Volume 219, number 1, 139–144 FEB 04904 July 1987

Catecholamine inhibition of Ca^{2+}-induced insulin secretion from electrically permeabilised islets of Langerhans

Peter M. Jones, Julie M. Fyles, Shanta J. Persaud and Simon L. Howell

MRC Secretory Mechanisms Group, Department of Physiology, King's College London, Campden Hill Road, Kensington, London W8 7AH, England

Received 18 May 1987

Noradrenaline (1–10 μM) inhibited Ca^{2+}-induced insulin secretion from electrically permeabilised islets of Langerhans with an efficacy similar to that for inhibition of glucose-induced insulin secretion from intact islets. The inhibition of insulin secretion from permeabilised islets was blocked by the α_2-adrenoreceptor antagonist, yohimbine. Adenosine 3',5'-cyclic monophosphate (cAMP) did not relieve the noradrenaline inhibition of Ca^{2+}-induced secretion from the permeabilised islets, although noradrenaline did not affect the secretory responses to cAMP at substimulatory (50 nM) concentrations of Ca^{2+}. These results suggest that catecholamines do not inhibit insulin secretion solely by reducing B-cell adenylate cyclase activity, and imply that one site of action of noradrenaline is at a late stage in the secretory process.

Insulin secretion; Electrical permeabilisation; Noradrenaline; cyclic AMP; (Islets of Langerhans)

1. INTRODUCTION

Catecholamines exert a profound inhibition of insulin secretion in vivo, from perfused pancreas in vitro and from isolated islets of Langerhans, suggesting that circulating levels of the hormone adrenaline or the release of noradrenaline from sympathetic nerve terminals may play an important role in the physiological regulation of insulin secretion (reviews [1,2]).

A number of mechanisms by which catecholamines could inhibit insulin secretion have been proposed, including effects on Ca^{2+} handling [3,4], modulation of prostaglandin synthesis [5], and regulation of intracellular concentrations of cAMP [6,7]. The intracellular mechanisms of the inhibition are still unclear, but the ability of catecholamines to inhibit insulin release in response to a wide variety of secretagogues suggests an action at an important point in the secretory pathway.

We have studied the involvement of Ca^{2+} and cAMP in the catecholamine inhibition of insulin secretion by comparing the effects of noradrenaline on insulin secretion from intact islets of Langerhans and from islets in which the cells have been permeabilised by high-voltage discharge to allow the introduction of ions and small molecules into the intracellular compartment. Electrically permeabilised islets do not respond to glucose [8] but secrete insulin in response to Ca^{2+} [9] or cAMP [10], and thus offer a useful model in which to study the involvement of these intracellular mediators in the control of insulin secretion.

2. MATERIALS AND METHODS

Islets of Langerhans were isolated from rat pancreas by collagenase digestion [11] and incubated for 60 min at 37°C in a bicarbonate-buffered (pH

Correspondence address: P.M. Jones, MRC Secretory Mechanisms Group, Department of Physiology, King's College London, Campden Hill Road, Kensington, London W8 7AH, England

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

7.4) physiological salt solution [12] containing 2 mM glucose, 2 mM $CaCl_2$ and 0.5 mg/ml bovine serum albumin (BSA, fraction V). In experiments using intact islets, groups of three islets were incubated for 60 min at 37°C in 0.6 ml of the physiological salt solution containing the test substances of interest. In other experiments, islets were permeabilised by exposure to a high-intensity electric field [9]. Briefly, the islets 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 permeation buffer of various Ca^{2+} concentrations containing the test substances of interest. Insulin secretion from intact and permeabilised islets was measured by radioimmunoassay as described [13]. ATP, BSA, noradrenaline, yohimbine, cAMP and 2′-*O*-dibutyryl adenosine 3′:5′-cyclic monophosphate (db-cAMP) were obtained from Sigma (Poole, England). Ascorbic acid (100 μM) was included in all incubations to inhibit the oxidation of noradrenaline. Differences between means were assessed by analysis of variance or Student's unpaired *t*-test, as appropriate.

3. RESULTS

Noradrenaline caused a marked inhibition of glucose-stimulated insulin secretion from intact islets, and of Ca^{2+}-induced insulin secretion from electrically permeabilised islets. Fig.1 shows the effects of noradrenaline (10 μM) on insulin secretion from intact islets in response to glucose (upper panel) and from permeabilised islets in response to Ca^{2+} (lower panel). Noradrenaline had no significant effects on the basal rate of insulin secretion by intact islets in 2 mM glucose, or on that of permeabilised islets in the presence of 50 nM Ca^{2+}. However, the presence of 10 μM noradrenaline totally abolished the 10-fold increase in insulin secretion by intact islets in response to 20 mM glucose (fig.1, upper panel) and the secretory response of permeabilised islets to 10 μM Ca^{2+} (fig.1, lower panel).

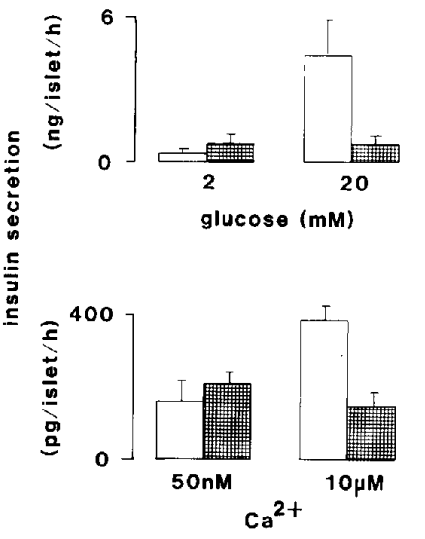

Fig.1. Noradrenaline inhibition of insulin secretion. Noradrenaline (10 μM, hatched bars) totally inhibited glucose-induced (20 mM) insulin secretion from intact islets (upper panel) and Ca^{2+}-induced (10 μM) insulin secretion from electrically permeabilised islets (lower panel). Bars show mean ± SE, n = 6–8.

Inhibition of insulin secretion by noradrenaline was dose-related, as shown in fig.2. In permeabilised islets, 50% inhibition of the maximum Ca^{2+}-induced response was observed at a noradrenaline concentration of approx. 2.5 μM, while maximum inhibition of secretion was produced by 10 μM noradrenaline (fig.2, upper panel). Inhibition of glucose-induced insulin secretion from intact islets showed a similar dose-response relationship, with 50% inhibition of secretion at around 2 μM noradrenaline and total inhibition of glucose-induced insulin secretion at noradrenaline concentrations of 5–10 μM (fig.2, lower panel).

Noradrenaline inhibition of insulin secretion was antagonised by the α_2-adrenoreceptor antagonist yohimbine. In intact islets, 10 μM yohimbine completely blocked the inhibition of glucose-induced insulin secretion by noradrenaline (20 mM glucose, 4.3 ± 0.3 ng/islet per h; + 10 μM noradrenaline, 0.5 ± 0.1; + 10 μM noradrenaline + 10 μM yohimbine, 3.8 ± 0.4, mean ± SE, n = 5). Similarly, in experiments using permeabilised islets 10 μM Ca^{2+} stimulated insulin secretion by 254 ± 33% (n = 9, $p < 0.01$), noradrenaline

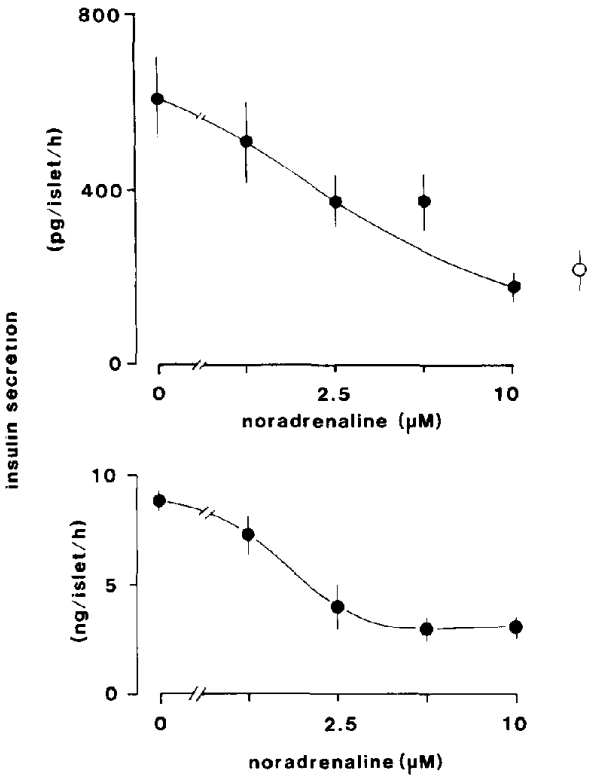

Fig.2. Dose responsiveness of noradrenaline inhibition of insulin secretion. (Upper panel) Increasing concentrations of noradrenaline caused a dose-related inhibition of insulin secretion from electrically permeabilised islets incubated in the presence of 10 μM Ca^{2+}. The open point shows basal secretion in the presence of 50 nM Ca^{2+} (mean ± SE, n = 7). (Lower panel) Noradrenaline inhibition of insulin secretion from intact islets incubated in the presence of 20 mM glucose showed a similar dose responsiveness (mean ± SE, n = 6).

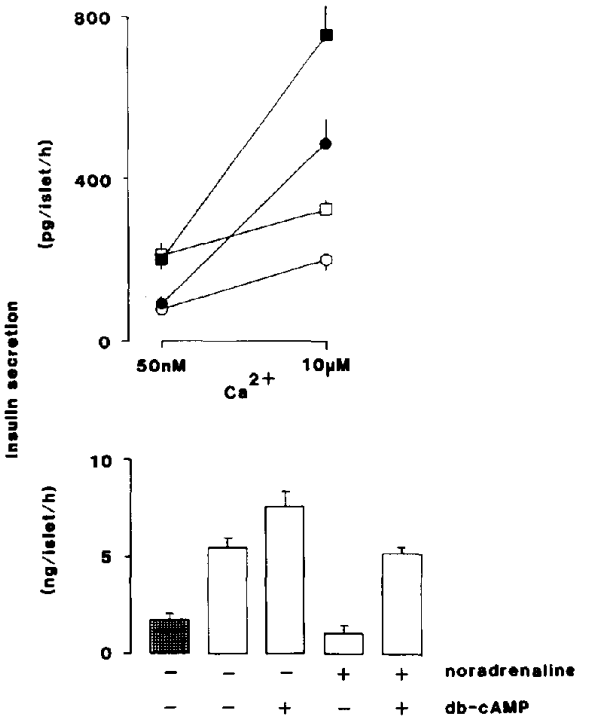

Fig.3. Effects of cAMP on noradrenaline inhibition of insulin secretion. (Upper panel) Ca^{2+}-induced insulin secretion from electrically permeabilised islets (●) was significantly ($p < 0.01$) enhanced by the presence of 100 μM cAMP (■). Noradrenaline (10 μM) markedly inhibited insulin secretion in response to Ca^{2+} alone (○). In the presence of 100 μM cAMP (□) noradrenaline had no effect on insulin secretion at sub-stimulatory Ca^{2+} concentration (50 nM), but significantly ($p < 0.01$) inhibited secretion in the presence of 10 μM Ca^{2+} (mean ± SE, n = 9). (Lower panel) Glucose-induced insulin secretion from intact islets (20 mM, open bars) was enhanced by 5 mM db-cAMP, and totally inhibited by 10 μM noradrenaline. Noradrenaline also caused a significant reduction ($p < 0.05$) of the secretory response to db-cAMP plus glucose, but there was still a significant ($p < 0.01$) stimulation of secretion above basal rates in the presence of 2 mM glucose (hatched bar, mean ± SE, n = 4–6).

(10 μM) totally inhibited Ca^{2+}-induced secretion (71 ± 8% basal, n = 9, N.S.) and this inhibition was abolished by the inclusion of 10 μM yohimbine in the incubation buffer (270 ± 27% basal, n = 9, $p < 0.01$).

The effects of cAMP on insulin secretion from permeabilised islets are shown in fig.3 (upper panel). cAMP stimulated insulin secretion from electrically permeabilised islets at both sub-stimulatory (50 nM) and stimulatory (10 μM) concentrations of Ca^{2+}. Noradrenaline (10 μM) inhibited the secretory response to 10 μM Ca^{2+} alone, and also reduced the insulin release evoked by cAMP in the presence of 10 μM Ca^{2+}. However, noradrenaline did not inhibit insulin secretion in response to cAMP at sub-stimulatory concentrations of Ca^{2+} (+ cAMP, 224 ± 38% basal; + cAMP + noradrenaline, 226 ± 46%, n = 9). Fig.3 (lower panel) shows the results of experiments using intact islets in which glucose-

induced (20 mM) insulin secretion was enhanced in the presence of 5 mM db-cAMP. Noradrenaline (10 μM) completely inhibited glucose-stimulated insulin secretion ($p > 0.2$ vs 2 mM glucose controls) and significantly reduced ($p < 0.05$) the secretory response to db-cAMP in the presence of 20 mM glucose. However, while noradrenaline totally abolished glucose-induced secretion, it did not completely inhibit the secretory response to db-cAMP, since a 3-fold increase in insulin release above basal levels ($p < 0.01$) was observed in the presence of both noradrenaline and db-cAMP.

4. DISCUSSION

Despite numerous reports that catecholamines inhibit insulin secretion from pancreatic B-cells (see [1,2]) the intracellular mechanisms of this inhibition remain obscure. The catecholamine inhibition of secretion is thought to be a direct effect on B-cells [7,14] and to be mediated by α_2-adrenoreceptors [15,16], as confirmed in the present studies by the abolition of the inhibitory effects of noradrenaline by the α_2-receptor antagonist, yohimbine. In addition to suppressing glucose-induced insulin secretion from intact islets, noradrenaline also had marked inhibitory effects on Ca^{2+}-induced secretion from electrically permeabilised islets, as has previously been reported in studies using digitonin-treated islets [17]. The similar inhibitory effects of noradrenaline on insulin secretion from both intact and permeabilised islets in the present study, and the antagonism of these effects by yohimbine, suggest a similar mode of action of the catecholamine in the two systems. These also suggest that the electrically permeabilised islet is a valid model for studying the noradrenaline inhibition of insulin secretion.

A number of conclusions can be drawn from the catecholamine inhibition of Ca^{2+}-induced insulin secretion from permeabilised islets. Firstly, electrically permeabilised islets retain functional α_2-adrenoreceptors which are still linked to their effector system. Secondly, the intracellular effector system through which noradrenaline inhibits insulin secretion can function in permeabilised cells in which the intracellular environment is in equilibrium with an extracellular medium of defined composition. Thirdly, the noradrenaline inhibition of insulin secretion from permeabilised islets in the presence of a maximum stimulatory concentration of Ca^{2+} [9] suggests that noradrenaline reduces the capacity of B-cells to respond to Ca^{2+}, and therefore implies that noradrenaline does not act primarily by affecting cellular Ca^{2+} handling but at some later stage in the secretory process. A similar conclusion has recently been drawn from less direct studies using intact tissue. Thus, in intact islets, the effects of catecholamines on Ca^{2+} uptake [16] or efflux [18] could be dissociated from the inhibition of insulin secretion, while fluorescence measurements of cytosolic Ca^{2+} in insulin-secreting tumour cells demonstrated that α_2-agonists could inhibit insulin secretion without measurably affecting intracellular concentrations of Ca^{2+} [19].

In other tissues α_2-agonists can act by inhibiting adenylate cyclase and thus reducing intracellular concentrations of cAMP [20]. It has been suggested that a similar mechanism is involved in the adrenergic inhibition of insulin secretion since cAMP is thought to play an important role in regulating the magnitude of the B-cell secretory response (review [21]). In support of such a mechanism, catecholamines have been reported to inhibit adenylate cyclase activity in islet homogenates [22,23] and to decrease the total cAMP content of isolated islets [24,25] and B-cells [7]. However, the effects of α_2-agonists on islet cAMP are variable and do not always parallel the inhibitory effects on insulin secretion [26,27]. Furthermore, there have been several reports that catecholamines inhibit insulin secretion in the presence of membrane-permeable analogues of cAMP [3,15,16], as was confirmed in the present studies using intact islets, suggesting that the inhibition of secretion cannot be solely attributed to a decrease in intracellular cAMP. Note, however, that in our experiments the noradrenaline inhibition of secretion in response to db-cAMP plus glucose was only partial, even at a concentration of noradrenaline well in excess of that required to inhibit totally secretion in response to glucose alone. Similar results have also been reported in other recent studies [16] in which the secretory responses to db-cAMP were only abolished at concentrations of noradrenaline at least an order of magnitude greater than those required to inhibit totally glucose-induced insulin secretion, in contrast to

earlier reports in which similar concentrations of catecholamines totally inhibited db-cAMP-induced secretion from intact islets [3,15].

The conclusion that decreases in intracellular cAMP cannot fully account for the inhibition of insulin secretion was further supported by our studies in permeabilised islets in which the intracellular concentrations of cAMP could be precisely controlled. Thus, supplying a stimulatory concentration of cAMP to the intracellular compartment did not reverse the noradrenaline inhibition of Ca^{2+}-induced insulin secretion, suggesting the involvement of factors other than cAMP in the inhibitory mechanism under these conditions. However, the small but significant secretory responses of permeabilised islets to cAMP at sub-stimulatory Ca^{2+} concentrations were not affected by noradrenaline, perhaps suggesting that catecholamines preferentially inhibit the normal secretory responses of permeabilised B-cells to Ca^{2+}, rather than to cAMP. cAMP is generally thought not to be an initiator of insulin secretion, but to modulate the magnitude of the B-cell secretory response to primary stimuli (see [21]), perhaps by increasing the sensitivity of the secretory process to Ca^{2+} [10,28]. It is therefore slightly surprising that cAMP stimulated insulin secretion from noradrenaline-treated permeabilised islets which no longer responded to Ca^{2+}. It may be that the responsiveness of noradrenaline-treated permeabilised islets to cAMP reflects two opposing effects on the secretory process, with cAMP increasing, and noradrenaline decreasing, the sensitivity of the secretory process to Ca^{2+}. Alternatively, it may be that a minor component of the secretory response to cAMP is independent of Ca^{2+} and is therefore unaffected by the catecholamine inhibition of Ca^{2+}-induced secretion.

In conclusion, our experiments using electrically permeabilised islets suggest that direct introduction of Ca^{2+} or cAMP into the cytosolic compartment does not fully relieve the catecholamine inhibition of insulin secretion, implying that at least one component of the inhibition occurs at a stage of the secretory pathway beyond changes in intracellular Ca^{2+} or cAMP. The mechanisms by which the adrenergic receptor-mediated inhibition of insulin secretion can occur at a late stage of the secretory pathway merit further investigation.

ACKNOWLEDGEMENTS

Financial assistance from the Medical Research Council and the British Diabetic association is gratefully acknowledged. J.M.F. was an SERC-CASE student. We thank the late Professor P.F. Baker for helpful discussions.

REFERENCES

[1] Miller, R.E. (1981) Endocr. Rev. 2, 471–494.
[2] Ahren, B., Taborsky, G.J. jr and Porte, D. jr (1986) Diabetologia 29, 827–836.
[3] Malaisse, W.J., Brisson, G. and Malaisse-Lagae, F. (1970) J. Lab. Clin. Med. 76, 895–902.
[4] Lorenz, R., Sharp, R. and Burr, I.M. (1979) Diabetes 28, 52–55.
[5] Metz, S.A. and Roberston, R.P. (1980) Am. J. Physiol. 239, E490–E500.
[6] Yamazaki, S., Katada, T. and Ui, M. (1982) Mol. Pharmacol. 21, 648–653.
[7] Schuit, F.C. and Pipeleers, D.G. (1986) Science 232, 875–877.
[8] Jones, P.M. and Howell, S.L. (1986) in: Biophysics of the Pancreatic B Cell (Atwater, I. et al. eds) Plenum, New York.
[9] Jones, P.M., Stutchfield, J. and Howell, S.L. (1985) FEBS Lett. 191, 102–106.
[10] Jones, P.M., Fyles, J.M. and Howell, S.L. (1986) FEBS Lett. 205, 205–209.
[11] Howell, S.L. and Taylor, K.W. (1968) Biochem. J. 108, 17–24.
[12] Gey, G.O. and Gey, M.L. (1936) Am. J. Cancer 27, 45–76.
[13] Hii, C.S.T., Stutchfield, J. and Howell, S.L. (1986) Biochem. J. 233, 287–289.
[14] Fyles, J.M., Cawthorne, M.A. and Howell, S.L. (1987) Biosci. Rep., in press.
[15] Nakaki, T., Nakadate, T., Yamamoto, S. and Kato, R. (1983) Life Sci. 23, 191–195.
[16] Morgan, N.G. and Montague, W. (1985) Biochem. J. 226, 571–576.
[17] Tamagawa, T., Niki, I., Niki, H. and Niki, A. (1985) Biomed. Res. 6, 429–432.
[18] Morgan, N.G., Rumford, G.M. and Montague, W. (1985) Biosci. Rep. 5, 1053–1060.
[19] Ullrich, S. and Wollheim, C.B. (1985) Mol. Pharmacol. 28, 100–106.
[20] Exton, J.H. (1985) Am. J. Physiol. 248, E633–E647.
[21] Malaisse, W.J. and Malaisse-Lagae, F. (1984) Experientia 40, 1068–1075.
[22] Howell, S.L. and Montague, W. (1973) Biochim. Biophys. Acta 320, 44–52.

[23] Kuo, W.-N., Hodgins, D.S. and Kuo, J.F. (1973) J. Biol. Chem. 248, 2705–2711.
[24] Katada, T. and Ui, M. (1981) J. Biochem. 89, 979–990.
[25] Garcia-Morales, P., Dufrane, S.P., Sener, A., Valverde, I. and Malaisse, W.J. (1984) Biosci. Rep. 4, 511–521.
[26] Rabinovitch, A., Cerasi, E. and Sharp, G.W.G. (1978) Endocrinology 102, 1733–1740.
[27] Ullrich, S. and Wollheim, C.B. (1984) J. Biol. Chem. 259, 4111–4115.
[28] Hughes, S.J., Christie, M.R. and Ashcroft, S.J.H. (1987) Mol. Cell. Endocr. 50, 231–236.