Concentration-dependent effects of adrenaline on the profile of insulin secretion from isolated human islets of Langerhans

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ABSTRACT

The effects of the mixed α/β-agonist adrenaline on insulin secretion from isolated human islets of Langerhans were studied. In static incubation experiments, adrenaline (0.1 nmol/l to 10 μmol/l) caused a concentration-dependent inhibition of glucose-induced insulin secretion from isolated human islets. However, perfusion experiments revealed that the time-course of the secretory changes induced by adrenaline was complex. When employed at a high concentration (1 μmol/l), adrenaline caused a sustained inhibition of glucose-induced insulin secretion, which could be relieved by the addition of the α2-agonist yohimbine (10 μmol/l). By contrast, infusion of adrenaline at a lower concentration (10 nmol/l), produced a large initial potentiation of glucose-induced insulin secretion. This response was, however, short-lived and followed by sustained inhibition of secretion, which could be relieved by yohimbine (10 μmol/l). The initial stimulation of insulin secretion provoked by 10 nmol adrenaline/l was abolished when islets were incubated in the presence of the β-antagonist, propranolol (1 μmol/l), consistent with activation of β-adrenoceptors. In support of this, treatment of human islets with the selective β2-agonist clenbuterol, was also associated with marked stimulation of insulin secretion. By contrast, each of two selective β3-agonists tested failed to alter insulin secretion from human islets. The results indicate that human pancreatic B-cells are equipped with both α2- and β2-adrenoceptors which can affect insulin secretion. Adrenaline interacts with both of these but the α2-response is predominant and can overcome the tendency of β2-adrenoceptors to potentiate insulin release.


INTRODUCTION

It is well established that adrenergic agonists (including naturally occurring catecholamines) alter the rate of insulin secretion when administered to experimental animals or to human volunteers in vivo, or when added to isolated islets of Langerhans incubated in vitro (reviewed by Woods & Porte, 1974; Miller, 1981; Ahren et al. 1986; Morgan et al. 1989). The final response obtained can be either an increase or a decrease in insulin secretion rate, and these opposing effects reflect the activation of different types of adrenoceptor. Adrenoceptors of the α2-subtype regulate pathways which result in inhibition of insulin secretion (Langer et al. 1983; Morgan & Montague, 1985; Persaud et al. 1989), while β-adrenoceptors control mechanisms that are associated with potentiation of the secretory response to glucose and other nutrients (Kaneto et al. 1975; Hermann & Deckert, 1977; Lacey et al. 1990). Hence, changes in insulin secretion mediated by catecholamines reflect the balance between the effects of α2- and β-adrenoceptor activation and their relative importance in modulating the secretory pathway.

There is now strong evidence that, in the rat, islet B-cells possess large numbers of α2-adrenoceptors (Cherksey et al. 1981; Fyles et al. 1987; Morgan et al. 1989) but do not express functional β-adrenoceptors,
since direct application of \( \beta \)-agonists to isolated rat B-cells or islets in vitro does not increase insulin secretion (Zielmann et al. 1985; Schuit & Pipeleers, 1986; Lacey et al. 1990, 1991). Thus, in this species the stimulatory effects of catecholamines observed in vivo must be mediated by indirect mechanisms. Conversely, \( \alpha_2 \)-adrenergic inhibition of insulin secretion (in vivo or in vitro) can result from direct interaction of agonists with \( \alpha_2 \)-adrenoceptors on the rat B-cell.

We have recently presented evidence that the situation in human islets is quite different (Lacey et al. 1990). In contrast to rat islets, isolated human islets respond to direct application of selective \( \beta \)-agonists with a large sustained stimulation of insulin secretion. The data indicate that human islets express \( \beta \)-adrenoceptors which can play a direct role in the control of insulin secretion (Lacey et al. 1990). However, since adrenergic agonists have also been reported to inhibit insulin secretion from isolated human islets (Harrison et al. 1985; Rhodes et al. 1985), it is important to determine how the net response to adrenergic stimulation is determined, and which of the two competing responses predominates.

In the present study we have examined the response of isolated human islets to the endogenous catecholamine, adrenaline, which can interact with both \( \alpha_2 \) and \( \beta \)-adrenoceptors. The aim was to establish the relative importance of islet \( \alpha_2 \) and \( \beta \)-adrenoceptors in the control of insulin secretion from human islets.

**MATERIALS AND METHODS**

Adrenaline, yohimbine and collagenase (Type XI) were purchased from Sigma (Poole, Dorset, U.K.). Bovine serum albumin (fraction V) was from Wilfred Smith plc (Edgware, Middx, U.K.). Clenbuterol, propranolol HCl and ICI 201651 were gifts from
cannulated in situ and the organ cooled on ice. Digestion was achieved by intraductal infusion of collagenase, followed by incubation at 37 °C for 20 min and disruption of the tissue with forceps. The islets were separated from contaminant acinar tissue by centrifugation on discontinuous density gradients of bovine serum albumin, immediately placed in tissue culture medium RPMI-1640 (Lake et al. 1989) and transported to our laboratory.

**Islet culture**

On arrival, the human islets were washed once in a bicarbonate buffered-physiological saline solution (pH 7.4; Gey & Gey, 1936) containing 4 mmol glucose/l, 1 mmol CaCl$_2$/l, and supplemented with 400 IU sodium penicillin G/ml and 200 μg streptomycin sulphate/ml. The islets were then resuspended in fresh culture medium RPMI-1640, containing 10% (v/v) fetal calf serum and antibiotics as described above, and cultured at 37 °C in a humidified atmosphere of O$_2$:CO$_2$ (95:5) for 1 to 3 days prior to use.

**Islet incubation**

After tissue culture, islets were resuspended in fresh bicarbonate buffer. The methods for studying islet insulin secretion have been described previously (Morgan & Montague, 1984; Morgan et al. 1985). Briefly, for static incubation experiments, islets were preincubated in bicarbonate buffer at 37 °C for 30 min. Groups of five islets were then selected individually under a dissecting microscope, and placed in tubes containing 0.5 ml bicarbonate buffer supplemented with bovine serum albumin (1 mg/ml) and test reagents. After incubation at 37 °C for 1 h, samples were removed for determination of insulin levels by radioimmunoassay. Crystalline biosynthetic human insulin was used as standard.

For perfusion studies, groups of up to 300 islets were transferred to perfusion chambers (male–female luer connectors) and perfused with bicarbonate buffer (containing 4 mmol glucose/l and 1 mg bovine serum albumin/ml) at a flow rate of 0.5 ml/min (37 °C). Islets were perfused for a 30-min stabilization period prior to each experiment. After addition of test reagents, samples of the perfusion buffer were collected at 2- or 5-min intervals and assayed for insulin content by radioimmunoassay.

In all experiments, islets from each individual organ donor were incubated separately from those of other donors. Results were pooled, where appropriate, only after assay and analysis of each individual response.
Statistical analysis

All data are presented as mean values ± S.E.M. Statistical significance was assessed by Student’s t-test for unpaired data.

RESULTS

Effects of adrenergic agonists on insulin secretion from isolated human islets incubated under static conditions

Incubation of isolated human islets in the presence of 20 mmol glucose/l and clenbuterol, an agonist which interacts selectively with β2-adrenoceptors, resulted in significant potentiation of glucose-induced insulin secretion (Fig. 1), confirming previous data (Lacey et al. 1990). By contrast, when islets were incubated with 20 mmol glucose/l in the presence of ICI 201651 (an agonist which shows selectivity for the newly described β3-subclass of adrenoceptors; Holloway et al. 1988; Fig. 1), no potentiation of the response was seen at any concentration of ICI 201651 between 10 nmol/l and 10 μmol/l. Incubation of human islets, isolated from a separate donor, with a different β3-agonist, BRL 37344, also failed to induce a rise in glucose-induced insulin secretion (20 mmol glucose/l, 0.54 ± 0.08 ng/islet per h; 20 mmol glucose/l + 35 μmol BRL 37344/l, 0.51 ± 0.08 ng/islet per h (not significant); 20 mmol glucose/l + 20 μmol clenbuterol/l, 1.18 ± 0.16 ng/islet per h (P<0.005 compared with 20 mmol glucose/l alone). Values are means ± S.E.M. of eight observations, using islets from a single donor.

Incubation of isolated human islets with increasing concentrations of the combined α- and β-agonist, adrenaline, was associated with a concentration-related inhibition of glucose-induced insulin secretion (Fig. 2). Addition of 10 μmol adrenaline/l was required for complete abolition of the secretory response to glucose, but no stimulatory effects were observed at any concentration tested (Fig. 2).
Effects of adrenaline on glucose-induced insulin secretion from perfused human islets

In order to study the effects of adrenergic agonists further, we performed a series of perfusion experiments to investigate the time-course of the responses mediated by adrenaline in islets. Isolated human islets responded to an increase in glucose concentration (from 4 to 20 mmol/l) with a biphasic insulin secretory response (Figs 3 and 4). Introduction of 1 μmol adrenaline/l during the second phase of the secretory response resulted in a very small rise in secretion rate, which did not achieve statistical significance and was followed by sustained inhibition of secretion (Fig. 3). Subsequent introduction of the α₂-antagonist yohimbine (55 min after commencement of the adrenaline infusion) resulted in a return to the stimulated secretion rate (Fig. 3).

Infusion of adrenaline at a lower concentration (10 nmol/l) during the second phase of glucose stimulation, resulted in a different pattern of secretion (Fig. 4). In this case, the initial response to the catecholamine was a rapid, large potentiation of insulin secretion which reached a peak within 5 min (Fig. 4). After this time, the secretory rate declined and net inhibition of secretion followed. This inhibition was then maintained until the introduction of yohimbine which resulted in a return to stimulated levels (Fig. 4).

To confirm the receptor specificity of the initial stimulation observed with 10 nmol adrenaline/l, islets were perfused in the presence of the β-antagonist, propranolol (1 μmol/l; Fig. 5a). Under these conditions, introduction of a low concentration of adrenaline (10 nmol/l) failed to potentiate secretion and only inhibition was observed (Fig. 5a). The control channel in this experiment, performed with islets from the same donor, confirmed that 10 nmol adrenaline/l did induce the expected rise in insulin secretion if propranolol was omitted (Fig. 5b).
Propranolol (1 μmol/l)
Glucose (20 mmol/l)
Adrenaline (10 nmol/l)

Time (min)

Insulin secretion (pg/islet per min)

Glucose (20 mmol/l)
Adrenaline (10 nmol/l)
DISCUSSION

The present results demonstrate that, in man, pancreatic B-cells possess functional $\alpha_2$- and $\beta$-adrenoceptors that can each regulate insulin secretion. Since clenbuterol preferentially activates $\beta_2$-adrenoceptors (Holloway et al. 1988; Lacey et al. 1991), the data also confirm that the adrenoceptors involved are of the $\beta_2$-subtype. It has been suggested on the basis of in-vivo evidence, that the newly characterized $\beta_3$-subtype of receptors may also be involved in mediating the insulin secretory response to $\beta$-agonists (Yoshida et al. 1991; Yoshida, 1992). We were unable to demonstrate any direct stimulation of insulin secretion by $\beta_3$-agonists in isolated human islets, under conditions when clenbuterol produced a marked potentiation of secretion (Fig. 1). Lack of direct stimulation by $\beta_3$-agonists has also been reported in rat islets (Yoshida et al. 1991). Thus, it appears that any effects mediated by $\beta_3$-agonists on insulin secretion in vivo do not result from direct interaction with receptors present on the pancreatic B-cell.

Activation of $\beta$-adrenoceptors leads to a rise in cyclic AMP (Lefkowitz & Caron, 1988) which is a potentiator of nutrient-induced insulin secretion (Sharp, 1979; Henquin, 1985). However, it has been shown in rat and mouse islets, that activation of $\alpha_2$-adrenoceptors leads to inhibition of insulin secretion even when cyclic AMP levels are elevated (Langer et al. 1983; Ullrich & Wollheim, 1984; Morgan & Montague, 1985; Debuyser et al. 1991). If this situation is also true in human islets, then stimulation of $\beta$-adrenoceptors should be ineffective as a means of potentiating insulin release under conditions when $\alpha_2$-adrenoceptor activation also occurs. Our results support this concept. Thus, in static incubation experiments, adrenaline did not potentiate glucose-induced insulin release at any concentration tested between 0.1 nmol/l and 10 $\mu$mol/l (Fig. 1), even though this agent can activate both $\alpha_2$- and $\beta$-adrenoceptors. Perfusion experiments performed with 1 $\mu$mol adrenaline/l also support this conclusion since the net effect was inhibitory (Fig. 3). Furthermore, inhibition of glucose-induced insulin secretion has been reported in human islets perfused with 100 $\mu$mol adrenaline/l (Rhodes et al. 1985). Therefore, as in rat islets, $\alpha_2$-inhibition of insulin secretion is predominant in human islet B-cells and can over-ride $\beta_2$-stimulation.

When islets were perfused with a lower concentration of adrenaline (10 nmol/l) the secretory profile was more complex (Fig. 4). Initially, a large stimulation of insulin secretion was observed which was not seen with the higher concentration of agonist. However, this stimulation was not sustained but converted rapidly into net $\alpha_2$-inhibition of secretion, which could be relieved by yohimbine (Fig. 4). A concentration-dependent difference in the insulin secretory response to adrenaline has also been reported from in-vivo studies in the dog where, paradoxically, a larger increment in immunoreactive insulin was seen after infusion of a lower dose of adrenaline (0.1 $\mu$g/kg per min) than after administration of a higher dose (0.2 $\mu$g/kg per min; Gray et al. 1980).

When islets were pretreated with propranolol (1 $\mu$mol/l), the capacity of adrenaline (10 nmol/l) to potentiate insulin secretion was abolished (Fig. 5a). Thus, it appears that the initial potentiation of glucose-induced insulin secretion seen in response to 10 nmol adrenaline/l is mediated by $\beta_2$-adrenoceptors and that the inhibitory response results from $\alpha_2$-receptor activation. These results emphasize that the ability of 10 nmol adrenaline/l to promote stimulation of insulin secretion cannot be ascribed to marked differences in islet $\alpha_2$- and $\beta_2$-receptor affinity, since it is evident that both types of receptor were activated by 10 nmol adrenaline/l. Rather, it appears that there is a difference in the temporal sequence of events mediated by each receptor when the catecholamine concentration is low. The transient profile of stimulation is not observed if the $\beta_2$-adrenoceptors are activated selectively (Lacey et al. 1990), demonstrating that it is not an intrinsic property of the $\beta$-system. Thus, rapid down-regulation of the response cannot account for the dynamic changes observed.

The profile of secretion resulting from introduction of adrenaline (Figs 3–5) also explains the failure to detect direct stimulation of insulin secretion in static incubation experiments (Fig. 2). When integrated over a relatively long period of time, any initial stimulation of insulin secretion is likely to be obscured by the later inhibition. Thus, the net result is that the presence of low concentrations of adrenaline produces no apparent change in the rate of secretion.

**Figure 5.** Time-course of changes in insulin secretion induced by 10 nmol adrenaline/l in the presence and absence of propranolol in perfused human islets. Groups of up to 300 human islets were perfused with bicarbonate buffer containing 4 nmol glucose/l at 37 °C for 30 min prior to the start of the experiment. (a) Ten minutes prior to the start of the experiment ($\tau$ = 0), 1 $\mu$mol propranolol/l was infused for the duration of the perfusion; (b) shows control data obtained in the absence of propranolol. Test reagents were infused continuously from the times shown until the end of the experiment. Data were obtained from (b) one or (a) two channels containing islets from a single organ donor.
Since the extent of $\beta_2$-stimulation is reduced at high concentrations of adrenaline (Fig. 3), only inhibition is observed under these conditions in static incubation experiments (Fig. 2).

In conclusion, the present study confirms that human pancreatic B-cells possess both $\alpha_2$- and $\beta_2$-adrenoceptors which can function in the control of insulin secretion. The results have also revealed that isolated human islets respond to adrenaline in a complex manner. At high concentrations of adrenaline the $\alpha_2$-inhibitory response is entirely predominant and there is no evidence for any $\beta_2$-stimulation of insulin secretion. At lower adrenaline concentrations the initial response is stimulatory but this is overcome by net inhibition of secretion.

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REFERENCES


