# ACTIVATION BY GLUCOSE OF ADENYL CYCLASE IN PANCREATIC ISLETS OF THE RAT

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Received 3 May 1973

## 1. Introduction

It is established that the adenyl cyclase–cyclic 3′,5′-monophosphate (cyclic AMP) system plays an important role in the release of hormones from a number of endocrine glands [1–3]. Indirect evidence has accumulated during recent years, indicating that cyclic AMP is important also for the release of insulin from the pancreatic beta cells [4, 5], although the precise manner in which this nucleotide participates in insulin secretion is obscure.

On the basis of *in vivo* studies on insulin secretion in man, we postulated that glucose acts on the beta cell by stimulating the formation of cyclic AMP which, in its turn, initiates the release of the hormone [6]. The present experiments give direct evidence that glucose stimulates adenyl cyclase in isolated islets of Langerhans.

## 2. Materials and methods

Male Sprague-Dawley rats, weighing 200–250 g were fed ad libitum until decapitation. The pancreases of 8 rats were quickly removed, trimmed free from fat, minced with scissors and incubated in Krebs–Henseleit bicarbonate buffer (KHB) containing 0.6 mg/ml of glucose, 20 mg/ml of bovine albumin, and 4–6 mg/ml of collagenase during 5–10 min with vigorous shaking. After complete digestion of the exocrine tissue, the islets were centrifuged and the pellet obtained was suspended in 4 ml of KHB containing 25% Ficoll above which 2 ml each of 23, 20.5 and 11% Ficoll were layered. After centrifugation at 800 *g* for 20 min the islets were removed, washed twice and transferred to a tube containing 10 ml of KHB with 2 mg/ml of bovine albumin, 0.6 mg/ml of glucose and 100 $\mu$Ci/ml of [$^3$H]adenine. The islets were incubated for 60 min at 37°C under continuous gasing with $CO_2$:$O_2$ (5:95), washed 4 times, and collected with a finely drawn Pasteur pipette under a stereomicroscope. Groups of 25 islets were transferred to test tubes containing KHB with 2 mg/ml of bovine albumin, the incubations being started after addition of the substances to be tested (final volume 1.0 ml). The incubation was stopped by boiling for 3 min after 200 $\mu$g of cyclic AMP had been added to each tube for later determination of recovery by $A_{260}$ measurement. The contents were subsequently subjected to sonification for 15 sec and kept at −20°C until the purification procedure was started.

The separation of [$^3$H]cyclic AMP from other radioactive substances was performed by ion-exchange chromatography and barium sulfate precipitation as described by Krishna [7]. The eventual presence of radioactive impurities were checked by subjecting incubates to i) paper chromatography followed by ii) phosphodiesterase treatment and iii) again paper chromatography, whereafter the spot coinciding with 5′-AMP was eluted and counted for radioactivity as described [8]. This method of purification yielded identical results as compared to the above described procedure routinely used.

In experiments where insulin was measured, islets were isolated and preincubated as above (except for the omission of adenine) and then transferred in groups of 10 to test tubes containing 1.0 ml of KHB with agents to be tested. Samples were withdrawn and assayed for insulin content using a double-antibody radioimmunoassay [9] with rat insulin as standard.

Crude collagenase was purchased from Worthington Co., bovine albumin (fraction V) from Armour Co., [$^3$H]adenine (specific activity 3.3 $\mu$Ci/mM) from NEN Chemicals, and insulin reagent kits from the Radiochemical Centre, Amersham. 3-Isobutyl-1-methylxanthine (IBMX) and rat insulin were generous gifts of Mr. Roger L. Bergstrom, Searle Co. and Dr. J. Schlichtkrull, Novo Research Institute, respectively.

## 3. Results

Fig. 1 presents the changes induced by glucose on radioactive cyclic AMP accumulation in the islets of Langerhans. In the absence of the phosphodiesterase inhibitor IBMX, increasing the glucose concentration of the medium from 0.6 to 5.0 mg/ml elicited a 77% increase in [$^3$H]cyclic AMP accumulation ($p < 0.05$). The addition of IBMX in concentrations up to 0.1 mM had no significant effect on the basal (0.6 mg/ml) [$^3$H]cyclic AMP levels, while the same concentration of the phosphodiesterase inhibitor markedly magnified the stimulation induced by high glucose (stimulation by 154 and 378% for 0.01 and 0.1 mM IBMX, respectively). When the concentration of IBMX was increased to 1.0 mM, the [$^3$H]cyclic AMP accumulation in the low glucose medium was raised by 179%. This level was further increased when the glucose concentration was raised to 5.0 mg/ml (145% over 0.6 mg/ml plus 1.0 mM IBMX).

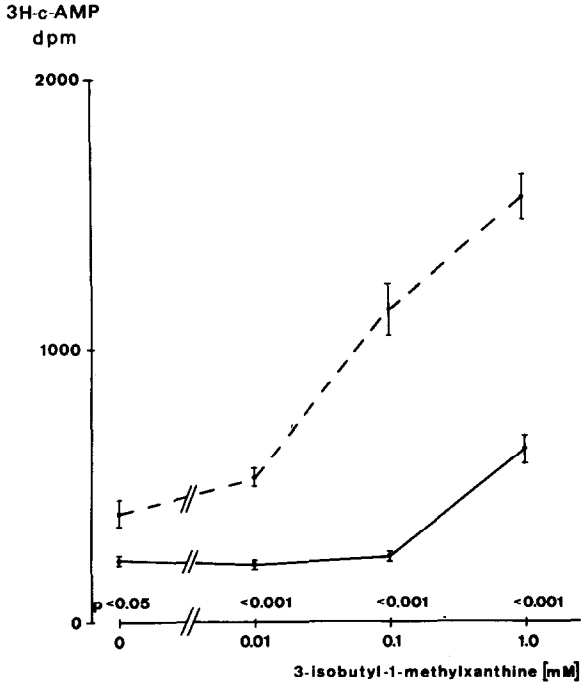


Fig. 1. Changes in [$^3$H]cAMP accumulation elicited by low and high glucose concentrations in the presence or absence of 3-isobutyl-1-methylxanthine (IBMX). Islets preincubated with [$^3$H]adenine were incubated for 3 min in groups of 25 in media containing glucose 0.6 mg/ml (——) or 5.0 mg/ml (– – –) in the presence or absence of 0.01–1.0 mM IBMX. Results are expressed as the mean ± S.E.M. of duplicate incubations from six complete experiments.

The effect of 0.1 mM IBMX on the insulin release induced by 0.6 or 5.0 mg/ml glucose was tested at 10 and 30 min of incubation. As shown in table 1, insulin secretion in the presence of IBMX was stimulated by high glucose already at 10 min. IBMX enhanced the effect of high glucose significantly at 30 min while the xanthine derivative had no effect on insulin secretion in the presence of low glucose concentration.

## 4. Discussion

Glucose is the main physiologic releaser of insulin. The mechanisms by which glucose regulates the rate of secretion of the hormone from the islets of Langerhans are not known. Traditionally, the dominating concept has been that one or several metabolic products of glucose in the beta cell trigger the cell structures that control the extrusion of the insulin granules from the cell (for review see [10]). However, we [6] and others [11] have recently questioned this concept. On the basis of kinetic studies on insulin secretion in man, and its modification by agents known to interfere with the intracellular metabolism of cyclic AMP, we proposed that in the beta cell two distinct functions of glucose should be considered: glucose as a substrate, and glucose as a stimulator of a membrane receptor. The latter would initiate a signal which induces rapid release of insulin. We also stated that the available experimental evidence suggested that this signal of glucose is mediated in the cell by stimulation of the formation of cyclic AMP [6]. This hypothesis has been criticized since several investigators have been unable to demonstrate any stimulatory effect of glucose on the cyclic AMP content of isolated is-

Table 1
Cumulative insulin release from isolated islets after 10 and 30 min of duplicate or triplicate incubations (μU insulin/islet; mean ± S.E.M. of 7 complete experiments).

| | Time of incubation (min) | | | | | |
|---|---|---|---|---|---|---|
| | 10 | | | 30 | | |
| | 3-Isobutyl-1-methylxanthine | | *p* | 3-Isobutyl-1-methylxanthine | | *p* |
| | – | + | | – | + | |
| Glucose | | | | | | |
| (0.6 mg/ml) | 14.2 ± 2.6 | 12.0 ± 1.8 | N.S. | 21.8 ± 3.4 | 25.9 ± 3.8 | N.S. |
| (5.0 mg/ml) | 19.5 ± 6.9 | 26.1 ± 6.2 | N.S. | 112.0 ± 20.7 | 204.2 ± 27.9 | <0.01 |
| *P* | N.S. | <0.05 | | <0.0025 | <0.001 | |

*p* Values on the vertical columns refer to significance of difference of experiments with and without IBMX (0.1 mM), on the horizontal columns to that of low and high glucose.

lets of the rat [12, 13], or on the adenyl cyclcase activity of membrane fractions from various types of islet tissue [14, 15].

The methods used in the present investigation were selected for the following reasons: it was judged necessary to work with intact islets, since the fate of the glucose receptor in fractionated cells is unknown; incorporation of [$^3$H]adenine into cyclic AMP (over ATP) provides a method of measuring changes in the nucleotide pool in response to stimulation, whereas measurement of total cyclic AMP would also include the "inert" pool of the second messenger [16].

The findings presented in this report demonstrate unequivocally that glucose does indeed influence the cyclic AMP accumulation in the islets of Langerhans. Such an effect was seen in the absence of other agents and was potentiated by the addition of the phosphodiesterase inhibitor IBMX in concentrations that, by themselves, had no apparent infuence on the accumulation of [$^3$H]cyclic AMP in low glucose medium. These results, in our opinion, indicate that glucose acts as a stimulator of the adenyl cyclase of the islets of Langerhans. An inhibitory effect of glucose on the phosphodiesterase enzymes seems less likely, since the glucose effect was most apparent when a potent inhibitor of phosphodiesterase was present. The rapidity of the stimulation (3 min) should speak against the possibility that glucose, by some unknown mechanism, increases the specific radioactivity of the ATP pool in the islet cells, thus imitating a real stimulation of the cyclase.

During the preparation of this work Charles and co-workers [17] presented data from perfused islets, showing that glucose increased the cyclic AMP content of the tissue, but that this effect was modest compared to the effect of theophylline (another xanthine derivative) alone. In our experiments, however, there was a preponderant effect of high glucose on [$^3$H]cyclic AMP when combined with IBMX; actually the phosphodiesterase inhibitor, in moderate doses seemed to induce only potentiation of the glucose effect.

There seems to be a certain degree of parallelism between the cyclic AMP and the insulin data in the present report, albeit with differences in the time scale. Incubation conditions which did not increase the cyclic AMP accumulation had no stimulatory effect on insulin release, while the highest hormone production rate was measured when high glucose was combined with IBMX. However, the present experimental design does not permit a detailed analysis of the cyclic AMP–insulin interrelation.

In summary, the results obtained in the present study strongly suggest that the primary action of glucose on the islet cells is mediated through stimulation of adenyl cyclase.

**Acknowledgements**

We gratefully acknowledge the excellent technical assistance offered by Miss Inga-Lena Wivall. Financial

support was obtained from the Swedish Medical Research Council (grant no 19X-34-08A), the Nordic Insulin Foundation and the Swedish Diabetes Association.

## References

[1] D.G. Grahame-Smith, R.W. Butcher, R.L. Ney and E.W. Sutherland, J. Biol. Chem. 242 (1967) 5535.
[2] A.G. Gilman and T.W. Rall, J. Biol. Chem. 243 (1968) 5867.
[3] A.L. Steiner, G.T. Peake, R.D. Utiger, I.E. Karl and D.M. Kipnis, Endocrinology 86 (1970) 1354.
[4] J.R. Turtle and D.M. Kipnis, Nature 213 (1967) 727.
[5] K.E. Sussman and G.D. Vaughan, Diabetes 16 (1967) 449.
[6] E. Cerasi and R. Luft, Horm. Metab. Res. 2 (1970) 246.
[7] G. Krishna, B. Weiss and B.B. Brodie, J. Pharmacol. Exptl. Ther. 163 (1968) 379.
[8] J.-B. Osnes, T. Christoffersen, J. Mørland and I. Øye, J. Chromatog. 67 (1972) 139.
[9] C.N. Hales and P.J. Randle, Biochem. J. 88 (1963) 137.
[10] D.A. Mayhew, P.H. Wright and J. Ashmore, Pharmacol. Rev. 21 (1969) 183.
[11] F.M. Matschinsky, R. Landgraf, J. Ellerman and J. Kotler-Brajtburg, Diabetes 21 Suppl. 2 (1972) 1555.
[12] W. Montague and J.R. Cook, Biochem. J. 122 (1971) 115.
[13] D.M. Kipnis, Acta Diabet. Lat. 7 Suppl. 1 (1970) 314.
[14] T. Atkins and A.J. Matty, J. Endocrinol. 51 (1971) 67–78.
[15] B. Davis and N.R. Lazarus, Biochem. J. 129 (1972) 373.
[16] J.L. Humes, M. Rounbehler and F.A. Kuehl, Anal. Biochem. 32 (1969) 210.
[17] M.A. Charles, R. Fanska, F.G. Schmid, P.H. Forsham and G.M. Grodsky, Science 179 (1973) 569.