates of 80–100 islets were prepared in a buffer consisting of 25mM-Tris–HCl, 5mM-$MgCl_2$, 1mM-EDTA, pH7.6, to give final protein concentrations of 8–15μg/50μl of homogenate. Portions (50μl) of an assay buffer containing 25mM-Tris–HCl, pH7.6, 5mM-$MgCl_2$, 1mM-3-isobutyl-1-methylxanthine, 0.1% albumin, 1mM-EDTA, 20mM-phosphocreatine, 1mg of creatine phosphokinase/ml and [α-^{32}P]ATP (5–30μM; specific radioactivity 0.5–1.8Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.). NaF (10mM) or glucagon (20μg/ml) was added to the assay buffer as appropriate. Incubations were initiated by the addition of homogenate and were continued for 30min at 30°C. The reaction was terminated by placing in a boiling-water bath for 3min. Cyclic [^{32}P]AMP formed was separated from the reaction mixture by the use of columns of neutral alumina eluted with 10mM-Tris–HCl buffer, pH7.6, by the method of Ramachandran (1971), and was measured by counting of Čerenkov radiation in a Beckman LS233 liquid-scintillation spectrometer (Braunsberg & Guyver, 1965). Proteins were measured by the method of Lowry *et al.* (1951) with crystalline albumin standards. Enzyme activities were expressed as nmol of cyclic AMP formed/30min per mg of protein.

Insulin secretion. Patterns of insulin secretion from isolated rat islets of Langerhans were determined by methods described in detail by Green & Taylor (1972). After a preincubation period of 30min in medium containing 2mM-glucose, islets were reincubated for a further 60min in medium containing various concentrations of glucose and other test substances. The insulin content of the media at the end of this incubation period was determined by radioimmunoassay, with reagents and human insulin standards obtained from Wellcome Research Laboratories, Beckenham, Kent, U.K.

Results

Basal, glucagon-stimulated and F^--stimulated adenylate cyclase activities obtained in islets of Langerhans isolated from rats after a variety of pretreatments are shown in Table 1, in which for simplicity all values of enzyme activity are compared with a basal value of 100 observed in untreated rats included in each experiment. Starvation of the rats for 48h before isolation of the islets clearly caused a dramatic fall of basal and glucagon-stimulated cyclase activity, although the F^--stimulated activity was not significantly affected (Table 1). By contrast, glucose loading of the rats, either acutely over 5h by intraperitoneal or intravenous injection of glucose, or by supplementation of the drinking water with glucose for a period of 5 days, each caused significant increases in basal, glucagon- and F^--stimulated cyclase activity. Administration of glibenclamide (1mg/kg) at 12h intervals for 36h before islet isolation produced no significant change in the observed activity of adenylate cyclase (Table 1).

To determine whether the effects of glucose were mediated via direct effects on the islets of Langerhans, or indirectly via other neural or humoral influences, incubations of isolated islets of Langerhans were performed in the presence of various concentrations of glucose for periods of from 1 to 17h. There was no significant difference in adenylate cyclase activity between islets incubated in the presence of 5.5mM- and 17mM-glucose for up to 1h, but thereafter there was an increase in the presence of 17mM-glucose, which was progressive up to 7h and which was maintained for up to 24h of incubation (Fig. 1). Experiments with mouse islets maintained in tissue culture (Andersson & Hellerström, 1972) for 6 days demonstrated the existence of increased adenylate cyclase activity in islets maintained in the presence of 3.0mg of glucose/ml compared with islets maintained in 0.6mg of glucose/ml. The magnitude of the increase

Table 1. *Adenylate cyclase activity in isolated islets of Langerhans after starvation or glucose loading of rats*

Basal, glucagon- and F^--stimulated adenylate cyclase activity was measured in homogenates of 80–100 islets of Langerhans after pretreatment of the animals as shown. Islets from fed animals were included as controls in every experiment, and all results have been expressed as the % change±S.E.M. from the basal activity (mean of 0.71 nmol of cyclic AMP/30 min per mg of protein) in fed rats in the appropriate experiments. * Indicates values not significantly different from the basal, glucagon- or F^--stimulated values obtained in control fed rats.

Treatment	No. of observations	Adenylate cyclase activity (% of basal value in fed animals)		
		Basal	Glucagon	F^-
Fed animals	39	100	181±8	338±12
Starved 48h	8	59±4	88±5	295±10*
Fed, glucose (2g/kg) intraperitoneally hourly for 4h	8	149±7	238±6	573±17
Fed, glucose (2g/kg) intravenously hourly for 4h	6	121±4	200±6*	406±9
Fed, glucose (5%) in drinking water, 4 days	6	125±5	219±9	395±8
Starved 48h, but with glucose (2g/kg) every 8h during the period of starvation	6	82±5	144±7	310±12*
Fed, actinomycin D (100μg/kg) then glucose (2g/kg) intraperitoneally hourly for 4h	4	136±6	201±9*	545±23
Fed, glibenclamide (1mg/kg) every 12h×4	6	117±6	195±8*	302±10*

Table 2. *Adenylate cyclase activity in homogenates of mouse islets maintained in tissue culture for 6 days*

Islets from normal mice were cultured for 6 days by the procedure of Andersson & Hellerström (1972). Groups of 80 islets were then homogenized for measurement of adenylate cyclase activity by methods described in the text. Each value is the mean ± S.E.M. of four observations. Differences between values obtained in islets cultured in medium containing 0.6 mg and 3.0 mg are significant ($P < 0.01$).

Glucose concentration of culture medium (mg/ml)	Adenylate cyclase activity (nmol of cyclic AMP formed/30 min per mg of protein)		
	Basal	Glucagon	F^-
0.6	0.53±0.1	0.99±0.13	2.91±0.44
3.0	1.32±0.14	4.12±0.39	6.98±0.71

was comparable with that observed in rat islets after 17h of incubation (Table 2).

To investigate the nature and specificity of the increase in cyclase activity caused by glucose, isolated rat islets were incubated *in vitro* for periods of up to 24h. To maintain adequate ATP concentrations in the islets, incubation media contained 1 mg of glucose/ml together with the additions shown in Table 3. 2-Deoxyglucose (20mM) or 20mM-galactose did not activate cyclase, and 20mM-pyruvate or 20mM-mannose seemed less effective than 20mM-glucose. Addition of cycloheximide (0.1mg/ml) or of actinomycin D (10μg/ml) to incubation media containing high glucose concentrations did not significantly inhibit the increase caused by incubation in the presence of 3mg of glucose/ml. These latter experiments were not continued after 4h because of the predicted widespread metabolic effects of interruption of protein biosynthesis during such an extended period.

The pattern of insulin secretion during incubation of islets from starved, fed or glucose-loaded rats in the presence of 2–8mM-glucose is shown in Fig. 2. The secretory response of islets from rats starved for 48h was diminished at all glucose concentrations, and the threshold to stimulation of secretion by glucose increased to a higher concentration. These effects were reversed in islets from rats previously loaded with intraperitoneal glucose (Fig. 2) in which the threshold concentration for glucose stimulation of secretion was lower, and the degree of stimulation was increased relative to that of fed rats. Addition of theophylline to islets from starved rats clearly restored the secretory response to 2–8mM-glucose to normal values, whereas with islets from fed rats 2–8mM-glucose plus theophylline produced a secretory pattern similar to that of islets from glucose-loaded rats in the absence of theophylline (Figs. 3*a*–*c*).

The secretory response to 2–8mM-glucose of islets

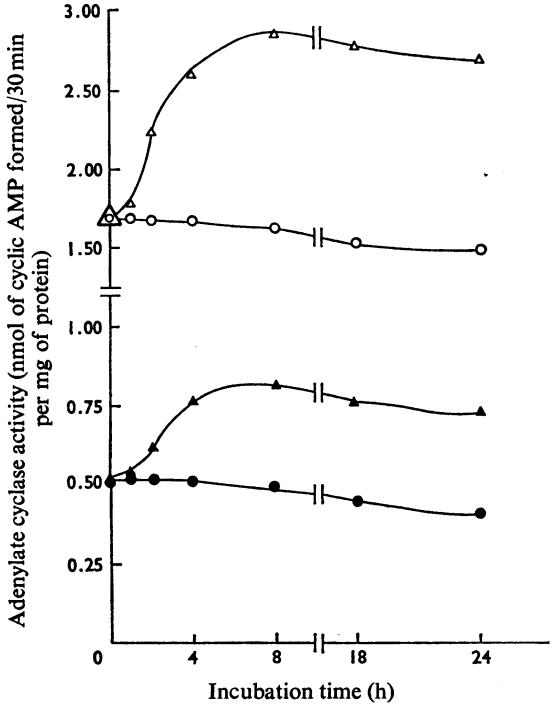

Fig. 1. *Time-course of activation of basal and F^--stimulated adenylate cyclase activity during incubation of isolated rat islets in the presence of* 5.5 mM- *or* 17 mM-*glucose*

Batches of islets were incubated in the conditions described in the text for periods of from 1–24h. At the appropriate intervals the incubation media were removed and the islets homogenized for determination of basal and F^--stimulated adenylate cyclase activity. ● and ▲, Basal activity after incubation in 5.5mM- and 17mM-glucose respectively; ○ and △, F^--stimulated activity after incubation in 5.5mM- and 17mM-glucose respectively.

incubated with 13 mM-imidazole (Fig. 3*d*) closely resembled that of islets from starved rats.

Discussion

Relationship between glucose and adenylate cyclase in the pancreatic β-cell

Despite a recent report to the contrary (Charles *et al.* 1973), the weight of available experimental evidence suggests that glucose does not stimulate the release of insulin from the β-cell via a direct increase of cyclic AMP concentrations. Thus glucose has no effect on islet cell adenylate cyclase in mouse or rat islets (Atkins & Matty, 1971; Davis & Lazarus, 1972;

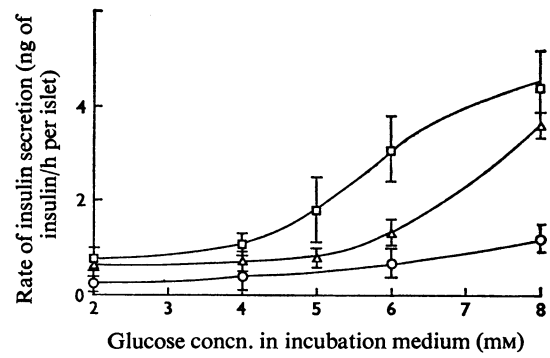

Fig. 2. *Insulin secretory responses of islets isolated from starved, fed or glucose-loaded rats to* 2–8 mM-*glucose*

Each value is the mean±S.E.M. of ten observations. Rats were either starved for 48h, allowed free access to food, or fed and loaded with four intraperitoneal injections of 2g of glucose/kg before isolation of the islets. Insulin secretory responses of the isolated islets were determined as described in the text. ○, Islets from starved rats; △, islets from fed rats; □, islets from glucose-loaded rats.

Miller *et al.*, 1972; Howell & Montague, 1973), or hamster islet tumour (Goldfine *et al.*, 1972), or on phosphodiesterase in mouse or rat islets (Ashcroft *et al.*, 1972; Sams & Montague, 1972). Further, cyclic AMP concentrations (Kipnis, 1970; Montague & Cook, 1971) and cyclic AMP-dependent protein kinase activity (Montague & Howell, 1972; Steiner, 1972) are unaltered after incubation of islets for up to 1h in medium containing high concentrations of glucose. There is, however, no doubt that the β-cell concentration of cyclic AMP may modulate the insulin secretory response to stimulatory concentrations of glucose and other secretagogues (Malaisse *et al.*, 1967*b*; Montague & Cook, 1971). In addition the results reported here indicate that there may exist long-term regulatory effects of glucose on the secretory response of the β-cell mediated by changes of adenylate cyclase activity, which may play a role in maintaining optimal cyclic AMP concentration for an appropriate secretory response to a given stimulus.

Relationship between adenylate cyclase activation and insulin secretory response

Although we have not measured cyclic AMP concentrations directly in this study, a number of observations suggest that they may correlate with the activity of adenylate cyclase. Thus Selawry *et al.* (1972, 1973) reported that starvation markedly decreased cyclic AMP concentrations in isolated

Table 3. *Adenylate cyclase activity in homogenates of rat islets of Langerhans after incubation of intact islets for 4 or 17h in the incubation media shown*

Measurements of basal adenylate cyclase activity were performed after incubation of isolated islets in the presence of additions to the incubation media as shown. Values shown are the % of the control value (0.67 and 0.64 nmol of cyclic AMP/30 min per mg of protein after 4 and 17 h incubation respectively), determined after incubation of islets in medium containing 5.5 mM-glucose for the same period. Each result is the mean ± S.E.M. of at least six observations. * indicates a significant difference for values observed in islets incubated for the same period in 5.5 mM-glucose alone ($P < 0.05$).

Additions to incubation medium	Adenylate cyclase activity (% of activity found after incubation of the same duration in 5.5 mM-glucose) 4h	17h
None	56±4*	43±3*
2.7 mM-Glucose	74±5*	67±4*
8 mM-Glucose	142±6*	189±8*
17 mM-Glucose	156±5*	215±7*
5.5 mM-Glucose + 20 mM-mannose	136±6*	177±7*
5.5 mM-Glucose + 20 mM-galactose	101±4	107±5
5.5 mM-Glucose + 20 mM-deoxyglucose	109±5	112±4
5.5 mM-Glucose + 20 mM-sodium pyruvate	126±6*	163±8*
5 mM-Glucose + 0.1 mg of cycloheximide/ml	94±8	—
17 mM-Glucose + 0.1 mg of cycloheximide/ml	148±9*	—
17 mM-Glucose + 10 μg of actinomycin D/ml	141±8*	—

islets of Langerhans, and the activity of cyclic AMP-dependent protein kinase, which may reflect cyclic AMP concentrations in the islets (Montague & Howell, 1972), decreases significantly during starvation (W. Montague, unpublished work).

There is also evidence to suggest that changes of cyclic AMP concentrations in the β-cell may be related to the altered patterns of insulin secretion observed during starvation or after glucose loading. Thus, the pattern of secretory response to 2–8 mM-glucose in islets from starved rats can be reproduced during incubation of islets from fed rats with imidazole, an agent which stimulates cyclic nucleotide phosphodiesterase (Sams & Montague, 1972; Ashcroft *et al.*, 1972) and may decrease cyclic AMP concentrations in the islets. By contrast, treatment of the islets with the phosphodiesterase inhibitor theophylline, which increases cyclic AMP (Turtle & Kipnis, 1967; Montague & Cook, 1971) as well as cyclic AMP-dependent protein kinase activity (Montague & Howell, 1972) in islets, causes a greater insulin secretory response at any given stimulatory glucose concentration, and also alters the pattern of secretion in response to 2–8 mM-glucose to resemble that seen in glucose-loaded rats.

Intensive glucose loading of rats for long periods causes glycogen deposition in the islets (Malaisse *et al.*, 1967*b*) and the breakdown of this glycogen store might be expected to augment the β-cell secretory responses to glucose in subsequent experiments. However, glycogen breakdown would not appear to be an important factor in determining secretory responses in our experiments because the islets were isolated in a medium containing only 2 mM-glucose and exposure to such a glucose concentration would be expected to result in degradation of existing glycogen stores during the isolation and preincubation period. Ultrastructural examination of islets from glucose-loaded rats immediately after isolation did not reveal the presence of glycogen. The observed lack of effect of glibenclamide, a potent insulin secretogogue, in increasing adenylate cyclase activity (Table 1) in islets isolated from treated animals makes it unlikely that the changes of activity are merely secondary to changes in rates of insulin secretion.

It may be argued that the changes of secretory pattern observed in starvation and after glucose loading are consistent with alterations of the enzymes of glucose metabolism in the islets, and Grey *et al.* (1970*b*) have suggested that the glucokinase–hexokinase system might be a candidate for a role of this type. However, no defect has been reported in glucose penetration in either of the first two phosphorylation stages of glycolysis or in the energy potential of the cell in islets from starved rats (Kipnis, 1972; Matschinsky, 1972). In addition, the defect in secretory

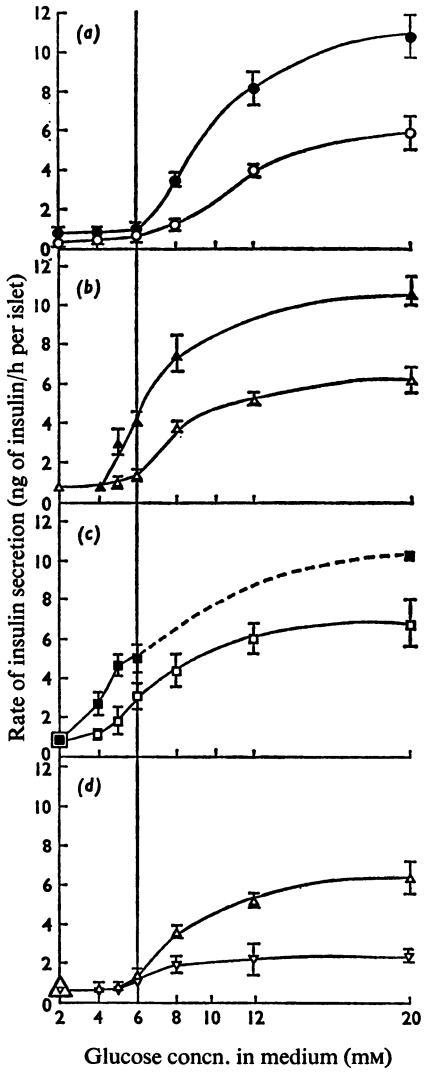

Fig. 3. *Effect of theophyllin* (5 mM) *on insulin secretory responses to* 2–20 mM-*glucose of islets isolated from starved, fed or glucose-loaded rats, and of imidazole* (13 mM) *on insulin secretion from islets isolated from fed rats*

Each value is the mean±S.E.M. of ten observations. The insulin secretory responses are of islets from: (*a*) starved rats in the presence (●) or absence (○) of theophylline; (*b*) fed rats in the presence (▲) or absence (△) of theophylline; (*c*) fed rats subjected to intraperitoneal injections of glucose (2g/kg) for 4h before islet isolation, in the presence (■) or absence (□) of theophylline; (*d*) fed rats the presence (▽) or absence (△) of imidazole (13 mM).

response to glucose seen in starving rats may be rapidly and completely overcome by injection of theophylline (Grey *et al.*, 1970*b*), or by addition of theophylline to the incubation medium (see above), suggesting that an acute defect in cyclic AMP metabolism, rather than in carbohydrate metabolism, might be responsible for the diminished secretory response observed.

Although detailed studies of glucose metabolism in islets from starved and glucose-loaded rats are clearly required, the present evidence is consistent with the concept that alterations of cyclic AMP concentrations within the β-cell may be responsible for the altered secretory responses to 2–8 mM-glucose seen after starvation or glucose loading.

Mechanism of increase of adenylate cyclase activity

The apparent lack of effect of cycloheximide or of actinomycin D on the increase of adenylate cyclase by glucose suggests that activation of existing enzyme, rather than induction of new enzyme might be responsible for the increased activity observed. The ineffectiveness of galactose and of 2-deoxyglucose suggests that a metabolic rather than a stereospecific or transport effect of glucose might be involved, and this conclusion was reinforced by the finding that 20 mM-pyruvate or 20 mM-mannose were at least partly able to reproduce the effects of glucose. The nature of the metabolite involved must remain a matter for speculation; one or more purine nucleotides might fulfil a role of this type because in acute experiments they can modulate the activity of both basal and stimulated activities of adenylate cyclase in islets (Howell & Montague, 1973) as well as in liver (Rodbell *et al.*, 1971) and thyroid (Wolff & Cook, 1973).

It is noteworthy that exactly comparable effects of starvation in lowering adenylate cyclase, and of glucose loading together with injection of secretin or pancreozymin on increasing adenylate cyclase, have been noted in exocrine pancreas (Rutten *et al.*, 1972). The effects of starvation or glucose loading on adenylate cyclase in rat islets are unlikely to be mediated solely via secretin or pancreozymin, since they can be observed after intravenous or intraperitoneal glucose injection. In addition, proteins and amino acids administered orally, which should provide an effective stimulus to secretion of these hormones, are ineffective in reversing the refractory response of the β-cell to glucose in the starved rat (Turner & Young, 1973).

Adenylate cyclase activity of the β-cell *in vivo* may be regulated by a range of hormonal and neural factors, only some of which have so far been identified. The results reported here suggest that glucose may exert long-term regulatory effects on β-cell adenylate cyclase activity. These effects may play a role in maintaining cyclic AMP concentrations at the values

required for optimal effectiveness of carbohydrate-stimulated insulin release over long periods in various physiological and nutritional states.

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References

Andersson, A. & Hellerström, C. (1972) *Diabetes* **21** *Suppl.* **2**, 546–554

Ashcroft, S. J. H., Randle, P. J. & Taljedal, I.-B. (1972) *FEBS Lett.* **20**, 263–266

Asplund, K. (1970) in *The Structure and Metabolism of the Pancreatic Islets* (Falkmer, S., Hellman, B. & Taljedal, I.-B., eds.), pp. 477–484, Pergamon Press, Oxford

Atkins, T. & Matty, A. J. (1971) *J. Endocrinol.* **51**, 67–78

Braunsberg, H. & Guyver, A. (1965) *Anal. Biochem.* **10**, 86–95

Cahill, G. F., Herrera, M. G., Morgan, A. P., Soeldner, J. S., Steinke, J., Levy, P. L., Reichard, G. N. & Kipnis, D. M. (1966) *J. Clin. Invest.* **45**, 1751–1769

Cerasi, E. & Luft, R. (1969) *Horm. Metab. Res.* **1**, 162–168

Cerasi, E. & Luft, R. (1970) in *Pathogenesis of Diabetes mellitus* (Cerasi, E. & Luft, R., eds.), pp. 17–40, Almqvist and Wicksell, Stockholm

Cerasi, E. & Luft, R. (1972) *Diabetes* **21**, *Suppl.* **2**, 685–694

Charles, M. A., Fanska, R., Schmid, F. G., Forsham, P. H. & Grodsky, G. M. (1973) *Science* **179**, 569–571

Davis, B. & Lazarus, N. R. (1972) *Biochem. J.* **129**, 373–379

Eagle, H. (1959) *Science* **120**, 432–434

Erlandsen, S. L., Wells, L. O. & Lazarow, A. (1968) *Metab. Clin. Exp.* **17**, 638–643

Gey, G. O. & Gey, M. K. (1936) *Amer. J. Cancer* **27**, 45–76

Goldfine, I. D., Roth, J. & Birnbaumer, L. (1972) *J. Biol. Chem.* **247**, 1211–1218

Green, I. C. & Taylor, K. W. (1972) *J. Endocrinol.* **54**, 317–325

Grey, N. J., Goldring, S. & Kipnis, D. M. (1970*a*) *J. Clin. Invest.* **49**, 881–889

Grey, N. J., Goldring, S. & Kipnis, D. M. (1970*b*) in *Pathogenesis of Diabetes mellitus* (Cerasi, E. & Luft, R., eds.), pp. 155–172, Almqvist and Wicksell, Stockholm

Hellerström, C., Howell, S. L., Edwards, J. C. & Andersson, A. (1972) *FEBS Lett.* **27**, 97–101

Howell, S. L. & Montague, W. (1973) *Biochim. Biophys. Acta* **320**, 44–52

Howell, S. L. & Taylor, K. W. (1966) *Biochim. Biophys. Acta* **130**, 519–521

Kipnis, D. M. (1970) *Acta Diabetolog. Lat.* **7**, *Suppl.* **1**, 314–334

Kipnis, D. M. (1972) *Diabetes* **21**, *Suppl.* **2**, 606–616

Lambert, A. E., Junod, A., Stauffacher, W., Jeanrenaud, B. & Renold, A. E. (1969) *Biochim. Biophys. Acta* **184**, 529–539

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Malaisse, W. J. (1972) in *Handbook of Physiology* (Steiner, D. F. & Freinkel, N., eds.), section 7, vol. 1, pp. 237–260, American Physiological Society

Malaisse, W. J., Malaisse-Lagae, F. & Wright, P. H. (1967*a*) *Amer. J. Physiol.* **213**, 843–848

Malaisse, W. J., Malaisse-Lagae, F. & Mayhew, D. (1967*b*) *J. Clin. Invest.* **46**, 1724–1734

Matschinsky, F. M. (1972) In *Handbook of Physiology* (Steiner, D. F. & Freinkel, N., eds.), section 7, vol. 1, pp. 199–214, American Physiological Society

Miller, E. A., Wright, P. H. & Allen, D. O. (1972) *Endocrinology* **91**, 1117–1119

Montague, W. & Cook, J. R. (1971) *Biochem. J.* **122**, 115–120

Montague, W. & Howell, S. L. (1972) *Biochem. J.* **129**, 551–560

Ramachandran, J. (1971) *Anal. Biochem.* **43**, 227–239

Rodbell, M., Birnbaumer, L., Pohl, S. L. & Krans, M. J. (1971) *J. Biol. Chem.* **246**, 1877–1882

Rutten, W. J., de Pont, J. J. H. M. & Bonting, S. L. (1972) *Biochim. Biophys. Acta* **274**, 201–213

Sams, D. J. & Montague, W. (1972) *Biochem. J.* **129**, 945–952

Selawry, H., Voyles, N., Gutman, R., Wade, A., Fink, G. & Recant, L. (1972) *Diabetes* **21**, 329

Selawry, H., Gutman, R., Fink, G. & Recant, L. (1973) *Biochem. Biophys. Res. Commun.* **51**, 198–204

Steiner, D. F. (1972) *Diabetes* **21**, *Suppl.* **2**, 571

Turner, D. S. & Young, D. A. B. (1973) *Acta Endocrinol.* **72**, 46–53

Turtle, J. R. & Kipnis, D. M. (1967) *Biochem. Biophys. Res. Commun.* **28**, 797–802

Vance, J. E., Buchanan, K. D. & Williams, R. H. (1968) *J. Lab. Clin. Med.* **72**, 290–297

Wolff, J. & Cook, G. H. (1973) *J. Biol. Chem.* **248**, 350–355