Differential effects of \( \beta \)-adrenergic agonists on insulin secretion from pancreatic islets isolated from rat and man

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ABSTRACT

The selective \( \beta_2 \)-adrenergic agonist clenbuterol was ineffective as a stimulus for insulin secretion when isolated rat pancreatic islets were incubated with glucose at concentrations between 4 and 20 mM. Inclusion of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine led to potentiation of glucose-induced insulin secretion, but did not facilitate stimulation by clenbuterol. Furthermore, maintenance of isolated rat islets for up to 3 days in tissue culture also failed to result in the appearance of a secretory response to \( \beta \)-agonists. By contrast, clenbuterol induced a dose-dependent increase in insulin release from isolated human islets incubated with 20 mM glucose. Clenbuterol did not increase the basal rate of insulin secretion (4 mM glucose) in human islets. Under perfusion conditions, the secretory response of human islets to clenbuterol was rapid, of similar magnitude to that seen under static incubation conditions and could be sustained for at least 30 min. The increase in insulin secretion induced by clenbuterol was inhibited by propranolol, indicating that the response was mediated by activation of \( \beta \)-receptors. In support of this, a similar enhancement of glucose-induced insulin secretion was elicited by a different \( \beta_2 \)-agonist, salbutamol, in human islets. The results indicate that the B cells of isolated rat islets are unresponsive to \( \beta \)-agonists, whereas those of human islets are equipped with functional \( \beta \)-receptors which can directly influence the rate of insulin secretion.

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INTRODUCTION

The secretion of insulin from the pancreatic B cell is controlled by a wide range of factors, including metabolites, hormones, paracrine regulators and neurotransmitters (Hedeskov, 1980; Miller, 1981). These can act either to increase or decrease the rate of secretion, and the interplay between these various regulators is likely to be important in the coordinated control of circulating insulin levels.

It has long been recognized that catecholamines may play a significant role in regulation of insulin secretion (Coore & Randle, 1964; Porte, 1967), and that the islets of Langerhans possess a rich adrenergic innervation (Ahren, Ericson, Lundquist et al. 1981; Miller, 1981). Indeed the islet catecholamine content is as high as that of any tissue in the body (Morgan, Hurst & Chan, 1989), and the release of islet noradrenaline is significantly increased following stimulation of the sympathetic innervation (Miller, 1981; Ahren, Taborsky & Porte, 1986).

Despite this evidence, it is still unclear precisely how catecholamines control insulin secretion and where their sites of interaction lie within the islet. Thus it is well established that administration of \( \beta \)-agonists can elicit a rise in circulating insulin levels in experimental animals and in man (Porte, 1967; Iversen, 1973; Loubatieres-Mariani, Chapal, Ribes & Loubatieres, 1977), but there is dispute concerning the location of the \( \beta \)-receptors which mediate this effect. Several studies have provided results which suggest that \( \beta \)-agonists can exert direct effects on the pancreatic B cell (Hermann & Deckert, 1977; Fyles, Cawthorne & Howell, 1986), and support the proposal that adrenergic stimulation of insulin secretion occurs as a consequence of binding of catecholamines to \( \beta \)-receptors present on the insulin-secreting cells within the islet. Indeed, the
presence of β-adrenergic binding sites has been demonstrated in rat islet preparations (Cherksey, Altszuler & Zadunaisky, 1981; Fyles et al. 1986). However, other data indicate that stimulation of insulin secretion by β-adrenergic agonists is mediated by an indirect mechanism (Ribes, Trimble, Blayac et al. 1984), perhaps involving a primary effect on glucagon release (Zielmann, Schutte, Lenzen & Panten, 1985). In support of this, experiments utilizing separate populations of rat A and B cells indicate that β-adrenergic responsiveness is confined to the A cell subpopulation in this species (Schutt & Pipeleers, 1986).

In addition, doubt has been cast on the in-vivo data by the demonstration that stimulation of central rather than peripheral β-receptors elicits insulin secretion in some species, and that the resultant rise in circulating insulin is mediated by vagal activation (Ribes, Hillaire-Buys, Gross et al. 1989).

In the present study we have addressed these discrepancies by examining whether treatment of isolated islets of Langerhans with β-agonists can elicit a direct increase in the rate of insulin secretion. The experiments were performed initially with isolated rat islets in order to clarify the possible involvement of β-receptors in the control of insulin secretion in this species. In addition, we have also carried out the first detailed examination of β-adrenergic responses in isolated human islets.

MATERIALS AND METHODS

Islet isolation

Islets of Langerhans were isolated from the pancreata of human organ donors and from male Wistar rats by collagenase digestion, as described in detail previously (Montague & Taylor, 1968; Lake, Basset, Larkins et al. 1989). Before removal of pancreata from human donors, full and informed consent was obtained from next of kin. All procedures were carried out with approval from the appropriate Ethical Committees. Rat islets were isolated in a bicarbonate-buffered physiological saline solution (Gey & Gey, 1936), gassed with O₂:CO₂ (95:5) and containing glucose (4 mM) and CaCl₂ (1 mM), and were used within 2 h of isolation.

Tissue culture

Rat islets to be maintained in tissue culture were isolated under aseptic conditions, washed with 5 × 10 ml sterile bicarbonate-buffered medium and suspended in 20 ml medium RPMI-1640, containing 10% (v/v) fetal calf serum, 400 IU sodium penicillin G/ml and 200 μg streptomycin sulphate/ml. The medium containing the islets was then transferred to sterile plastic Petri dishes and incubated at 37°C under an atmosphere of air:CO₂ (95:5) at 100% humidity for up to 3 days.

Human islets were isolated and transported to the laboratory in medium RPMI-1640, where they were cultured at 37°C for 1–6 days before use.

After tissue culture, rat or human islets were hand-picked into fresh bicarbonate-buffered medium and preincubated at 37°C for 30 min before each experiment.

Insulin secretion studies

Both static incubation and perfusion studies were carried out at 37°C using a bicarbonate-buffered medium (pH 7.4) containing glucose (4 mM) and bovine serum albumin (1 mg/ml). The methods for studying islet insulin secretion have been described previously (Morgan & Montague, 1984; Morgan, Rumford & Montague, 1985). Briefly, for static incubation studies, groups of three islets were hand-picked into 0.5 ml bicarbonate-buffered medium. After incubation for 1 h at 37°C in the presence of test reagents, samples of the medium were removed and their insulin contents measured by radioimmunoassay using crystalline biosynthetic human insulin as standard.

For perfusion experiments, groups of up to 200 islets were placed in perfusion chambers and perfused with medium containing glucose (4 mM) for a 30-min stabilization period at a flow rate of 1 ml/min. After addition of test reagents, samples of medium were collected sequentially and insulin contents determined by radioimmunoassay.

Reagents

Clenbuterol and propranolol-HCl were kindly donated by ICI Pharmaceuticals (Macclesfield, Cheshire, U.K.). Bovine serum albumin (fraction V) was from Wilfred Smith plc (Edgware, Middx, U.K.), and RPMI-1640 from Northumbria Biologicals (Cramlington, Cumbria, U.K.). Crystalline biosynthetic human insulin was a gift from Eli Lilly & Co. (Indianapolis, IN, U.S.A.). 3-Isobutyl-1-methylxanthine (IBMX), salbutamol, streptomycin, penicillin and collagenase (type XI) were all from Sigma Chemical Co. (Poole, Dorset, U.K.).

Statistical analysis

All data are presented as means ± S.E.M. Statistical significance was assessed by Student's t-test for unpaired data.
RESULTS

Effects of ß-agonists on insulin secretion from isolated rat and human islets

Insulin secretion from both isolated rat and human islets of Langerhans incubated under static conditions was stimulated by glucose in a concentration-dependent manner (Table 1). In agreement with a previous study (Harrison, Christie & Gray, 1985), the dose-response curve to glucose for human islets appeared to lie to the left of that for the rat, indicating that human islets were more sensitive to glucose than those of the rat.

In freshly isolated rat islets, stimulation of insulin secretion by glucose was not significantly altered by addition of the ß-agonist clenbuterol (10 μM; Table 1). Similar results were also obtained if the incubation period was reduced to 30 min, or if the islets were treated with clenbuterol in a perfusion system to allow the dynamics of insulin secretion to be monitored continuously (results not presented). Furthermore, incubation of freshly isolated rat islets in the presence of the phosphodiesterase inhibitor IBMX (50 μM) and clenbuterol (10 μM) failed to raise the rate of insulin secretion above that seen with IBMX alone (IBMX: 7.51 ± 0.47 ng/islet per h; IBMX plus clenbuterol: 6.57 ± 0.22 ng/islet per h).

In contrast to the situation in rat islets, treatment of isolated human islets with 10 μM clenbuterol provoked a significant stimulation of insulin secretion in the presence of 20 mM glucose (Table 1).

One difference between the studies described so far is that human islets were cultured for various periods of time before use, whereas rat islets were used within 2 h of isolation. To account for any possible changes in the response of human islets to clenbuterol during culture, rat islets were also maintained in tissue culture for periods up to 3 days. However, in these islets, glucose-induced insulin secretion was again not increased by the presence of clenbuterol (Table 2). Furthermore, treatment of cultured rat islets with another ß-agonist, salbutamol, did not lead to a significant increase in secretion rate (Table 2). Forskolin, however, was still able to increase the rate of glucose-induced insulin secretion from cultured rat islets (Table 2).

Characterization of the ß-adrenergic stimulation of insulin secretion in human islets

In perifusion studies, raising the glucose concentration from 4 to 20 mM provoked a biphasic secretory response from isolated human islets (Fig. 1). The increase in secretion was detected within 3 min, and the peak first-phase response was reached within a further 2 min. Thereafter, the secretion rate declined to a steady-state value which could be maintained for at least 35 min in the continued presence of 20 mM glucose (Fig. 1). Infusion of 10 μM clenbuterol during the second phase of glucose-induced insulin secretion elicited an immediate and significant rise in hormone secretion rate (Fig. 1). This was evident within 2 min and resulted in an overall twofold enhancement of the rate of secretion. During the period of continuous clenbuterol infusion (30 min) the increased rate of insulin secretion was maintained at a constant level throughout.

The secretion rate declined when the ß-agonist D,L-propranolol (10 μM) was introduced during the period of stimulation with clenbuterol (Fig. 1), consistent with the blockade of ß-receptors. Thus, in the presence of clenbuterol (10 μM), insulin secretion

<table>
<thead>
<tr>
<th>Glucose concentration (mM)</th>
<th>Insulin secretion (ng/islet per h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>No clenbuterol</td>
</tr>
<tr>
<td>4</td>
<td>0.80 ± 0.13</td>
</tr>
<tr>
<td>8</td>
<td>3.10 ± 0.27</td>
</tr>
<tr>
<td>20</td>
<td>4.78 ± 0.27</td>
</tr>
<tr>
<td>Man</td>
<td>0.67 ± 0.09</td>
</tr>
<tr>
<td>20</td>
<td>1.46 ± 0.08</td>
</tr>
</tbody>
</table>

*P < 0.02 compared with the corresponding value in the absence of clenbuterol (Student's t-test for unpaired data).

Groups of three isolated rat or human islets were hand-picked into 0.5 ml incubation medium containing increasing concentrations of glucose, in the presence or absence of clenbuterol.

| Table 2. Effects of forskolin (10 μM) and the ß-agonists clenbuterol (10 μM) and salbutamol (10 μM) on glucose-induced insulin secretion from cultured rat islets. Values are means ± S.E.M. of eight observations |
|-------|------------------|------------------|
| Glucose concentration (mM) | Test agent | Insulin secretion (ng/islet per h) |
| 4 | | 0.09 ± 0.04 |
| 20 | | 0.89 ± 0.18** |
| 20 | Clenbuterol | 0.71 ± 0.21 |
| 20 | Salbutamol | 0.85 ± 0.26 |
| 20 | Forskolin | 1.78 ± 0.13* |

*P < 0.01 compared with 20 mM glucose alone; **P < 0.001 compared with 4 mM glucose. (Student's t-test for unpaired data).

Isolated rat islets were cultured for 3 days and then incubated in the presence of glucose alone, or glucose and 10 μM clenbuterol, salbutamol or forskolin.

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averaged 11.1 ± 0.41 pg/islet per min and this was reduced significantly (P<0.001) to 6.5 ± 0.07 pg/islet per min by propranolol.

Examination of the effect of different concentrations of clenbuterol on glucose-induced insulin secretion revealed that the response was dose dependent and saturable (Fig. 2). Half the maximal increase in secretion was elicited by approximately 50 nM clenbuterol, while maximal secretion required 1 μM. A similar dose-dependent rise in insulin secretion was also observed with a different β-agonist, salbutamol (Fig. 2). However, in this case the response tended to be smaller in magnitude, and the islets were less sensitive to the agonist. Thus half the maximal response was obtained at 5 μM salbutamol, and maximal rates of hormone secretion were achieved only in the presence of 10 μM salbutamol.

**DISCUSSION**

The results obtained in the present work strongly support the view that treatment of isolated rat islets with selective β2-agonists does not result in an increase in insulin secretion. Thus, neither clenbuterol nor salbutamol was able to induce an increase in insulin secretion, irrespective of the prevailing glucose concentration. Moreover, inclusion of the phosphodiesterase inhibitor IBMX also failed to reveal any direct stimulation of insulin secretion by...
these agonists. The effect of IBMX would be to amplify any increase in intracellular cyclic AMP resulting from agonist-induced adenylate cyclase activation. Since increases in cyclic AMP within the B cell lead to potentiation of insulin release (Sharp, 1979), it is clear that no direct effect of the β-agonists was evident in this study. These results, therefore, confirm that isolated rat islets do not respond to β-agonists with a direct increase in insulin output (Zielmann et al. 1985). This conclusion is also in accord with in-vivo studies in the rat, which have failed to demonstrate an increase in circulating insulin levels following chronic administration of clenbuterol (Emery, Rothwell, Stock & Winter, 1984).

In some previous in-vitro studies, evidence has been presented that the insulin secretary response to β-agonists is relatively transient and, as a consequence, is difficult to detect under static incubation conditions (Fyles et al. 1986). We have excluded the possibility that clenbuterol elicits a short-lived enhancement of insulin secretion in rat islets by monitoring the effects of this agent in perifused islets. Under these conditions, clenbuterol also failed to increase significantly the rate of glucose-induced insulin secretion (N. G. Morgan & N. S. Berrow, unpublished observations).

In marked contrast to the situation observed in isolated rat islets, this study provides the first direct demonstration that isolated human islets respond to β-adrenergic agonists with a significant increase in insulin secretion. This response was observed following incubation of islets with two structurally unrelated β-agonists, clenbuterol and salbutamol, and was dose dependent in each case. Previous work has shown that salbutamol infusion can elicit a rise in plasma insulin in man (Massara, Fassio, Camanni & Molinatt, 1975), and the present data suggest that this may reflect a direct effect of the agent at the level of the endocrine pancreas.

The relative potencies of clenbuterol and salbutamol were in accord with the pharmacology of these two drugs at β-receptors in other tissues (Tschan, Perruchoud & Herzog, 1979), and indicate that the observed enhancement of insulin secretion was due to β-receptor agonism. In further support of this, the ability of clenbuterol to increase insulin secretion from human islets was significantly inhibited by the β-antagonist DL-propranolol. The extent of inhibition, under these conditions, was not total, reflecting the fact that the active isomer of propranolol was present at only 5 μM, whereas clenbuterol was used at 10 μM. We have observed (R. J. Lacey & N. G. Morgan, unpublished observations) that higher concentrations of propranolol exert deleterious effects on insulin secretion in human islets and, therefore, higher concentrations were not used in the present study.

We cannot completely exclude the possibility that the β responses observed in our experiments were secondary to the release of a paracrine regulator (e.g. glucagon) within the islets. However, the fact that β-adrenergic enhancement of insulin secretion could be observed under both static and perifusion conditions argues against this possibility. In the latter case, it would be anticipated that the high flow rate of the perifusion system (1 ml/min) would rapidly remove any released glucagon, such that paracrine influences on insulin secretion would have been minimized. The observation that the extent of stimulation of secretion by clenbuterol was similar under both static and perifusion conditions indicates that the response was not mediated by a paracrine mechanism. These considerations do not imply that glucagon release was not stimulated by the β-agonists, but they do indicate that this could not account for the effects on insulin secretion.

The present results point to a significant difference between rat and human islets, in that the former are not equipped with β-receptors which can directly regulate the rate of insulin secretion. We have excluded the possibility that the effects of clenbuterol in human islets resulted from maintenance of the tissue in culture, as reported for hepatocytes (Kunos, Hirata, Ishac & Tchakarov, 1984), since culture of rat islets under similar conditions failed to induce any β response. These results also show that the lack of response to β stimulation in freshly isolated islets was not due to damage of the receptors during isolation. If this had been the case, the response would have returned during culture, as the receptor population was replenished.

In conclusion, the present results confirm that isolated rat islets do not respond to β-agonists with an increase in insulin secretion. This implies that the observed effects of such agents on circulating insulin levels in vivo are not mediated by any direct action at the level of the pancreatic B cell. In contrast, isolated human islets possess a functional β-adrenergic system which could play a direct role in the regulation of insulin secretion by catecholamines.

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