Regulation of insulin secretion by cAMP in rat islets of Langerhans permeabilised by high-voltage discharge

Peter M. Jones, Julie M. Fyles and Simon L. Howell

Department of Physiology, King's College London (KQC), Campden Hill Road, Kensington, London W8 7AH, England

Received 16 July 1986

Adenosine 3',5-cyclic monophosphate (cAMP) was shown to stimulate insulin secretion from electrically permeabilised islets of Langerhans incubated in Ca^{2+}/EGTA buffers. cAMP-induced insulin secretion occurred in the presence of either sub-stimulatory (50 nM) or stimulatory (> 100 nM) concentrations of Ca^{2+}. Similar effects on secretion were obtained in response to 8-bromo-cAMP (8-Br-cAMP) or the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine. Forskolin (0.2–20 μM) increased adenylate cyclase activity and enhanced insulin secretion from the permeabilised islets. These results suggest that, in electrically permeabilised islets, cAMP-induced insulin secretion is not dependent on changes in cytosolic Ca^{2+}.

Insulin secretion (Islets of Langerhans) Electrical permeabilization cyclic AMP Forskolin Isobutylmethylxanthine

1. INTRODUCTION

There is considerable evidence that the intracellular concentration of cAMP is involved in the regulation of insulin secretion from pancreatic B-cells [1,2]. B-cells possess plasma membrane-associated adenylate cyclase [3–5] and cAMP-dependent protein kinases [6,7], through which it is assumed cAMP exerts its intracellular actions. Numerous studies have shown that insulin release is enhanced in the presence of analogues of cAMP, or by increasing intracellular cAMP using agents which activate adenylate cyclase or inhibit phosphodiesterases ([1,2,8] for references). Several hormones and neurotransmitters which affect insulin secretion are thought to act by receptor-mediated alteration of adenylate cyclase activity [3,5], and glucose or other metabolic secretagogues can elevate intracellular cAMP concentrations [2,9], perhaps as a response to increases in cytosolic Ca^{2+} [2,8,9].

We have now studied the effects of changes in cAMP concentration on insulin secretion from islets of Langerhans which have been permeabilised by exposure to a high-intensity electrical field [10]. These electrically permeabilised islets respond to increases in Ca^{2+} concentration over the range 10^{-7}–10^{-5} M with dose-related increases in insulin secretion [11]. Since low-M_r solutes can be introduced directly into the cytosolic compartment, and the intracellular Ca^{2+} can be clamped at predetermined concentrations, permeabilised islets offer a useful model in which to study the relationships between Ca^{2+} and other intracellular mediators of insulin secretion.

2. MATERIALS AND METHODS

Islets of Langerhans were isolated from rat pancreas by collagenase digestion [12] and incubated for 60 min at 37°C in a bicarbonate-buffered physiological salt solution [13] containing 2 mM glucose, 2 mM $CaCl_2$ and 0.5 mg/ml bovine serum albumen (BSA, fraction V, Sigma). Isolated islets were permeabilised by exposure to a high-intensity electric field, as described [11]. Briefly, the islets

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

were thoroughly washed in a Ca^{2+}/EGTA buffer (permeation buffer) containing 140 mM K-glutamate, 15 mM Hepes, 7 mM $MgSO_4$, 5 mM adenosine 5'-triphosphate (ATP), 0.5 mg/ml BSA, 1–5 mM EGTA, pH 6.6, with $CaCl_2$ added to produce a Ca^{2+} concentration of 50 nM, and permeabilised by 5 exposures to an electric field of 3.4 kV/cm. Groups of 10 permeabilised islets were incubated at 37°C for 30 min in 1.0 ml of permeation buffer of various Ca^{2+} concentrations containing the test substances of interest. 3-Isobutyl-1-methylxanthine (IBMX, Sigma) was dissolved in dimethyl sulphoxide; forskolin (Sigma) was dissolved in 95% ethanol. Controls received the appropriate concentration of vehicle alone. Insulin secretion by the permeabilised islets was measured by radioimmunoassay [14]. Adenylate cyclase activity in homogenates of permeabilised islets was assessed by measuring the formation of cyclic [α-^{32}P]AMP from [α-^{32}P]ATP, as described [3]. The cyclic [α-^{32}P]AMP was separated on neutral alumina columns [3], and radioactivity measured by liquid scintillation spectroscopy. Differences between means were assessed by analysis of variance or Student's unpaired t-test, as appropriate.

3. RESULTS

In the experiments shown in fig.1, increasing the Ca^{2+} concentration of the incubation buffer from 50 nM to 10 μM produced a 2.4-fold increase in insulin secretion by electrically permeabilised islets in the absence of cAMP. Addition of cAMP to the incubation medium produced dose-related increases in insulin secretion in the presence of both sub-stimulatory (50 nM) or stimulatory (>100 nM) concentrations of Ca^{2+} (fig.1). Although the absolute amount of cAMP-stimulated insulin secretion was greater at higher concentrations of Ca^{2+}, the magnitude of the cAMP stimulation (i.e. % increase over control) was very similar over a wide range of Ca^{2+} concentrations. For example, 100 μM cAMP evoked a stimulation of 188 + 14, 202 + 21 and 211 + 14% at 50 nM, 1 μM and 10 μM Ca^{2+}, respectively (mean ± SE, n = 6–8). The metabolically stable analogue 8-Br-cAMP had similar, but more marked, effects on insulin secretion by the permeabilised islets.

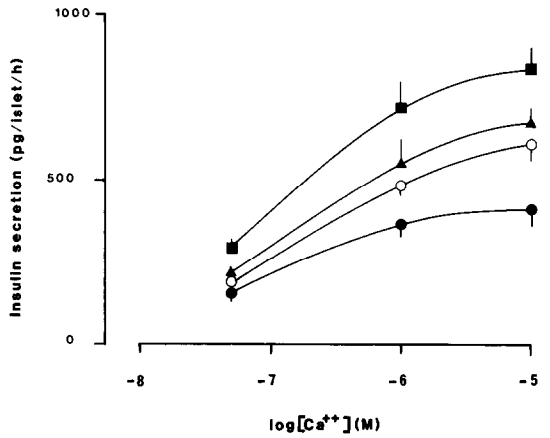

Fig.1. Stimulation of insulin secretion by cAMP in electrically permeabilised islets. The figure shows insulin secretion by permeabilised islets incubated in a buffer containing various concentrations of Ca^{2+} and cAMP. In the absence of cAMP (●) increasing concentrations of Ca^{2+} stimulate secretion. The presence of 10 μM (○), 50 μM (▲) or 100 μM (■) cAMP produced dose-related increases in insulin secretion at all concentrations of Ca^{2+}. Points show mean ± SE, n = 7.

Fig.2 shows the results of experiments in which permeabilised islets were incubated in buffers containing 10 μM Ca^{2+} in the presence of 10 and 100 μM cAMP or 8-Br-cAMP. In these experiments, 10 μM cAMP had no effect on insulin

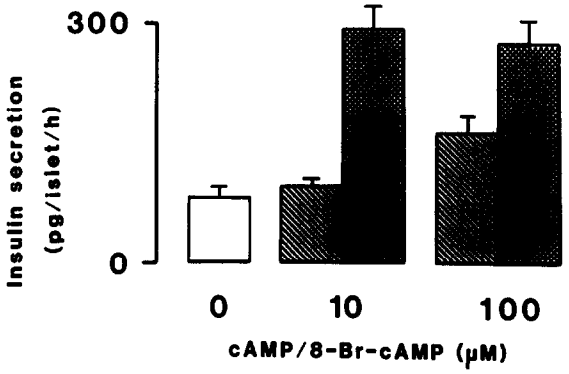

Fig.2. Effects of cAMP and 8-Br-cAMP on insulin secretion by permeabilised islets. cAMP (hatched bars) and 8-Br-cAMP (stippled bars) stimulated insulin secretion above that induced by 10 μM Ca^{2+} alone (open bar). 8-Br-cAMP produced a maximum effect at a concentration of 10 μM ($p < 0.05$), whereas 100 μM cAMP was required to enhance significantly secretion ($p < 0.05$). Bars show mean ± SE, n = 4.

release, but 100 μM cAMP produced a significant ($p < 0.05$) stimulation of secretion. 10 μM 8-Br-cAMP had a greater stimulatory effect than 100 μM cAMP, and this appeared to be a maximum response since no additional stimulation of secretion was observed in the presence of 100 μM 8-Br-cAMP.

Electrically permeabilised islets possessed a functional adenylate cyclase system. Thus, the adenylate cyclase activator forskolin (0.2–20 μM) produced dose-related increases in cAMP formation in homogenates of permeabilised islets, as shown in fig.3 (upper panel). In parallel experiments, the same concentrations of forskolin caused a significant stimulation of insulin secretion from electrically permeabilised islets (fig.3, lower panel). Insulin secretion by permeabilised islets was also stimulated by the phosphodiesterase inhibitor, IBMX. Fig.4 (upper panel) shows the significant ($p < 0.02$) stimulation of insulin secretion by IBMX (1 mM) at a range of Ca^{2+} concentrations. Note that IBMX stimulated insulin

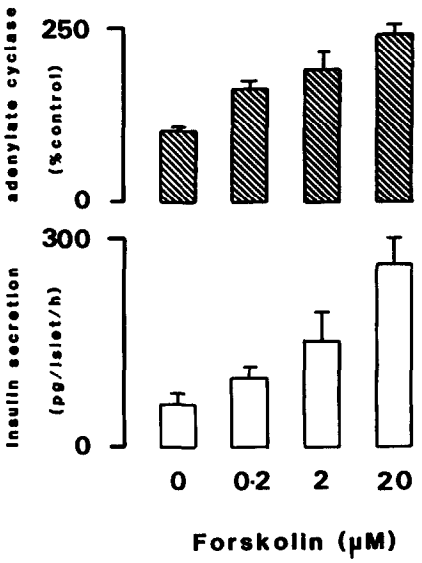

Fig.3. Effects of forskolin on adenylate cyclase and insulin secretion. Upper panel: forskolin (0.2–20 μM) produced dose-related increases in cAMP formation by homogenates of permeabilised islets, from a basal value of 12.6 ± 0.6 pmol cAMP/mg protein per 30 min (mean ± SE, $n = 3$). Lower panel: forskolin also stimulated insulin secretion by permeabilised islets incubated in a buffer containing 10 μM Ca^{2+} ($p < 0.05$, $n = 5$).

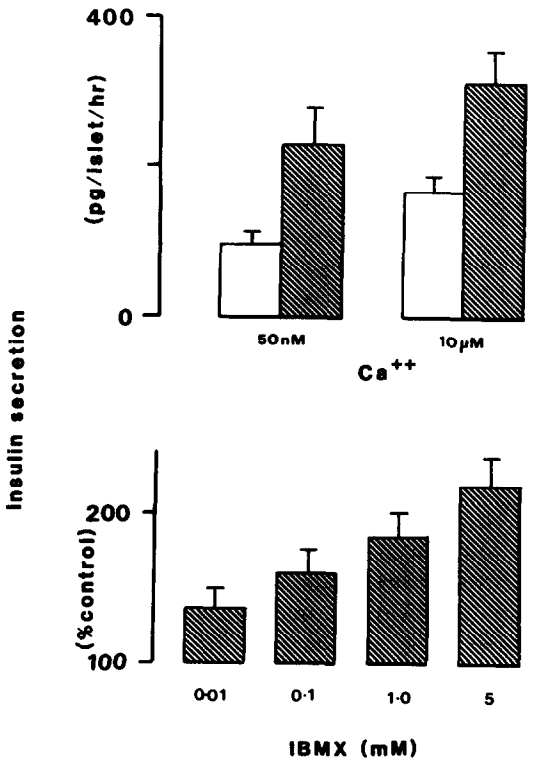

Fig.4. IBMX stimulation of insulin secretion by permeabilised islets. Upper panel: the addition of 1 mM IBMX (hatched bars) to the incubation buffers stimulated insulin secretion at both sub-stimulatory (50 nM) and stimulatory (10 μM) concentrations of Ca^{2+} (mean ± SE, $n = 5$). Lower panel: the effects of IBMX in the presence of 50 nM Ca^{2+} were dose-related. Values are expressed as a percentage of secretion in the absence of IBMX (mean ± SE, $n = 6$).

secretion at sub-stimulatory concentrations of Ca^{2+} (50 nM). The effects of IBMX were dose-related, with significant stimulation of secretion at concentrations of 0.1 mM and above (fig.4, lower panel, $p < 0.05$).

4. DISCUSSION

A number of studies have suggested that elevations in intracellular cAMP produce an increased secretion of insulin from B-cells [1,2], but there is still some uncertainty as to the importance of cAMP in the B-cell secretory response [1,2,8,9]. In general, the majority of available experimental evidence suggests that cAMP does not initiate in-

sulin secretion, but acts to modulate the secretory response to initiators of secretion such as glucose [2], perhaps by direct actions on Ca^{2+} fluxes into [15] or within [16] the B cell or, alternatively, by changing the sensitivity of the exocytotic mechanisms to Ca^{2+} [17–19].

In this study the use of electrically permeabilised islets ensured that intracellular Ca^{2+} could be fixed at known and pre-determined concentrations. Under these conditions, cAMP produced significant increases in insulin secretion by permeabilised islets at sub-stimulatory Ca^{2+} concentrations and increased the maximum secretory response to Ca^{2+}. In this respect the effects of cAMP on insulin secretion were similar to those previously reported for phorbol esters [11], which are thought to activate protein kinase C [20]. The concentrations of cAMP effective in promoting secretion from the permeabilised islets were somewhat higher than those measured in intact islets (unstimulated, approx. 1–10 μM [6,8,9]) but similar responses could be elicited using lower concentrations of metabolically stable analogues of cAMP, suggesting that intracellular phosphodiesterase activity was degrading the cAMP, thus limiting its effectiveness. Indeed, insulin secretion from the permeabilised islets was stimulated by the phosphodiesterase inhibitor, IBMX, presumably as a response to accumulation of endogenous cAMP within the permeabilised cells, although IBMX may affect insulin secretion by mechanisms other than inhibition of phosphodiesterase activity [15]. Increasing cAMP by using the adenylate cyclase activator forskolin also stimulated secretion from electrically permeabilised islets at similar concentrations to those which increase secretion from intact [18] and digitonin-permeabilised islets [21].

It is unlikely that these effects of cAMP on insulin secretion are a response to cAMP-induced fluxes of Ca^{2+} or other ions [15] across the B-cell plasma membrane, since in electrically permeabilised islets the intracellular and extracellular spaces are in ionic equilibrium. Similarly, the cAMP-induced secretion is unlikely to be caused by mobilisation of Ca^{2+} from intracellular stores [2,16], since changes in intracellular Ca^{2+} should be buffered by the chelator present in the medium, although the possibility of transient local changes in intracellular Ca^{2+} cannot be entirely ruled out. These observations do not preclude cAMP having effects on insulin secretion by altering Ca^{2+} handling in intact tissue, but they do suggest that at least part of the secretory response to cAMP is not dependent on changes in cytosolic Ca^{2+}. Similar conclusions were reached in studies using fluorescent indicators to measure cytosolic Ca^{2+} in islets from obese mice [22] and in insulin-secreting tumour cells [23], in which the secretory responses to dibutyryl cAMP [22], phosphodiesterase inhibitors [22], or forskolin [22,23] were not accompanied by measurable increases in cytosolic Ca^{2+}.

The effects of cAMP on insulin secretion by permeabilised islets could perhaps be explained by a sensitisation of the secretory mechanism to Ca^{2+}, thus promoting the exocytosis of insulin at otherwise sub-stimulatory concentrations of Ca^{2+}. Such an action of cAMP has previously been suggested from studies on secretion using intact [17–19] or digitonin permeabilised [21] islets, and from cytosolic Ca^{2+} measurements in intact tissues [22,23]. It is also possible that cAMP may affect insulin secretion by altering intracellular concentrations of second messengers other than Ca^{2+}. The inhibitory effects of cAMP on serotonin secretion from platelets are thought to reflect a decreased availability of 1,2-diacylglycerol (DAG) [24], presumably by inhibition of phospholipase C [24,25]. The possibility that cAMP affects DAG production in islets cannot at present be excluded. Further studies are required to identify the precise mechanisms through which cAMP stimulates insulin secretion, and to determine the relationships between cAMP and other intracellular regulators of exocytosis in the B cell.

ACKNOWLEDGEMENTS

Financial assistance from the Medical Research Council and the British Diabetic Association is gratefully acknowledged.

REFERENCES

[1] Sharp, G.W. (1979) Diabetologia 16, 287–296.
[2] Malaisse, W.J. and Malaisse-Lagae, F. (1984) Experientia 40, 1068–1075.
[3] Howell, S.L. and Montague, W. (1973) Biochim. Biophys. Acta 320, 44–52.

[4] Kuo, W.-N., Hodkins, D.S. and Kuo, J.F. (1973) J. Biol. Chem. 248, 2705–2711.
[5] Schuit, F.C. and Pipeleers, D.G. (1985) Endocrinology 117, 834–840.
[6] Montague, W. and Howell, S.L. (1973) Biochem. J. 134, 321–327.
[7] Harrison, D.E., Ashcroft, S.J.H., Christie, M.R. and Lord, J.M. (1984) Experientia 40, 1075–1084.
[8] Woolheim, C.B. and Sharp, G.W. (1981) Physiol. Rev. 61, 914–973.
[9] Hedeskov, C.J. (1980) Physiol. Rev. 60, 442–509.
[10] Knight, D.E. and Scrutton, M.C. (1986) Biochem. J. 234, 497–506.
[11] Jones, P.M., Stutchfield, J. and Howell, S.L. (1985) FEBS Lett. 191, 102–106.
[12] Howell, S.L. and Taylor, K.W. (1968) Biochem. J. 108, 17–24.
[13] Gey, G.O. and Gey, M.L. (1936) Am. J. Cancer 27, 45–76.
[14] Yaseen, M.A., Pedley, K.C. and Howell, S.L. (1982) Biochem. J. 206, 81–87.
[15] Henquin, J.C. (1985) Arch. Int. Physiol. Biochim. 93, 37–48.
[16] Brisson, G.R., Malaisse-Lagae, F. and Malaisse, W. (1972) J. Clin. Invest. 51, 232–241.
[17] Hellman, B. (1975) Endocrinology 97, 392–398.
[18] Malaisse, W.J., Garcia-Morales, P., Dufrane, S.P., Sener, A. and Valverde, I. (1984) Endocrinology 115, 2015–2020.
[19] Christie, M.R. and Ashcroft, S.J.H. (1985) Diabetes Res. Clin. Practice Suppl.1, S102.
[20] Castanaga, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, A. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847–7851.
[21] Tamagawa, T., Niki, H. and Niki, A. (1985) FEBS Lett. 183, 430–432.
[22] Rorsman, P. and Abrahamsson, H. (1985) Acta Physiol. Scand. 125, 639–647.
[23] Wollheim, C.B., Ullrich, S. and Pozzan, T. (1984) FEBS Lett. 177, 17–22.
[24] Rittenhouse-Simmons, S. (1979) J. Clin. Invest. 63, 580–587.
[25] Knight, D.E. and Scrutton, M.C. (1984) Nature 309, 66–68.