Selective stimulation of glucagon secretion by β_2 -adrenoceptors in isolated islets of Langerhans of the rat

Rosemary J. Lacey, Nicholas S. Berrow, *John H.B. Scarpello & ¹Noel G. Morgan

Cellular Pharmacology Group, Department of Biological Sciences, and *Department of Postgraduate Medicine, University of Keele, Keele, Staffordshire ST5 5BG

- 1 In rat isolated islets of Langerhans the selective β_2 -adrenoceptor agonist, clenbuterol (1 to 20 μM), significantly increased the level of adenosine 3':5'-cyclic monophosphate (cyclic AMP) within 2 min of incubation.
- 2 The cyclic AMP response to clenbuterol was inhibited in the presence of the selective β_2 adrenoceptor antagonist, ICI 118551 (0.1 or 10 μM) but remained unchanged when the β_1 -antagonist, atenolol (0.1 μM) was administered.
- 3 Despite causing an elevation in cyclic AMP, clenbuterol (up to 20 μM) failed to influence insulin secretion at any glucose concentration tested, even in the presence of a phosphodiesterase inhibitor.
- 4 By contrast, clenbuterol elicited a dose-dependent rise in the rate of glucagon secretion; the maximal agonist-induced increase in secretion was two fold, a response equivalent to that observed with 20 mM L-arginine.
- 5 ICI 118551 significantly inhibited the rise in glucagon secretion induced by clenbuterol (up to 20 μM).
- 6 The results indicate that the rat islet A cell population is equipped with functional β_2 -adrenoceptors which influence glucagon secretion via the second messenger cyclic AMP, but that the B cells are deficient in functional β -receptors.

Keywords: β_2 -adrenoceptor; clenbuterol; cyclic AMP; glucagon secretion; insulin secretion

Introduction

The adrenergic regulation of insulin secretion has been reported to consist of separate components, defined as inhibitory (α_2) and stimulatory (β) (Miller, 1981). It is well established that islet cells possess functional α_2 -adrenoceptors (Nakaki *et al.*, 1983; Morgan & Montague, 1985; Schuit & Pipeleers, 1986), however, the role of β -adrenoceptors is controversial. Although it has been reported consistently that β -agonists cause a rise in plasma insulin levels in many species (Porte, 1967; Iversen, 1973; Massara *et al.*, 1975; Miller, 1981), evidence from studies with isolated islet preparations is equivocal (Cherksey *et al.*, 1981; Zielmann *et al.*, 1985; Fyles *et al.*, 1986). This may be due in part to significant species differences in the response of isolated pancreatic islets to β -adrenoceptor agonists (Lacey *et al.*, 1990a). Thus it remains unclear whether responses observed *in vivo* can be attributed to direct interaction with islet cell β -receptors or whether the effects result from indirect mechanisms such as the secondary stimulation of glucagon release. Furthermore, there is still conflicting evidence as to the subtype of β -receptor present in islets, since both β_1 and β_2 -receptors have been implicated in the control of hormone secretion (Furman & Tayo, 1974; Miller, 1981).

It is well established that β -receptors of both the β_1 and β_2 subtypes normally mediate their effects via the second messenger adenosine 3':5'-cyclic monophosphate (cyclic AMP) (Lefkowitz & Caron, 1988). The physiological consequences of β -agonism should therefore follow from an increase in intracellular cyclic AMP levels. In order to clarify the situation in the endocrine pancreas, we have studied the effect of the selective β_2 adrenoceptor agonist, clenbuterol, on cyclic AMP levels, insulin and glucagon secretion in rat isolated islets. We have also employed selective β -antagonists to characterize the β -receptor subtype involved. Our data demonstrate that whilst clenbuterol raises cyclic AMP levels and stimulates glucagon secretion from rat islets, it is ineffective on insulin secretion. This indicates that rat islet A cells possess β -adrenoceptors which influence glucagon secretion, but that

the insulin-secreting B cells are deficient in functional β -receptors. Part of this work has recently been published in abstract form (Lacey *et al.*, 1990b).

Methods

Islet isolation

Isolation of islets of Langerhans from the pancreata of male Wistar rats was carried out by collagenase digestion, as previously described (Montague & Taylor, 1968). Islets were isolated with a bicarbonate-buffered physiological saline solution (Gey & Gey, 1936), equilibrated to pH 7.4 by gassing with $\text{O}_2:\text{CO}_2$ (95:5) and containing glucose (4 mM) and CaCl_2 (1 mM). Islets were handpicked under a dissecting microscope and used within 2 h of isolation.

Secretion studies

For insulin and glucagon secretion studies, groups of 3 or 5 islets, respectively, were placed in 0.5 ml of the bicarbonate-buffered medium described above, also containing bovine serum albumin (1 mg ml⁻¹). After incubation at 37°C for 1 h in the presence of the appropriate test reagents, samples of the medium were removed for the measurement of insulin or glucagon content by radioimmunoassay.

Cyclic AMP measurement

Groups of 50 isolated islets were placed in microfuge tubes, to which 0.5 ml of the bicarbonate-buffered medium containing 4 mM glucose, 100 μM 3-isobutyl-1-methylxanthine (IBMX) and the test reagents was added. After an incubation period of 5 min at 37°C, the tubes were centrifuged briefly (9000 *g*) and the supernatant replaced with 200 μl of 80% ethanol. Samples were sonicated for 5 s at low power and then freeze dried. The amount of cyclic AMP in the resultant pellets was initially determined by use of a protein binding assay (Brown *et al.*, 1971). The sensitivity of this assay was such that small changes

¹ Author for correspondence.

in islet cyclic AMP were difficult to quantitate and later experiments made use of a more sensitive commercially obtained [¹²⁵I]-cyclic AMP radioimmunoassay (RIA) kit. For these experients, fewer islets were required (40/tube), and pellets were reconstituted in sodium acetate buffer (pH 6.2), provided in the RIA kit.

Materials

Clenbuterol, atenolol and ICI 118551 (erythro-d,l-1 (7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) were kindly donated by ICI Pharmaceuticals, and crystalline biosynthetic human insulin was a gift from Eli Lilly & Co. Forskolin, 3-isobutyl-1-methylxanthine, L-arginine and collagenase (type XI) were obtained from Sigma Chemical Co. Guinea-pig anti-glucagon serum was a gift from Dr P.M. Jones, Biomedical Sciences Division, King's College, London. [¹²⁵I]-cyclic AMP RIA kit was from Du Pont (UK) Ltd.

Statistical analysis

All data are presented as mean values ± s.e.mean. Statistical significance was assessed by Student's *t* test for unpaired data.

Results

At a concentration of 1 μM, the β₂-adrenoceptor agonist clenbuterol significantly increased the level of cyclic AMP in rat isolated islets incubated in the presence of 4 mM glucose and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; 100 μM; Table 1). It was noted that in different experiments the basal level of cyclic AMP was somewhat variable, but the magnitude of the response to 1 μM clenbuterol was consistent, and the extent of elevation of cyclic AMP was not further increased when 20 μM clenbuterol was used (control: 1.39 ± 0.26; 1 μM clenbuterol: 2.69 ± 0.31; 20 μM clenbuterol: 2.69 ± 0.33 fmol cyclic AMP/islet). The response to clenbuterol (20 μM) was transient, since a three fold increase in cyclic AMP could be detected as early as 2 min but this increase was no longer evident after 15 or 30 min of incubation (Figure 1). The stimulation of cyclic AMP by 1 μM clenbuterol was totally blocked by addition of the β₂-adrenoceptor antagonist, ICI 118551, at both concentrations tested (0.1 and 10 μM), whilst the β₁-antagonist atenolol did not significantly alter the cyclic AMP response to clenbuterol (Table 1). The adenylate cyclase activator forskolin was used as a positive control in these experiments and was able to increase cyclic AMP to levels greater than those observed with clenbuterol alone (Table 1). Neither ICI 118551 nor atenolol altered islet cyclic AMP in the absence of agonist (data not presented).

Whilst raising the glucose concentration from 4 to 20 mM

Table 1 Effects of selective β-adrenoceptor antagonists on clenbuterol-induced changes in cyclic AMP levels in rat isolated islets

Clenbuterol (μM)	ICI 118551 (μM)	Atenolol (μM)	Forskolin (μM)	Cyclic AMP (fmol/islet)
—	—	—	—	7.59 ± 0.67 (15)
1	—	—	—	11.25 ± 0.77 (23) ^a
1	0.1	—	—	5.60 ± 0.93 (8) ^b
1	10	—	—	7.80 ± 0.67 (19) ^c
1	—	0.1	—	10.74 ± 1.03 (4) ^{NS}
—	—	—	10	30.80 ± 2.90 (11) ^b

Islets were incubated for 5 min in the presence of 4 mM glucose, 100 μM 3-isobutyl-1-methylxanthine and test reagents as shown, prior to measurement of cyclic AMP levels by RIA. Data are presented as mean values ± s.e.mean. Numbers of observations are shown in parentheses. ^a*P* < 0.001 relative to control; ^b*P* < 0.001 relative to clenbuterol alone; ^c*P* < 0.005 relative to clenbuterol alone. NS, not significant relative to clenbuterol alone.

Figure 1 Time course of the effect of the selective β-adrenoceptor agonist, clenbuterol, on cyclic AMP levels in rat isolated islets. Islets were incubated for 2, 5, 15 or 30 min at 37°C, in the presence of 4 mM glucose, 100 μM 3-isobutyl-1-methylxanthine and 20 μM clenbuterol. The amount of cyclic AMP in the resultant pellets was determined by a protein binding assay. Results are expressed as % of control of 6–12 observations; s.e.mean shown by vertical bars. Control values, incubated in the absence of clenbuterol, were as follows: 2 min: 0.71 ± 0.09; 5 min: 1.28 ± 0.15; 15 min: 11.5 ± 0.9; 30 min: 13.2 ± 1.4 fmol cyclic AMP/islet. ^a*P* < 0.005 relative to respective control value; ^b*P* < 0.001 relative to respective control value, NS, not significant relative to respective control value.

caused a marked rise in the rate of insulin secretion, addition of the β₂-adrenoceptor agonist at 20 μM did not further influence either basal or glucose-induced insulin secretion (Figure 2). Forskolin (10 μM) significantly potentiated insulin secretion induced by 20 mM glucose (Figure 2). Incubation of rat isolated islets in the presence of glucose 8 mM and IBMX 50 μM elicited a large increase in the release of insulin compared to the response observed with 8 mM glucose alone (Figure 3). However, even in the presence of IBMX, clenbuterol (10 μM) failed to raise the insulin secretory rate above that seen with 8 mM glucose and IBMX alone (Figure 3). In contrast, addi-

Figure 2 Effect of clenbuterol and forskolin on glucose-induced insulin secretion. Islets were incubated in the presence of 4 or 20 mM glucose and 20 μM clenbuterol (solid columns) or 10 μM forskolin (hatched columns) as shown. After 1 h, samples of the medium were assayed for their insulin contents. Data are presented as mean values for 7–8 observations; vertical bars show s.e.mean. ^aNot significant relative to 4 mM glucose alone. ^b*P* < 0.001 relative to 4 mM glucose alone; ^cnot significant relative to 20 mM glucose alone; ^d*P* < 0.005 relative to 20 mM glucose alone.

Figure 3 Effect of 3-isobutyl-1-methylxanthine (IBMX), clenbuterol and forskolin on insulin secretion. Rat isolated islets were incubated in the presence of glucose alone, or glucose and IBMX (50 μ M) as shown. In addition, the medium containing 8 mM glucose and IBMX was supplemented with either 10 μ M clenbuterol (solid columns) or 1 μ M forskolin (hatched columns). After 1 h, samples of the medium were removed and their insulin content determined. Data are presented as mean values of 8 observations; s.e.mean shown by vertical bars. * $P < 0.02$ relative to 4 mM glucose; ^b $P < 0.001$ relative to 8 mM glucose in the absence of IBMX; ^c $P < 0.01$ relative to 8 mM glucose and IBMX in the absence of forskolin.

tion of forskolin (1 μ M), under these conditions, significantly augmented insulin release (Figure 3).

By contrast with its lack of effect on insulin secretion, clenbuterol (20 μ M) caused a significant increase in the rate of glu-

Table 2 Effects of clenbuterol, arginine and forskolin on glucagon secretion in rat isolated islets

Clenbuterol (20 μ M)	Arginine (20 mM)	Forskolin (25 μ M)	Glucagon secretion (ng/islet h ⁻¹)
-	-	-	0.25 $\pm$ 0.02 (40)
+	-	-	0.51 $\pm$ 0.06 (18) ^a
-	+	-	0.52 $\pm$ 0.04 (14) ^a
-	-	+	0.56 $\pm$ 0.06 (19) ^a

Islets were incubated for 1 h in the presence of 4 mM glucose and test reagents as shown, prior to measurement of glucagon release by RIA. Data are presented as mean rates of glucagon secretion $\pm$ s.e.mean. Numbers of observations are shown in parentheses. ^a $P < 0.001$ relative to control.

Table 3 Effect of increasing concentrations of clenbuterol on glucagon secretion

Clenbuterol (μ M)	Glucagon secretion (ng/islet h ⁻¹)
0	0.48 $\pm$ 0.03 (7)
0.002	0.46 $\pm$ 0.07 (5)
0.02	0.48 $\pm$ 0.08 (8)
0.2	0.70 $\pm$ 0.04 (7) ^a
2	0.90 $\pm$ 0.07 (7) ^a
20	0.84 $\pm$ 0.05 (6) ^a

Islets were incubated as described in Table 2. Data are presented as mean rates of glucagon secretion $\pm$ s.e.mean. Numbers of observations are shown in parentheses. ^a $P < 0.001$ relative to absence of clenbuterol.

Table 4 Effect of the selective β_2 -adrenoceptor antagonist, ICI 118551, on clenbuterol-induced glucagon secretion

Clenbuterol (μ M)	ICI 118551 (μ M)		
	0	25	50
0	0.41 $\pm$ 0.04 (14)	0.51 $\pm$ 0.08 (4)	0.47 $\pm$ 0.06 (8)
1	0.82 $\pm$ 0.08 (10)	0.49 $\pm$ 0.05 (7) ^a	0.43 $\pm$ 0.02 (8) ^b
10	0.97 $\pm$ 0.10 (6)	0.59 $\pm$ 0.09 (5) ^a	ND
20	0.76 $\pm$ 0.10 (13)	0.85 $\pm$ 0.06 (4)	0.47 $\pm$ 0.05 (8) ^b

Islets were incubated under the conditions shown for 1 h, and the medium sampled for assay of glucagon secretion. Data are presented as mean rates of glucagon secretion (ng/islet h⁻¹) $\pm$ s.e.mean. Numbers of observations are shown in parentheses. ^a $P < 0.02$; ^b $P < 0.001$ relative to clenbuterol in the absence of ICI 118551. ND, not determined.

cagon release from islets incubated in the presence of 4 mM glucose (Table 2). The maximal extent of stimulation elicited by clenbuterol resulted in a two fold increase in secretion (Tables 2 and 3). Two other reported stimulators of glucagon secretion, L-arginine (20 mM) and forskolin (25 μ M) also produced increases in the rate of glucagon release of a similar magnitude (Table 2). The stimulation of glucagon secretion by clenbuterol was dose-dependent (Table 3) with a significant increase observed when islets were incubated with $\geq 0.2 \mu$ M clenbuterol.

The β_2 -adrenoceptor antagonist ICI 118551 (50 μ M) completely inhibited the glucagon secretory response to clenbuterol (1 or 20 μ M; Table 4). At a lower concentration (25 μ M) ICI 118551 was less effective as an inhibitor of the response induced by 20 μ M clenbuterol, but was still able to suppress completely secretion induced by 1 or 10 μ M clenbuterol (Table 4).

Discussion

There has been a considerable amount of work investigating the relationships between β - and α -adrenoceptor mechanisms in the regulation of insulin secretion from the endocrine pancreas. Most studies now concur that the α_2 -inhibitory mechanism is predominant (Morgan, 1987). However, a substantial body of evidence has also been presented to support a direct role for β -adrenoceptors in the stimulation of insulin secretion in many species (Porte, 1967; Iversen, 1973; Hermann & Deckert, 1977; Fyles *et al.*, 1986). Many of these results have been obtained from *in vivo* studies (Porte, 1967; Iversen, 1973; Ribes *et al.*, 1984; John *et al.*, 1990), where paracrine and other secondary influences may complicate the interpretation of the observed responses.

Studies on the direct effects of β -agonists on isolated islets have been most controversial. Two groups have reported the presence of binding sites having the pharmacological characteristics of β -adrenoceptors in islet preparations (Cherksey *et al.*, 1981; Fyles *et al.*, 1986), whereas others have concluded that islet B cells are totally unresponsive to β -agonism (Schuit & Pipeleers, 1986). Furthermore, it has been suggested that the limited stimulation of insulin secretion by β -adrenoceptor agonists in some reports may reflect a paracrine response to increased glucagon output (Zielmann *et al.*, 1985). In this context, β -agonists have been shown to increase adenylate cyclase activity in islet homogenates (Howell & Montague, 1973; Fyles *et al.*, 1986) suggesting that at least some of the cells present in rat islets do possess β -receptors that are functionally coupled to an intracellular messenger system (cyclic AMP). Our present study aimed to clarify the situation by investigating the effects of a selective β -adrenoceptor agonist on cyclic AMP levels, insulin and glucagon secretion in rat isolated islets.

We have shown that in rat islets the β_2 -agonist clenbuterol significantly increased the level of cyclic AMP, a response that

was totally inhibited by the β_2 -selective antagonist ICI 118551, but remained unaltered in the presence of an equivalent concentration of atenolol (β_1 -selective antagonist; Table 1). These results clearly demonstrate the presence of β_2 -adrenoceptors on islet cells that are functionally coupled to the adenylate cyclase system. Information as to the cellular distribution of these receptors can be deduced from the insulin and glucagon secretion studies.

We have confirmed our own (Lacey *et al.*, 1990a) and others (Schuit & Pipeleers, 1986) previous findings that rat isolated islets do not respond to β -agonists with a direct increase in insulin release, even in the presence of IBMX (Figures 2 and 3). The inclusion of IBMX would serve to enhance any intracellular rise in cyclic AMP provoked by agonist-induced adenylate cyclase activation. Since in most cells, a physiological response can be induced by a very modest increase in cyclic AMP, our failure to detect any direct effect of the β -agonist on insulin release, despite the ability of isolated islets to respond to a rise in cyclic AMP, indicates that functional β -receptors are not present on islet B cells.

In contrast, clenbuterol induced a significant increase in islet glucagon secretion which was of similar magnitude to that in response to 20 mM arginine, a previously reported stimulator of glucagon release (Faure *et al.*, 1988; Bjaaland *et al.*, 1988). This response was dose-dependent (Table 3) and could be inhibited by inclusion of the β_2 -antagonist ICI 118551 (Table 4). The sensitivity of clenbuterol-induced glucagon secretion to inhibition by ICI 118551 appeared to be lower than that of the cyclic AMP response (Table 2). This may reflect the difference in time course of the two processes. Cyclic AMP elevation in response to clenbuterol was transient (Figure 1) and was determined in the islet cell pellet at a single time point (5 min). In contrast, glucagon secretion was estimated by measuring the total hormone release integrated over a 60 min period. Despite these differences, it is clear that both responses were susceptible to inhibition by ICI 118551.

Taken together, our observations support the view that the A cell population is selectively responsive to β -agonists in isolated rat islets and that the observed β -mediated rise in cyclic AMP occurs in the A and not the B cells. It should be emphasised that we cannot conclude from these results that the cyclic AMP response is confined exclusively to the islet A cells, since a number of other cell types are also present in rat islets (Orci, 1985; Miller, 1981) which could express β -receptors. We are confident, however, in drawing the conclusion that the insulin secreting B-cells do not express β_2 -receptors that directly control insulin secretion in the rat.

It is also noteworthy that, under the conditions of our experiments, the increase in glucagon output resulting from stimulation by clenbuterol, was insufficient to cause a secondary stimulation of insulin secretion.

Our results support the findings of several other studies, both *in vivo* and *in vitro*, that an increase in glucagon secretion can be elicited via β -adrenoceptor agonism (Iversen, 1973; Gerich *et al.*, 1974; Itoh & Gerich, 1982); we now suggest that this is a β_2 -adrenoceptor response. It should be noted that this may not be an exclusive mechanism since *in vivo* studies in the mouse have suggested that glucagon secretion can also be mediated via α_2 -adrenoceptors (Ahren & Lundquist, 1987). Samols & Weir (1979) obtained similar results in the isolated perfused pancreas of the dog, but reported only a moderate rise in glucagon release following α -agonism, with a much larger increase due to β -adrenoceptor stimulation. However, the situation is not entirely clear since other studies have shown α -adrenoceptor stimulation to decrease plasma glucagon in man (Gerich *et al.*, 1974). Thus, the receptor-mediated control of glucagon secretion is likely to be very complex. α -Adrenoceptor, β_2 -adrenoceptor, muscarinic cholinergic and somatostatin receptors may all be present on A cells to control glucagon release (Kaneto *et al.*, 1974; Itoh & Gerich, 1982; Schuit & Pipeleers, 1986; Schuit *et al.*, 1989).

The discrepancies in the findings of different workers indicate to us that there is marked species variation in the adrenergic regulation of pancreatic hormone secretion. Indeed, Loubatières-Mariani *et al.* (1977) observed differential effects of β -agonists on insulin secretion *in vitro* in the dog and the rat. We have also recently shown that β -adrenoceptor agonists stimulate insulin secretion from human isolated islets, which contrasts sharply with the situation in rat (Lacey *et al.*, 1990a). It is not yet clear whether glucagon secretion is also under direct β -adrenoceptor control in man.

In conclusion, our results provide evidence to support the view that direct β -adrenoceptor stimulation of B cells does not occur in rat isolated islets. Our data strongly indicate that it is the A cells which possess functional β_2 -adrenoceptors and that the observed rise in glucagon secretion rate is mediated via a concomitant elevation in cyclic AMP levels.

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References

- AHREN, B. & LUNDQUIST, I. (1987). α -Adrenoceptor blockade by phentolamine inhibits β -adrenergically and cholinergically induced glucagon secretion in the mouse. *Horm. Metab. Res.*, **19**, 600–603.
- BJAALAND, T., HIL, C.S.T., JONES, P.M. & HOWELL, S.L. (1988). Role of protein kinase C in arginine-induced glucagon secretion from isolated rat islets of Langerhans. *J. Mol. Endocrinol.*, **1**, 105–110.
- BROWN, B.L., ALBANO, J.D.M., EKINS, R.P., SGHERZI, A.M. & TAMPION, W. (1971). A simple and sensitive saturation assay method for the measurement of adenosine 3':5'-cyclic monophosphate. *Biochem. J.*, **121**, 561–562.
- CHEKSEY, B., ALTSZULER, N. & ZADUNAISKY, J. (1981). Preponderance of β -adrenergic binding sites in pancreatic islet cells of the rat. *Diabetes*, **30**, 172–174.
- FAURE, A., HAOUARI, M. & SUTTER, B.C.J. (1988). Short term and direct influence of oestradiol on glucagon secretion stimulated by arginine. *Diabet. Metab.*, **14**, 452–454.
- FURMAN, B.L. & TAYO, F.M. (1974). Effect of some β -adrenoceptor blocking drugs on insulin secretion in the rat. *J. Pharm. Pharmacol.*, **26**, 512–517.
- FYLES, J.M., CAWTHORNE, M.A. & HOWELL, S.L. (1986). The characteristics of β -adrenergic binding sites on pancreatic islets of Langerhans. *J. Endocrinol.*, **111**, 263–270.
- GERICH, J.E., LANGLOIS, M., NOACCO, C., SCHNEIDER, V. & FORSHAM, P.E. (1974). Adrenergic modulation of pancreatic glucagon secretion in man. *J. Clin. Invest.*, **53**, 1441–1446.
- GEY, G.O. & GEY, M.K. (1936). Maintenance of human normal cells in continuous culture: preliminary report; cultivation of mesoblastic tumors and normal cells and notes on methods of cultivation. *Am. J. Cancer*, **27**, 45–76.
- HERMANN, L.S. & DECKERT, T. (1977). The effect of epinephrine and isoproterenol on insulin secretion and glucose utilization in isolated islets of Langerhans from mice. *Acta Endocrinol.*, **84**, 105–114.
- HOWELL, S.L. & MONTAGUE, W. (1973). Adenylate cyclase activity in isolated rat islets of Langerhans: effects of agents which alter rates of insulin secretion. *Biochim. Biophys. Acta*, **320**, 44–52.
- ITO, H. & GERICH, J.E. (1982). Adrenergic modulation of pancreatic somatostatin, insulin and glucagon secretion: evidence for differential sensitivity of islet A, B and D cells. *Metabolism*, **31**, 715–720.
- IVERSEN, J. (1973). Adrenergic receptors and the secretion of glucagon and insulin from the isolated, perfused canine pancreas. *J. Clin. Invest.*, **52**, 2102–2116.
- JOHN, G.W., DOXEY, J.-C., WALTER, D.S. & REID, J.L. (1990). The role of α - and β -adrenoceptor subtypes in mediating the effects of catecholamines on fasting glucose and insulin concentrations in the rat. *Br. J. Pharmacol.*, **100**, 699–704.
- KANETO, A., MIKI, E. & KOSAKA, K. (1974). Effects of vagal stimulation on glucagon and insulin secretion. *Endocrinology*, **95**, 1005–1010.
- LACEY, R.J., BERROW, N.S., LONDON, N.J.M., LAKE, S.P., JAMES, R.F.,

- SCARPELLO, J.H.B. & MORGAN, N.G. (1990a). Differential effects of β -adrenergic agonists on insulin secretion from pancreatic islets isolated from rat and man. *J. Mol. Endocrinol.*, **5**, 49–54.
- LACEY, R.J., BERROW, N.S., SCARPELLO, J.H.B. & MORGAN, N.G. (1990b). Activation of β -adrenoceptors in rat islets of Langerhans leads to enhanced glucagon but not insulin secretion. *Diabetologia*, **33** Suppl., A100.
- LEFKOWITZ, R.J. & CARON, M.G. (1988). Adrenergic receptors: models for the study of receptors coupled to guanine nucleotide regulatory proteins. *J. Biol. Chem.*, **263**, 4993–4996.
- LOUBATIERES-MARIANI, M.M., CHAPAL, J., RIBES, G. & LOUBATIERES, A.L. (1977). Discrepancies in the response of the insulin secreting cells of the dog and rat to different adrenergic stimulating agents. *Acta Diabet. Latina*, **14**, 144–155.
- MASSARA, F., FASSIO, V., CAMANNI, F. & MOLINATT, G.M. (1975). Salbutamol-induced increase in plasma insulin in man. *Horm. Metab. Res.*, **7**, 94–96.
- MILLER, R.E. (1981). Pancreatic neuroendocrinology: peripheral neural mechanisms in the regulation of the islets of Langerhans. *Endocrine Rev.*, **2**, 471–494.
- MONTAGUE, W. & TAYLOR, K.W. (1968). Pentitols and insulin release by isolated rat islets of Langerhans. *Biochem. J.*, **109**, 333–370.
- MORGAN, N.G. (1987). Regulation of insulin secretion by α_2 -adrenergic agonists. *Trends Pharmacol. Sci.*, **8**, 369–370.
- MORGAN, N.G. & MONTAGUE, W. (1985). Studies on the mechanism of inhibition of glucose-stimulated insulin secretion by noradrenaline in rat islets of Langerhans. *Biochem. J.*, **226**, 571–576.
- NAKAKI, T., NAKADATE, T., YAMAMOTO, S. & KATO, R. (1983). Inhibition of dibutyryl cyclic AMP-induced insulin release by α_2 -adrenergic stimulation. *Life Sci.*, **32**, 191–195.
- ORCI, L. (1985). The insulin factory: a tour of the plant surroundings and a visit to the assembly line. *Diabetologia*, **28**, 528–546.
- PORTE, D. (1967). β -Adrenergic stimulation of insulin release in man. *Diabetes*, **16**, 150–155.
- RIBES, G., TRIMBLE, E.R., BLAYAC, J.P., WOLLHEIM, C.B. & LOUBATIERES-MARIANI, M.M. (1984). *In vivo* stimulation of pancreatic hormone secretion by norepinephrine infusion in the dog. *Am. J. Physiol.*, **246**, E339–E343.
- SAMOLS, E. & WEIR, G.C. (1979). Adrenergic modulation of pancreatic A, B and D cells: α -adrenergic suppression and β -adrenergic stimulation of somatostatin secretion, α -adrenergic stimulation of glucagon secretion in the perfused dog pancreas. *J. Clin. Invest.*, **63**, 230–238.
- SCHUIT, F.C. & PIPELEERS, D.G. (1986). Differences in adrenergic recognition by pancreatic A and B cells. *Science*, **232**, 875–877.
- SCHUIT, F.C., DERDE, M.-P. & PIPELEERS, D.G. (1989). Sensitivity of rat pancreatic A and B cells to somatostatin. *Diabetologia*, **32**, 207–212.
- ZIELMANN, S., SCHUTTE, G., LENZEN, S. & PANTEN, U. (1985). Effects of isoprenaline and glucagon on insulin secretion from pancreatic islets. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **329**, 299–304.

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