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Interactions between imidazoline compounds and sulphonylureas in the regulation of insulin secretion

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1 Imidazoline α_2-antagonist drugs such as efaroxan have been shown to increase the insulin secretory response to sulphonylureas from rat pancreatic B-cells. We have investigated whether this reflects binding to an islet imidazoline receptor or whether α_2-adrenoceptor antagonism is involved.

2 Administration of (±)-efaroxan or glibenclamide to Wistar rats was associated with a transient increase in plasma insulin. When both drugs were administered together, the resultant increase in insulin levels was much greater than that obtained with either drug alone.

3 Use of the resolved enantiomers of efaroxan revealed that the ability of the compound to enhance the insulin secretory response to glibenclamide resided only in the α_2-selective-(+)-enantiomer; the imidazoline receptor-selective-(−)-enantiomer was ineffective.

4 *In vitro*, (+)-efaroxan increased the insulin secretory response to glibenclamide in rat freshly isolated and cultured islets of Langerhans, whereas (−)-efaroxan was inactive. By contrast, (+)-efaroxan did not potentiate glucose-induced insulin secretion but (−)-efaroxan induced a marked increase in insulin secretion from islets incubated in the presence of 6 mM glucose.

5 Incubation of rat islets under conditions designed to minimize the extent of α_2-adrenoceptor signalling (by receptor blockade with phenoxybenzamine; receptor down-regulation or treatment with pertussis toxin) abolished the capacity of (+)-and (±)-efaroxan to enhance the insulin secretory response to glibenclamide. However, these manoeuvres did not alter the ability of (±)-efaroxan to potentiate glucose-induced insulin secretion.

6 The results indicate that the enantiomers of efaroxan exert differential effects on insulin secretion which may result from binding to effector sites having opposite stereoselectivity. Binding of (−)-efaroxan (presumably to imidazoline receptors) results in potentiation of glucose-induced insulin secretion, whereas interaction of (+)-efaroxan with a second site leads to selective enhancement of sulphonylurea-induced insulin release.

Keywords: Endocrine pancreas; efaroxan; glibenclamide; diabetes mellitus; anti-hyperglycaemic drugs; pancreatic β-cell

Introduction

It is well established that many compounds which inhibit the outward flow of potassium ions through ATP-sensitive potassium (K-ATP) channels in pancreatic B-cells are able to potentiate nutrient-induced insulin secretion. Among these agents, sulphonylureas are the best studied group and currently represent the drugs of choice for treatment of the insulin secretory defect frequently associated with non-insulin-dependent diabetes mellitus (NIDDIM) (Henquin, 1992; Satin, 1996). However, recent studies have shown that a second group of compounds, characterized by the possession of an imidazoline ring (or a closely related moiety) can also elicit an increase in insulin secretion by inducing closure of K-ATP channels (Schulz & Haselblatt, 1988; Chan & Morgan, 1990; Dunne, 1991; Jonas *et al*., 1992; Brown *et al*., 1993a, b; Chan *et al*., 1993; Chan, 1993; Berdeu *et al*., 1995). Such molecules may, therefore, represent a useful new class of therapeutic drugs in NIDDM and several imidazoline compounds have already shown promise as anti-hyperglycaemic agents in man (Broadstone *et al*., 1987; Kawazu *et al*., 1987; Berlin *et al*., 1994).

While there is good evidence that imidazoline and sulphonylurea compounds are effective insulin secretagogues in their own right, it has also been demonstrated that combinations of these drugs can represent particularly efficacious formulations. For example in rat, administration of high doses of sulphonylurea in combination with the imidazoline efaroxan leads to a much greater rise in circulating insulin than can be obtained with either drug alone (Berridge *et al*., 1992). These results imply that imidazoline and sulphonylurea drugs can interact functionally at the level of the pancreatic B-cell, a concept strengthened by the recent observation that a molecule having imidazoline antagonist activity in islets (RX801080) also inhibits the insulin secretory response to glibenclamide (Brown *et al*., 1993a).

Many of the imidazoline compounds which promote an increase in insulin secretion are also α_2-antagonists and their effects *in vivo* were initially thought to be mediated by the blockade of endogenous α_2-adrenoceptor-mediated tone which acts to restrain the rate of insulin secretion (Broadstone *et al*., 1987; Berlin *et al*., 1994). While this mechanism may account for part of their effects, the more recent discovery of a second mode of action for imidazoline compounds (which involves binding to an 'imidazoline receptor' associated with K-ATP channels) has prompted the suggestion that their insulin secretagogue activity may result primarily from agonist activity at an islet imidazoline receptor rather than antagonism at α_2-adrenoceptors (Brown *et al*., 1993b; Chan 1993; Berdeu *et al*., 1995; Morgan *et al*., 1995; Wang *et al*., 1996). However, it is not known which of these two possible mechanisms (i.e. imidazoline receptor agonism or α_2-adrenoceptor antagonism) accounts for the ability of imidazoline compounds to enhance the insulin secretory effects of sulphonylureas.

In the present study we have addressed this question by taking advantage of the finding that one of the most effective imidazoline α_2-antagonist insulin secretagogues, efaroxan, exists in two enantiomeric forms which display opposite

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selectivity for imidazoline receptors and α_2-adrenoceptors (Chan *et al*., 1993). (+)-Efaroxan is a potent α_2-antagonist but has minimal imidazoline receptor-mediated insulin-secretagogue activity *in vitro*, whereas (−)-efaroxan is a weakly potent α_2-antagonist but stimulates insulin secretion strongly (Chan *et al*., 1993; Morgan *et al*., 1995). We have now investigated the effects of these two enantiomeric forms of efaroxan on insulin secretion induced by the sulphonylurea glibenclamide, to establish whether α_2-antagonism or imidazoline receptor activation is the more important determinant for the enhancing effect of efaroxan.

Methods

in vivo *studies*

For assessment of the effects of imidazoline drugs and sulphonylureas on plasma insulin levels in rat, blood samples were removed from conscious male Wistar rats (200–300g body weight) via indwelling catheters permitting arterial sampling. Animals were treated with antibiotic (Synulox; 0.1 ml 100 g^{-1}) 1 h before the induction of anaesthesia by intramuscular injection of Dormitor (30 μl 100 g^{-1}) and intraperitoneal administration of Sublimase (0.6 ml 100 g^{-1}). Catheters were introduced into the femoral artery and fed to the abdominal portion of the aorta. The catheters were exteriorized at the back of the neck. The anaesthesia was reversed with Antisedan & Nubain (20 μl 100 g^{-1}) and the animals housed individually for a period of 7 days before experimentation.

For each experiment, animals were fasted overnight and placed in restraint cages. A zero time blood sample was removed and the animals were then dosed orally with test reagents or saline vehicle. Further blood samples were removed at indicated time points, for measurement of insulin levels by radioimmunoassay by use of a commercial guinea-pig anti-insulin serum (Sigma, U.K.) and human insulin standards.

in vitro *studies*

Effects of test reagents on insulin secretion *in vitro* were measured in islets of Langerhans isolated from male Wistar rats by collagenase digestion. Islets were selected by hand under a dissecting microscope to minimize the contaminating exocrine tissue. Islets were either used immediately ('fresh islets') or were cultured in RPMI-1640 for 18 h with reagents, as described in the Results section, before the assessment of their secretory function. These methods have been described in detail previously (Brown *et al*., 1993a, b; Chan *et al*., 1993).

Insulin secretion experiments involved incubation of groups of 3 isolated islets of Langerhans in 0.5 ml of a bicarbonate-buffered solution (Gey & Gey, 1936) gassed with $O_2:CO_2$ (95:5) and supplemented with varying concentrations of glucose, 1 mM calcium chloride and 1 mg ml^{-1} bovine serum albumin (fraction V). Incubation was performed at 37°C for 1 h, after which time samples of medium were removed for assay of insulin secretion by radioimmunoassay.

Statistical analysis

Results were processed by analysis of variance and differences between experimental groups were considered significant when $P<0.05$.

Materials

(±)-Efaroxan, its resolved enantiomers and glibenclamide were kindly provided by SmithKline Beecham Pharmaceuticals (Welwyn, Herts, U.K.). Idazoxan, phenoxybenzamine, antazoline, noradrenaline and pertussis toxin were purchased from Sigma Chemicals (Poole, Dorset, U.K.). UK14304 (5-bromo-6-[2-imidazolin-2-ylanino]-quinoxalene) was a gift from Pfizer Pharmaceuticals, Sandwich, Kent, UK. All other reagents were of analytical reagent grade.

Results

Effects of efaroxan and glibenclamide on circulating insulin levels in vivo

Initially, experiments were carried out *in vivo* in rats, to confirm that (±)-efaroxan and glibenclamide can interact functionally to elicit a potentiated rise in plasma insulin levels and to establish the time course of this response. Oral administration of a single dose of (±)-efaroxan (10 mg kg^{-1} body weight) to fasted rats, resulted in a significant rise in plasma insulin with 30 min, compared to saline treated animals. This was maintained for up to 1 h, after which time the insulin levels declined towards the basal value (Figure 1). Treatment of rats with glibenclamide also resulted in a rise in plasma insulin levels, and the extent and time course of this increase was identical to that seen after administration of (±)-efaroxan (Figure 1). When both drugs were administered together, the resultant rise in insulin was much larger than that observed with either agent alone (Figure 1) and circulating insulin was maintained at a high level for the entire duration of the experiment.

The effects of the enantiomers of efaroxan on plasma insulin levels were then explored, but lower doses were used (3.5 mg kg^{-1}) because of the limited availability of these reagents (Figure 2). In common with the racemate (3.5 mg kg^{-1}) each enantiomer of efaroxan induced a small increase in insulin levels within 45 min of administration to fasted rats (Figure 2a) and this effect was lost by 90 min (Figure 2b). However, a marked difference between the enantiomers was observed when the drugs were administered in combination with glibenclamide. As expected, racemic (±)-efaroxan caused a significant potentiation of the glibenclamide response (Figure 2a and b) and a similar effect was also seen when animals were treated with (+)-efaroxan in the presence of glibenclamide. However, the combination of (−)-efaroxan and glibenclamide did not elicit any increase in insulin levels beyond that induced by each drug alone (Figure 2a and b).

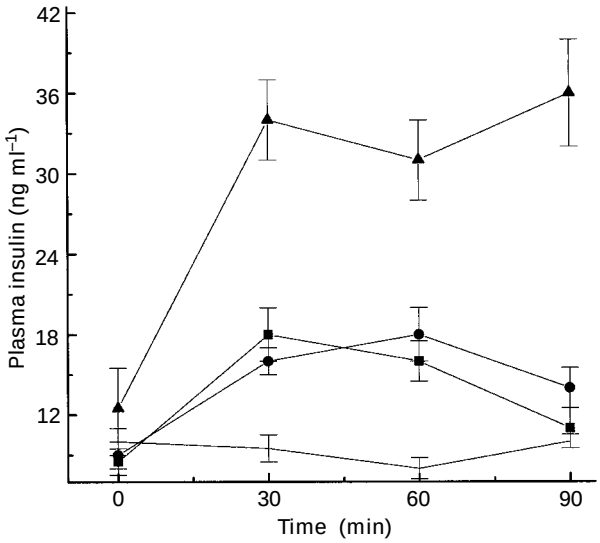

Figure 1 Effects of efaroxan and glibenclamide on plasma insulin levels in fasted rats. Groups of experimental animals were fasted overnight then treated orally with saline (control, no symbol) racemic efaroxan (10 mg kg^{-1}, ■), glibenclamide (2.5 mg kg^{-1}, ●) or efaroxan plus glibenclamide (▲). Blood samples were removed at the times indicated and assayed for insulin by radioimmunoassay. Data are presented as mean values and vertical lines show s.e.mean ($n=8$ animals per group). Combinations of (±) efaroxan and glibenclamide induced a statistically significant increase in insulin levels beyond that with each agent alone ($P<0.001$) at the 30, 60 and 90 minute time points.

Effects of the enantiomeric forms of efaroxan on insulin secretion in vitro

The actions of the enantiomers of efaroxan on insulin secretion were explored further in *in vitro* experiments employing isolated islets of Langerhans. As expected, stimulation of insulin secretion (in the presence of 6 mM glucose) by the two enantiomers revealed a difference in potency (Figures 3 and 4) with the (−)-enantiomer being far more effective than (+)-efaroxan over the dose range 10–50 μM. Examination of the interaction between the racemate and each isomer of efaroxan (25 μM) with glibenclamide *in vitro* revealed a surprising result (Table 1). Racemic efaroxan increased the insulin secretory response to glibenclamide significantly and a similar response was observed with the (+)-enantiomer, even though this drug did not induce any significant increase in insulin secretion, alone. By contrast, despite causing an increase in insulin secretion itself, (−)-efaroxan did not potentiate the response to glibenclamide (Table 1).

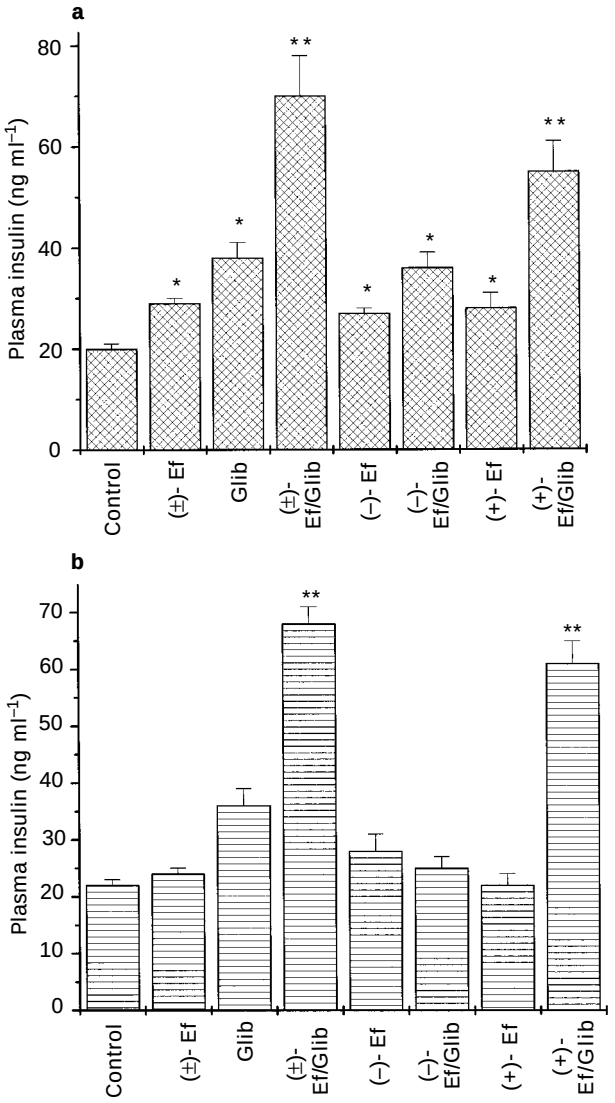

Figure 2 Effects of racemic efaroxan and the resolved enantiomers on plasma insulin levels in fasted rats. Groups of experimental animals were fasted overnight then dosed orally with racemic efaroxan (3.5 mg kg^{-1}, (±)-Ef), (−)-efaroxan (3.5 mg kg^{-1}), (+)-efaroxan (3.5 mg kg^{-1}) or glibenclamide (2.5 mg kg^{-1}, Glib) as shown. Blood samples were removed after 45 min (a) or 90 min (b) and assayed for insulin by radioimmunoassay. Results are presented as mean values ±s.e.mean for 8 animals per group. *$P<0.01$ relative to saline control. **$P<0.001$ relative to either efaroxan or glibenclamide alone.

The interactions of (+)-efaroxan with glibenclamide were then investigated over a wider range of concentrations (Figure 4). (+)-Efaroxan did not directly promote insulin secretion from rat isolated islets at concentrations up to 50 μM (Figure 4). Glibenclamide (1 μM) induced a significant rise in insulin secretion from rat isolated islets and addition of increasing concentrations of (+)-efaroxan resulted in further enhancement of the secretory response to glibenclamide, in a dose-dependent manner (Figure 4). (−)-Efaroxan did not produce a significant effect over this concentration range (not shown).

Effects of other imidazoline and α_2-antagonist drugs on insulin secretion from rat islets

The study was extended by investigation of the effects of two further imidazoline drugs on the insulin secretory response to glibenclamide in rat isolated islets. Idazoxan is a potent α_2-

Table 1 Effects of racemic efaroxan and its resolved enantiomers on glibenclamide-induced insulin secretion from rat isolated islets of Langerhans

Efaroxan (25 μM)	*Glibenclamide* (1 μM)	*Insulin secretion* (ng/islet h^{-1})
None	Absent	0.70±0.05
None	Present	1.25±0.15*
(±)	Absent	1.15±0.15*
(±)	Present	1.85±0.08**
(−)	Absent	1.40±0.15*
(−)	Present	1.50±0.18*,NS
(+)	Absent	0.95±0.09
(+)	Present	1.90±0.20**

Groups of 3 isolated islets were incubated in the presence of 6mM glucose in the presence or absence of test reagents, as shown, for 1 h, after which time the medium was sampled and assayed for insulin secretion. Efaroxan was used as the racemate ((±)) or as the resolved enantiomers, shown as (−) or (+). Results are presented as mean values±s.e.mean for 12–16 observations in each case. *$P<0.01$ relative to absence of efaroxan or glibenclamide. **$P<0.01$, relative to efaroxan or glibenclamide alone. NSNot significantly different from (−)-efaroxan in the absence of glibenclamide.

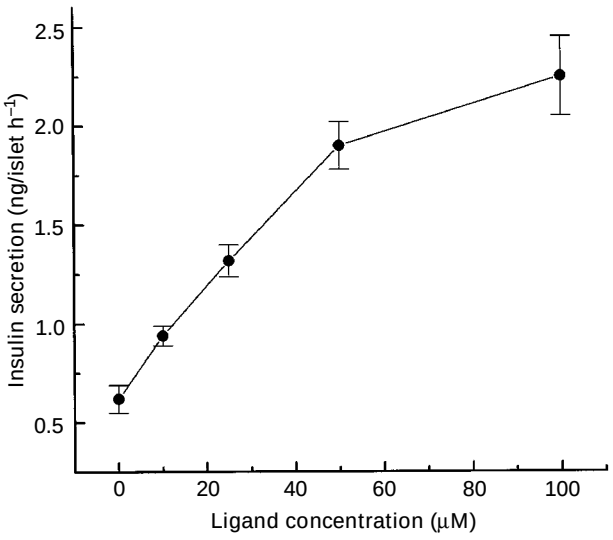

Figure 3 Stimulation of insulin secretion by (−)-efaroxan. Groups of rat isolated islets were treated with (−)-efaroxan at increasing concentrations in the presence of 6 mM glucose. Following incubation for 1 h, samples of the incubation medium were removed and assayed for insulin secretion. Data represent mean rates of insulin secretion ($n=12$); vertical lines show s.e.mean.

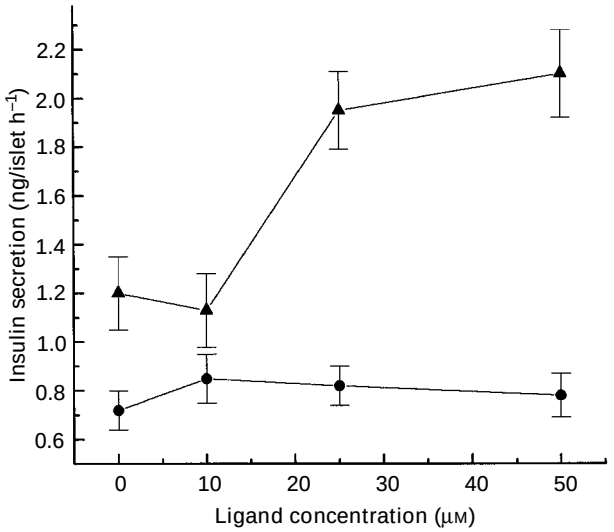

Figure 4 Effects of (+)-efaroxan on insulin secretion in the presence and absence of glibenclamide. Groups of rat isolated islets were treated with (+)-efaroxan at increasing concentrations in the presence of 6 mM glucose (●) or 6 mM glucose plus 1 μM glibenclamide (▲). Following incubation for 1 h samples of the incubation medium were removed and assayed for insulin secretion. Data represent mean rates of insulin secretion ($n=12-18$); vertical lines show s.e.mean.

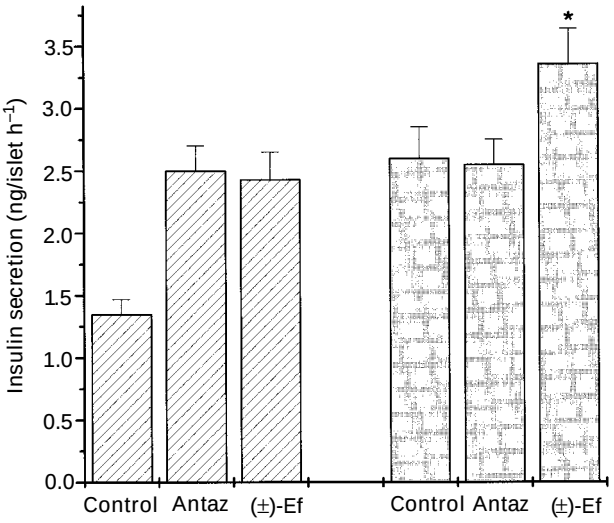

Figure 6 Effects of antazoline and racemic efaroxan on insulin secretion in the presence and absence of glibenclamide. Groups of rat isolated islets were treated with antazoline (50 μM; Antaz) or (±)-efaroxan (50 μM; Ef) in the presence of 6 mM glucose alone (hatched columns) or 6 mM glucose plus 1 μM glibenclamide (stippled columns). Following incubation for 1 h, samples of the incubation medium were removed and assayed for insulin secretion. Data represent mean rates of insulin secretion ±s.e.mean ($n=12$). *$P<0.01$ relative to glibenclamide alone.

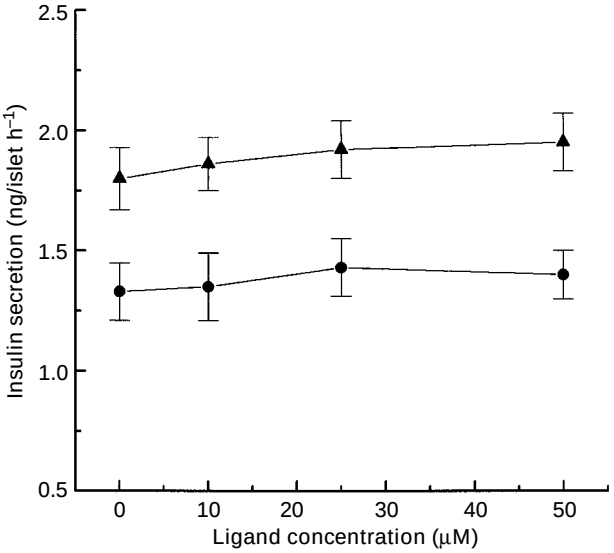

Figure 5 Effects of idazoxan on insulin secretion in the presence and absence of glibenclamide. Groups of rat isolated islets were treated with idazoxan at increasing concentrations in the presence of 6 mM glucose (●) or 6 mM glucose plus 1 μM glibenclamide (▲). Following incubation for 1 h, samples of the incubation medium were removed and assayed for insulin secretion. Data represent mean rates of insulin secretion ($n=12-18$); vertical lines show s.e.mean.

antagonist which, in rat islets, lacks the ability to stimulate insulin secretion via imidazoline receptor-mediated blockade of K-ATP channels (Figure 5; Chan & Morgan, 1990; Berdeu *et al*., 1995). Despite its potency as an α_2-antagonist (which is similar to (±)-efaroxan; Stillings *et al*., 1985) idazoxan failed to enhance the secretory response to glibenclamide in rat isolated islets (Figure 5). Similarly, antazoline (an imidazoline insulin secretagogue which has very low affinity for α_2-adrenoceptors; Berdeu *et al*., 1995) did not enhance the secretory response to glibenclamide under conditions where it caused a direct stimulation of insulin secretion in the absence of the sulphonylurea (Figure 6).

Effect of manoeuvres designed to inhibit α_2-adrenoceptor responses, on the ability of efaroxan to potentiate the insulin secretory response to glibenclamide

In the final section of the work, a number of different experimental paradigms were devised to minimize the extent of a α_2-adrenoceptor signalling in rat isolated islets of Langerhans. The consequences of these manoeuvres on the ability of efaroxan to potentiate the insulin secretory response to glibenclamide were studied.

Initially, the covalent α_2-antagonist phenoxybenzamine (Pbz) was used to inactive irreversibly islet α_2-adrenoceptors. Rat isolated islets were treated with either saline (control) or Pbz (0.1 μM) during a 30 min preincubation period, then exposed to test reagents (Figure 7). In control islets, glibenclamide alone increased insulin secretion and the addition of (+)-efaroxan, resulted in an additional enhancement of insulin secretion (Figure 7). In islets treated with Pbz, glibenclamide still elicited an increase in insulin secretion when added alone, but the combination of (+)-efaroxan and glibenclamide failed to enhance further insulin secretion under these conditions (Figure 7). Control experiments with the α_2-agonist noradrenaline confirmed that Pbz treatment resulted in complete α_2-blockade. Noradrenaline 1 μM failed to inhibit significantly glucose-induced insulin secretion after Pbz treatment, whereas it abolished insulin secretion from untreated islets (Figure 7).

In the next series of experiments, we exploited the results of previous studies showing that culture of rat islets in the presence of the selective α_2-agonist UK14304 leads to loss of α_2-responsiveness (Chan *et al*., 1993). In support of this, prior exposure of islets to 10 μM UK14304 for 18 h, resulted in complete loss of the ability of 1 μM noradrenaline to inhibit glucose-induced insulin secretion in subsequent incubation experiments (Figure 8). The response to noradrenaline was maintained in control islets not exposed to UK14304 during the culture period. The ability of either (±)-efaroxan or glibenclamide to promote insulin secretion was unaffected by culture with UK14304 (Figure 8), whereas the enhancement of

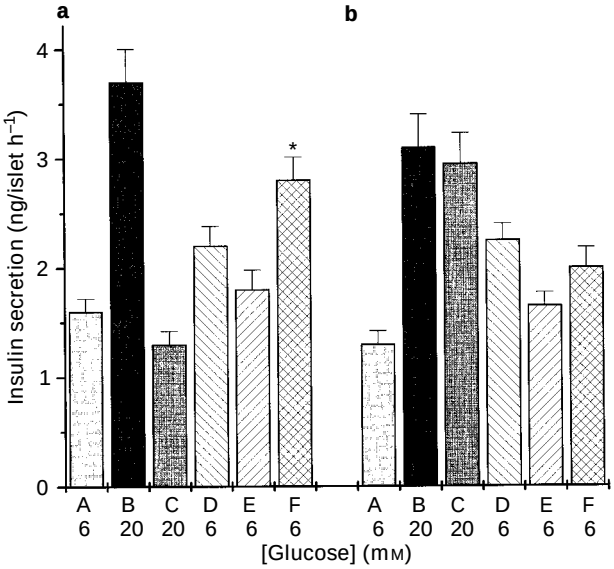

Figure 7 Effect of α_2-adrenoceptor blockade on the ability of efaroxan to increase glibenclamide-induced insulin secretion. Groups of isolated islets of Langerhans were preincubated for 30 min in the absence (a) or presence (b) of 0.1 μM phenoxybenzamine. They were then washed and exposed to test reagents: (B) 20 mM glucose; (C) 20 mM glucose +1 μM noradrenaline; (D) 1 μM glibenclamide; (E) 25 μM (+)-efaroxan; (F) (+)-efaroxan + glibenclamide, during a further incubation period of 60 min (A, control response to glucose). After this time the medium was sampled and assayed for insulin content. Data are presented as mean values ± s.e.mean ($n=12$). *$P<0.001$ relative to glibenclamide or efaroxan alone.

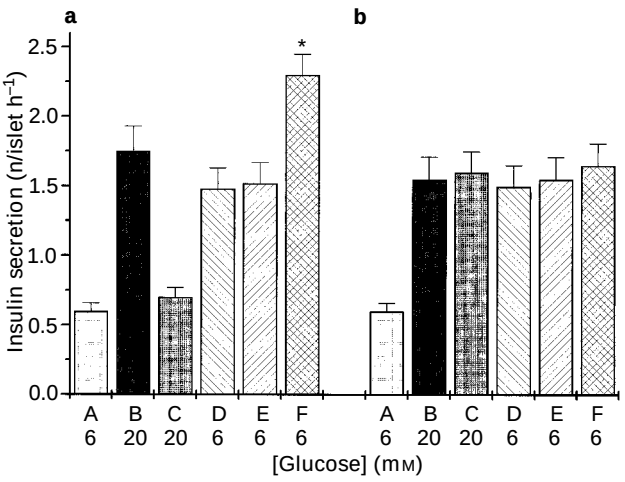

Figure 8 Effect of α_2-adrenoceptor downregulation on the ability of efaroxan to increase glibenclamide-induced insulin secretion. Groups of isolated islets of Langerhans were cultured for 18 h in the absence (a) or presence (b) of 1 μM UK14304. They were then washed and exposed to test reagents: (B) 20 mM glucose; (C) 20 mM glucose +1 μM noradrenaline; (D) 1 μM glibenclamide; (E) 50 μM (±)-efaroxan; (F) (±)-efaroxan + glibenclamide; during an incubation period of 60 min (A, control response to glucose). After this time the medium was sampled and assayed for insulin content. Data are presented as mean values ± s.e.mean ($n=24$). *$P<0.001$ relative to glibenclamide or efaroxan alone.

insulin secretion induced by the combination of (±)-efaroxan and glibenclamide in control islets, was lost after exposure to UK14304 (Figure 8).

Finally, groups of rat isolated islets were cultured for 18 h under either control conditions or in the presence of 200 ng ml^{-1} pertussis toxin. In the latter circumstances, subsequent exposure of the islets to noradrenaline (1 μM) did not lead to inhibition of glucose-induced insulin secretion (Figure 9), confirming that the α_2-adrenoceptors had become uncoupled from the control of insulin secretion by the pertussis toxin treatment. In these islets, the combination of (±)-efaroxan and glibenclamide did not enhance insulin secretion beyond that induced by either drug alone. As expected, the combination of (±)-efaroxan and glibenclamide did further enhance insulin secretion from the same batches of islets cultured in the absence of pertussis toxin (Figure 9).

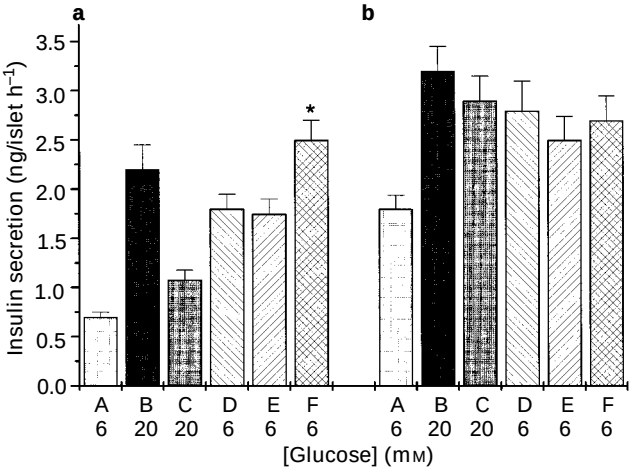

Figure 9 Effect of culture with pertussis toxin on the ability of efaroxan to increase glibenclamide-induced insulin secretion. Groups of isolated islets of Langerhans were cultured for 18 h in the absence (a) or presence (b) of 200 ng ml^{-1} pertussis toxin. They were then washed and exposed to test reagents: (B) 20 mM glucose; (C) 20 mM glucose +1 μM noradrenaline; (D) 1 μM glibenclamide; (E) 50 μM (±)-efaroxan; (F) efaroxan + glibenclamide, during an incubation period of 60 min (A, control response to glucose). After this time the medium was sampled and assayed for insulin content. Data are presented as mean values ± s.e.mean ($n=24$). *$P<0.001$ relative to efaroxan or glibenclamide alone.

Discussion

The results presented here demonstrate that, both *in vivo* and *in vitro*, combinations of (±)-efaroxan and glibenclamide cause a greater release of insulin from rat islets than when either drug is given alone. These data confirm the observations of Berridge *et al*. (1992) in fed rats, but extend the work to fasted animals and also reveal the isomeric specificity of the response. The results indicate that there may be a crucial mechanistic difference between the ability of imidazoline α_2-antagonists to potentiate glucose-induced insulin secretion and to enhance the secretory response to glibenclamide. In the former case, the response probably results from binding of drugs to imidazoline receptors associated with K-ATP channels, as suggested in previous work (Schulz & Haselblatt, 1988; Chan & Morgan, 1990; Dunne, 1991; Jonas *et al*., 1992; Brown *et al*., 1993a, b; Chan, 1993; Chan *et al*., 1993; Berdeu *et al*., 1995). By contrast, in the presence of glibenclamide, the (+) enantiomer of efaroxan (which is only very weakly potent as an imidazoline receptor ligand but is a potent α_2-antagonist; Chan *et al*., 1993) promoted a further rise in insulin secretion, despite the fact that it was ineffective in the absence of the sulphonylurea. Taken together, therefore, these observations suggest that the potentiation of glibenclamide-induced insulin secretion may involve the binding of imidazoline drugs to α_2-adrenoceptors rather than to imidazoline receptors.

In support of this conclusion, we have found that any one of a series of procedures designed to prevent or to minimize α_2-adrenoceptor signalling in islet cells also prevented the potentiation of glibenclamide-induced insulin secretion by efaroxan. This was true when the α_2-receptor was blocked

covalently with a low dose of phenoxybenzamine (Figure 7), when the receptor was down-regulated by exposure to the agonist UK14304 for 18 h (Figure 8) and when receptor-effector coupling was interrupted by culture in the presence of pertussin toxin (Figure 9). In each case the loss of α_2-responsiveness was confirmed by performing control experiments with noradrenaline. It is important to note that these experimental manipulations did not prevent the direct stimulation of insulin secretion by (±)-efaroxan in the presence of 6 mM glucose, suggesting that the islet imidazoline receptor is insensitive to UK14304 and that it is not coupled to K-ATP channels via a pertussis toxin-sensitive G-protein. Overall, therefore, the data imply that there may be a specific interaction between α_2-adrenoceptors and sulphonylurea responses in pancreatic B-cells, which can result in potentiation of sulphonylurea-induced insulin secretion.

This conclusion is at variance with the findings of Garrino and Henquin (1990) who argued that interactions between adrenoceptor ligands and sulphonylureas result from undefined 'non-specific' events. However, evidence for an interaction between sulphonylurea receptors and islet α_2-adrenoceptors has been provided in other work. For example, Sirek *et al*. (1974) showed that the α_2-antagonist dihydroergotamine could amplify the insulin secretory response to sulphonylureas in conscious dogs. In addition, Cherksey & Altszuler (1984) have suggested that sulphonylureas may influence the binding of ligands to α_2-adrenoceptors on pancreatic islet cells and Rustenbeck *et al*. (1995) have shown that treatment of islets with the α_2-antagonist phentolamine, in the presence of tolbutamide, results in an additive increase in cytosolic calcium concentration.

Despite the considerations above, there also remain a number of anomalies which do not fit readily with the concept that α_2-adrenoceptor blockade is important for the enhancement of glibenclamide-induced insulin secretion. For example, it is clear that blockade of the α_2-receptor with the drugs idazoxan or phenoxybenzamine did not directly promote any increase in the response to glibenclamide (Figure 7), whereas occupation of the receptor by phenoxybenzamine did prevent the enhancing effect of efaroxan. Furthermore, the concentrations of (±)-efaroxan required to enhance the secretory response to glibenclamide were in the range 10–50 μM (Figures 4 and 5). These levels seem unusually high given the potency of this drug as an α_2-adrenoceptor ligand.

Nevertheless, when taken as a whole, the results indicate that α_2-adrenoceptors exert a level of control over sulphonylurea-induced insulin secretion such that occupation of the receptors by certain antagonists results in enhancement of the response to sulphonylureas. It is conceivable that this might reflect the ability of selected antagonists to stabilize α_2-adrenoceptors in an inactive conformation and thereby limit their basal level of signalling activity. Such 'negative' agonism has been shown for some antagonists of G-protein coupled receptors (Schutz & Freissmuth, 1992) and could account for the present results. However, if this is the case, it remains to be explained why the response exhibits selectivity for sulphonylureas relative to glucose.

An alternative explanation for the present results cannot be excluded; namely that (+)-efaroxan acts as a selective agonist at a previously undefined 'imidazoline' receptor which can influence the functional activity of the sulphonylurea receptor. This site would need to be sensitive to phenoxybenzamine and UK14304 and coupled to a pertussis toxin-sensitive G-protein. No such receptor has yet been defined in islets, although it is noteworthy that the new insulin secretagogue S21663 displays properties which have led its developers to propose that it may interact with a novel 'imidazoline' receptor in the pancreatic B-cell (Wang *et al*., 1996). In addition, Zaitsev *et al*. (1996) have postulated that an undefined imidazoline receptor may control a distal step in B-cell exocytosis which lies beyond the regulation of potassium permeability.

In spite of the mechanistic ambiguities, the present data suggest that further consideration should be given to the development of therapeutic formulations in which selected α_2-antagonists are combined with sulphonylureas for the treatment of NIDDM. In this context, one additional point also merits comment. During the *in vivo* experiments with efaroxan, it was observed that both enantiomers caused a small, but statistically significant, rise in circulating insulin levels in the absence of glibenclamide. This implies that, *in vivo*, it may be possible to promote an increase in insulin release by either of two mechanisms – administration of an imidazoline receptor agonist (such as (−)-efaroxan) or treatment with a selective α_2-antagonist (e.g. (+)-efaroxan). Only in the latter case does co-administration of glibenclamide then lead to a still greater increase in secretion rate.

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