Br. J. Pharmacol. (1992), **107**, 8–14

Imidazoline antagonists of α_2-adrenoceptors increase insulin release *in vitro* by inhibiting ATP-sensitive K^+ channels in pancreatic β-cells

J.C. Jonas, *T.D. Plant & [1]J.C. Henquin

Unité de Diabétologie et Nutrition, University of Louvain, UCL 54.74, B-1200 Brussels, Belgium and *I. Physiologisches Institut, University of Saarland, D-6650 Homburg/Saar, Germany

1 Islets from normal mice were used to study the mechanisms by which imidazoline antagonists of α_2-adrenoceptors increase insulin release *in vitro*.

2 Alinidine, antazoline, phentolamine and tolazoline inhibited ^{86}Rb efflux from islets perifused with a medium containing 3 mM glucose, i.e. under conditions where many adenosine 5'-triphosphate (ATP)-sensitive K^+ channels are open in the β-cell membrane. They also reduced the acceleration of ^{86}Rb efflux caused by diazoxide, an opener of ATP-sensitive K^+ channels.

3 ATP-sensitive and voltage-sensitive K^+ currents were measured in single β-cells by the whole-cell mode of the patch-clamp technique. Antazoline more markedly inhibited the ATP-sensitive than the voltage-sensitive current, an effect previously observed with phentolamine. Alinidine and tolazoline partially decreased the ATP-sensitive K^+ current.

4 The four imidazolines reversed the inhibition of insulin release caused by diazoxide (through opening of ATP-sensitive K^+ channels) or by clonidine (through activation of α_2-adrenoceptors) in a concentration-dependent manner. Only the former effect correlated with the ability of each drug to increase control insulin release stimulated by 15 mM glucose alone.

5 It is concluded that the ability of imidazoline antagonists of α_2-adrenoceptors to increase insulin release *in vitro* can be ascribed to their blockade of ATP-sensitive K^+ channels in β-cells rather than to their interaction with the adrenoceptor.

Keywords: Imidazolines; K^+ channels; α_2-adrenoceptors; pancreatic β-cells; insulin release; alinidine; antazoline; phentolamine; tolazoline; diazoxide

Introduction

Several *in vivo* studies have shown that phentolamine augments basal and glucose-increased plasma insulin levels in normal subjects and animals (Cerasi *et al.*, 1969; Buse *et al.*, 1970; Misbin *et al.*, 1970; Lundquist, 1972; Ahren & Lundquist, 1985). Since insulin release is inhibited by activation of α_2-adrenoceptors in β-cells (Nakaki *et al.*, 1980), these observations have been interpreted as evidence for a suppression of β-cell function by a constant adrenergic tone. It has been further suggested that this tone could be excessive in non-insulin-dependent diabetic patients and contribute to their impaired β-cell function (Robertson *et al.*, 1976; Broadstone *et al.*, 1987). This concept, therefore, recently led to the development of α_2-adrenoceptor blockers as potential anti-diabetic agents (Kawazu *et al.*, 1987; Ortiz-Alonso *et al.*, 1991; Gautier *et al.*, 1991).

A number of *in vitro* studies have shown that phentolamine increases insulin release even in the absence of any agonist of α_2-adrenoceptors (Efendic *et al.*, 1975; Smith & Furman, 1988; Schulz & Hasselblatt, 1988; Garrino & Henquin, 1990). They suggested that the drug could have effects unrelated to blockade of α-adrenoceptors and possibly linked to its imidazoline structure (Schulz & Hasselblatt, 1989). The insulin-releasing property of phentolamine has recently been attributed to a blockade of ATP-sensitive K^+ channels in pancreatic β-cells (Plant & Henquin, 1990). These channels are indeed a key site of control of insulin release by glucose and several drugs: their closure accounts for the stimulation by tolbutamide, whereas their opening underlies the inhibition by diazoxide (Henquin & Meissner, 1982; Trube *et al.*, 1986). Besides phentolamine, it has also been suggested that two other imidazoline antagonists of α_2-adrenoceptors, efaroxan and midaglizole, increase insulin release by acting on adenosine 5'-triphosphate (ATP)-sensitive K^+ channels (Chan *et al.*, 1991a,b).

In the present study, we evaluated the effects of imidazoline derivatives structurally related to phentolamine on glucose-induced insulin release by pancreatic islets from normal mice. To determine whether their action on release is better explained by a blockade of α_2-adrenoceptors or of ATP-sensitive K^+ channels, we compared the ability of the drugs to relieve the inhibitions produced by clonidine and diazoxide respectively. The effects of these imidazoline derivatives on K^+ channels were also determined by measurements of ^{86}Rb efflux and by patch-clamp techniques.

Methods

Animals

All experiments were carried out with islets obtained by collagenase digestion of the pancreas of fed female NMRI mice.

^{86}Rb efflux experiments

These experiments were carried out at 37°C. After isolation, the islets were loaded with ^{86}Rb (Rb used as tracer for K) for 90 min in a medium containing 15 mM glucose and supplemented with $^{86}RbCl$ (1.5 to 3 MBq ml^{-1}; sp.act. 7.4 to 18.5 TBq mol^{-1}). The Rb concentration never exceeded 0.4 mM (Garrino & Henquin, 1988). ^{86}Rb efflux was then monitored in a dynamic perifusion system (Henquin, 1978). The

[1] Author for correspondence at: Unité de Diabétologie et Nutrition, UCL 54.74, Avenue Hippocrate, 54, B-1200 Brussels, Belgium.

radioactivity lost by the islets was measured immediately by the Cerenkov radiation (Henquin, 1978) in the effluent fractions collected at 2 min intervals. From the sum of the radioactivity remaining in the islets at the end of the experiments and the accumulated effluent radioactivity, the fractional efflux rate was calculated for each period (radioactivity lost by tissue during the time interval/radioactivity present in the tissue during that time interval) and expressed as percent per min. The solutions used had the following ionic composition (in mM): NaCl 120, KCl 4.8, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 24 and were gassed with 94% O_2/6% CO_2 to maintain a pH of 7.4. They were supplemented with 1 mg ml^{-1} bovine serum albumin fraction V (Boehringer, Mannheim, Germany).

Insulin release measurements

These experiments were also performed at 37°C, with solutions similar to those used for ^{86}Rb efflux experiments. After isolation, the islets were first preincubated for 60 min in a medium containing 15 mM glucose. They were then incubated for 60 min, in batches of 3, in 1 ml of medium containing 15 mM glucose and appropriate concentrations of test substances. At the end of the incubation, a portion of the medium was withdrawn and diluted before insulin assay. Insulin was measured by a double-antibody radioimmunoassay with rat insulin as standard (Novo Research Institute, Bagsvaerd, Denmark).

Electrical recordings

After isolation of the islets, islet cells were dispersed and cultured for 1–2 days as previously described (Plant, 1988). Patch-clamp experiments were performed at room temperature (20–24°C) on single β-cells. For measurements of ATP-sensitive and voltage-dependent K^+ currents the bath solution contained (in mM): NaCl 135, KCl 5.6, $CaCl_2$ 2.6, $MgCl_2$ 1.2, HEPES 10, and was titrated to pH 7.4 with NaOH. Pipettes were filled with a solution containing (in mM): KCl 135, $MgCl_2$ 4, $CaCl_2$ 2, EGTA 10, Na_2ATP 0.65, HEPES 20, and titrated to pH 7.15 with KOH. Details of the recording technique and the separation of ATP-sensitive and voltage-dependent currents from other membrane currents have been described previously (Garrino *et al.*, 1989; Plant & Henquin, 1990). In brief, ATP-sensitive K^+ currents were measured by recording the currents at 15 s intervals at the holding potential (− 70 mV) and during 100 ms pulses to − 60 and − 80 mV which were separated by an interval of 100 ms. Under the conditions used, the current which develops with time during dialysis is almost entirely ATP-sensitive K^+ current. To measure voltage-dependent K^+ currents, cells were held at − 70 mV and depolarized at 15 s intervals to 0 mV to activate K^+ currents in a bathing solution which was supplemented with 100 μM tolbutamide to block ATP-sensitive K^+ currents. A short (50 ms) hyperpolarization to − 100 mV, applied 100 ms before the test pulse, was used to estimate the leakage current.

Drugs

The following drugs were used: alinidine hydrobromide, ST 91 (2,6-diethyl-n-2 imidazolidinylidenebenzenamine) hydrochloride and clonidine hydrochloride (Boehringer-Ingelheim, Germany); phentolamine mesylate (Ciba-Geigy, Basel, Switzerland), tolazoline hydrochloride and antazoline hydrochloride (Sigma Chemical Co, St Louis, MO, U.S.A.); tramazoline hydrochloride (Thomae GmbH, Biberach, Germany); yohimbine hydrochloride (Aldrich-Chemie, Steinheim, Germany); diazoxide (Schering-Plough Avondale, Rathdrum, Ireland); tolbutamide-sodium salt (Hoechst AG, Frankfurt, Germany); tetraethylammonium chloride (Buchs, Switzerland). Stock solutions of diazoxide (50 mM) were prepared in 0.1 M NaOH. Other substances were dissolved in water before being added to the appropriate solutions. $^{86}RbCl$ was purchased from the Radiochemical Centre (Amersham, Bucks, U.K.).

Statistical analysis of results

Results are usually presented as means with s.e.mean for the indicated number of experiments. The statistical significance of differences between means was assessed by analysis of variance of the data, followed by a Dunnett's test.

Results

Effects on ^{86}Rb efflux

In the presence of 3 mM glucose, many ATP-sensitive K^+ channels in the β-cell membrane are open and the potassium permeability is high (Rorsman & Trube, 1985; Misler *et al.*, 1986; Ashcroft *et al.*, 1988). Under these conditions, the rate of ^{86}Rb efflux is high and declines slightly with time (Figure 1a). It was rapidly and reversibly decreased by tolbutamide, a selective blocker of ATP-sensitive K^+ channels (Trube *et al.*, 1986). Addition of 10 μM antazoline to the medium also inhibited ^{86}Rb efflux. In the steady state this inhibition was similar to that produced by 10 μM tolbutamide, but its onset was slower and it was not reversible (Figure 1a).

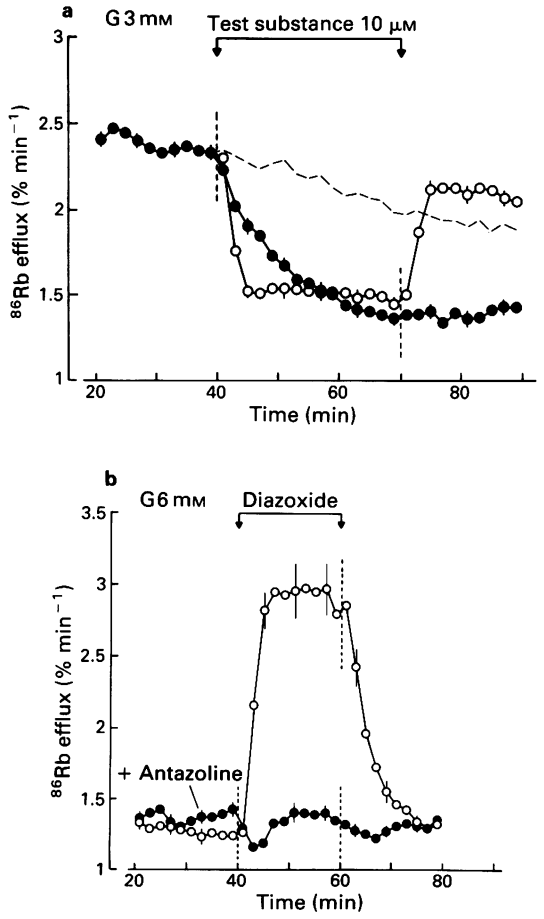

Figure 1 Effects of antazoline, tolbutamide and diazoxide on ^{86}Rb efflux from perifused mouse islets. (a) Antazoline (●) or tolbutamide (○) was added at a concentration of 10 μM to a medium containing 3 mM glucose (G). Controls without test substance are shown by the broken line. (b) Diazoxide (100 μM) was added to a medium containing 6 mM glucose (G) alone (○) or 6 mM glucose plus 10 μM antazoline (●). Values are means for 4–5 experiments with s.e.mean shown by vertical lines.

In similar experiments, five other imidazolines (alinidine, phentolamine, ST 91, tramazoline and tolazoline) produced a slow and irreversible inhibition of ^{86}Rb efflux. Yohimbine also produced a similar effect, which, however, was reversible. A quantitative assessment of these data is presented in Table 1. When the drugs were used at a concentration of 10 μM the order of potency was tolbutamide ⩾ antazoline ⩾ alinidine ⩾ phentolamine > ST 91 ⩾ tramazoline > tolazoline = yohimbine. The two weakest substances were also tested at 100 μM and yohimbine proved to be slightly more potent than tolazoline (Table 1).

Opening of ATP-sensitive K^+ channels by diazoxide (Trube *et al.*, 1986) in the presence of 6 mM glucose causes a marked acceleration of ^{86}Rb efflux that can be inhibited by phentolamine (Plant & Henquin, 1990). This effect of diazoxide was also antagonized by antazoline (Figure 1b) and by other imidazolines tested here (data not shown).

Effects on K^+ currents

As shown previously (Trube *et al.*, 1986; Garrino *et al.*, 1989), ATP-sensitive K^+ currents activate with time during dialysis of the cell interior with a low ATP concentration (Figure 2b). Addition of antazoline to the bathing solution inhibited the ATP-sensitive K^+ current. Effects of antazoline were visible at a concentration of 10 μM, but were difficult to differentiate from run-down of the current because the rate of block was slow. At a concentration of 100 μM, antazoline rapidly blocked the current, by 92 ± 1% (mean ± s.e.mean; $n = 9$). This effect of antazoline, in contrast to that of tolbutamide, was only poorly reversible (Figure 2). Little recovery of the current was observed after applications of antazoline for more than 3–4 min.

In similar experiments, 100 μM alinidine inhibited ATP-sensitive K^+ currents by 58 ± 5% ($n = 4$). Tolazoline had no detectable effect at 50 μM, but produced a small inhibition (by about 20%; $n = 3$) at 500 μM.

The effects of antazoline were also tested on the voltage-dependent K^+ current. In most experiments, currents were recorded in the presence of tolbutamide (100 μM) but without inhibition of the voltage-dependent Ca^{2+} current. With the intracellular Ca^{2+} buffering used in these experiments, a small rapidly-inactivating (100–200 ms) component of the current was probably Ca^{2+}-activated K^+ current activated by Ca^{2+}-entry (Smith *et al.*, 1990). This component is lost as Ca^{2+} currents run down during an experiment and accounts for the disappearance of the inactivating component (compare records A and C in Figure 3a). Antazoline (100 μM) caused a slow block of the current that reached a maximum after 3.5–4 min, but was almost completely reversible even after application of the drug for more than 10 min (Figure 3). This blockade was 69 ± 2% for the peak current and 68 ± 2% for the current at the end of the pulse, when the contamination by the Ca^{2+}-activated current is minimal. The inhibition by 100 μM antazoline was similar to that by tetraethylammonium ions (TEA^+) at a concentration of 4 mM (Figure 3d). The latter inhibition was, however, faster (within 1 min) than that produced by antazoline.

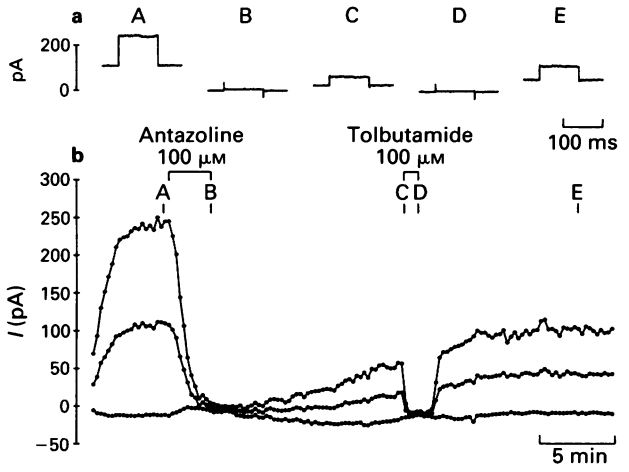

Figure 2 Inhibition of whole-cell ATP-sensitive K^+ currents by antazoline. (a) Currents recorded during 100 ms pulses from − 70 to − 60 mV at the times indicated by the letters in the experiment illustrated in (b). Outward currents are positive and shown as upward deflections. (b) Current values recorded at 15 s intervals at − 60, − 70 and − 80 mV (upper, middle and lower traces respectively). Antazoline (100 μM) was applied for 3 min as indicated by the bar. During recovery from the effects of antazoline, tolbutamide (100 μM) was applied for 1 min.

Table 1 Effects of various imidazolines, of yohimbine and of tolbutamide on ^{86}Rb efflux from mouse islets perifused with 3 mM glucose

Test substance (μM)	*Decrease in ^{86}Rb efflux*
—	5.1 ± 0.4
Antazoline 10	20.5 ± 1.5[b]
Alinidine 10	19.6 ± 2.6[b]
Phentolamine 10	17.9 ± 1.6[b]
ST91 10	14.9 ± 2.1[b]
Tramazoline 10	12.7 ± 1.3[b]
Tolazoline 10	8.1 ± 0.2[a]
Tolazoline 100	13.7 ± 0.7[b]
Yohimbine 10	8.0 ± 0.4[a]
Yohimbine 100	18.7 ± 1.0[b]
Tolbutamide 10	22.7 ± 1.2[b]

All substances were tested in experiments similar to those shown in Figure 1(a). The inhibition of ^{86}Rb efflux was calculated by integrating the area between a hypothetical horizontal line at the level of the average rate of efflux between 36 and 40 min (i.e. before drug addition) and the curve corresponding to the actual rate of efflux between 40 and 70 min (i.e. in the presence of the drug). This integration, which gives a dimensionless value, was calculated for each individual experiment. The value obtained in the absence of test substance reflects the small decrease of the control efflux rate between 40 and 70 min. Values are means ± s.e.mean for 4–5 experiments with each substance. [a]$P < 0.05$; [b]$P < 0.01$ versus controls in 3 mM glucose alone.

Effects on insulin release

Control insulin release by islets incubated in a medium containing 15 mM glucose alone amounted to 6.1 ± 0.1 ng per islet 60 min^{-1} ($n = 271$).

At a concentration of 0.1 μM, tramazoline and compound ST 91 inhibited glucose-induced insulin release by 71 ± 4% ($n = 16$) and 69 ± 3% ($n = 18$) respectively. These inhibitions were antagonized by yohimbine. Tramazoline and ST 91, therefore, behave like α_2-adrenoceptor agonists in pancreatic β-cells.

Figure 4a illustrates the concentration-dependent increase in insulin release brought about by 4 other imidazolines and by tolbutamide and yohimbine in the presence of 15 mM glucose alone. The relative potencies of the drugs were evaluated by determining the concentration at which they doubled insulin release (Table 2). The order of potency was tolbutamide > antazoline > phentolamine > alinidine >> yohimbine >> tolazoline. Interestingly, larger maximal increases in insulin release were produced by antazoline ($P < 0.05$) and phentolamine ($P < 0.01$) than by tolbutamide (Figure 4a).

Diazoxide inhibits glucose-induced insulin release by hyperpolarizing the β-cell membrane through a selective opening of ATP-sensitive K^+ channels (Henquin & Meissner, 1982; Trube *et al.*, 1986). The concentration of 40 μM diazoxide was chosen to achieve 85% inhibition of insulin release induced by 15 mM glucose (1.0 ± 0.1 ng per islet 60 min^{-1} vs

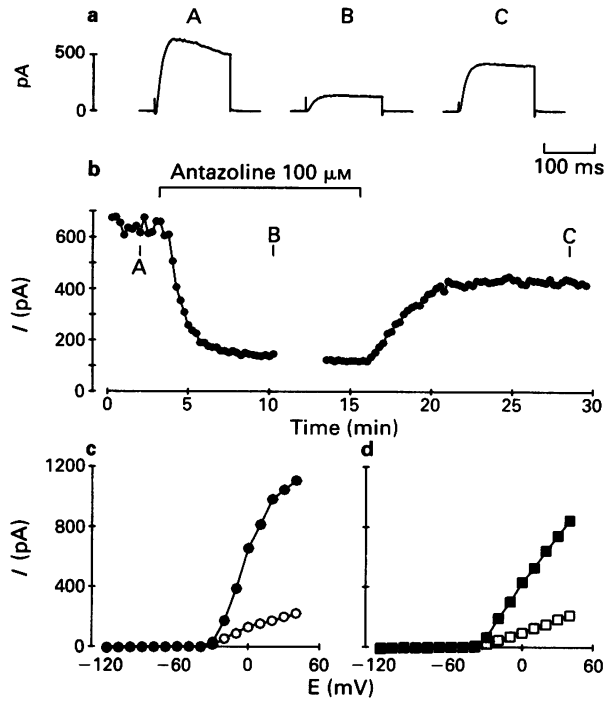

Figure 3 Effects of antazoline on voltage-dependent K^+ currents. (a) Mean currents determined from six consecutive records at the times indicated by the letters in (b). (b) Time course of the effects of antazoline (100 μM) on the maximum value of the voltage-dependent K^+ current during pulses from − 70 mV to 0 mV applied at intervals of 15 s. During the break in the measurements the current-voltage relation was recorded. (c) Current-voltage relations under control conditions (●) and about 8 min after the addition of antazoline (○). (d) Current-voltage relations after wash-out of antazoline (■), and following the subsequent addition of tetraethylammonium chloride (4 mM; □). During all experiments illustrated in this figure the bath solution contained tolbutamide (100 μM) to block ATP-sensitive K^+ currents.

6.5 ± 0.3 ng per islet 60 min^{-1} in controls; $n = 73$). Figure 4b shows that the inhibitory effect of diazoxide was antagonized in a concentration-dependent manner by tolbutamide > antazoline > phentolamine > alinidine. This order of potency was established by determining the concentration of each drug that was necessary to reverse the effect of diazoxide completely (Table 2). On the other hand, the inhibitory effect of diazoxide (85%) was only reduced to $63 \pm 4\%$ by 250 μM yohimbine ($P < 0.01$) and to $72 \pm 2\%$ by 250 μM tolazoline ($P < 0.05$).

Clonidine inhibits glucose-induced insulin release by activating α_2-adrenoceptors. The concentration of 100 nM clonidine was chosen to achieve 85% inhibition of insulin release induced by 15 mM glucose (0.85 ± 0.04 ng per islet 60 min^{-1} vs 5.8 ± 0.2 ng per islet 60 min^{-1} in controls; $n = 96$). This concentration is at least 50-fold lower than those at which clonidine directly affects ATP-sensitive K^+ channels in β-cells (Plant *et al.*, 1991).

Figure 4c shows that, with the exception of tolbutamide, all tested drugs antagonized the inhibition by clonidine in a concentration-dependent manner. Based on the drug concentration required to antagonize the effect of clonidine completely (Table 2), the order of potency was phentolamine > yohimbine > antazoline > tolazoline >> alinidine.

As shown in Figure 5, a strong correlation was found between the ability of tolbutamide, antazoline, phentolamine and alinidine to double control insulin release (in 15 mM glucose alone) and to reverse the inhibition by diazoxide ($r^2 = 0.98$ or 0.95 with and without tolbutamide). On the other hand, there was no correlation between the ability of the tested imidazolines to increase control insulin release and to reverse the inhibitory effect of clonidine.

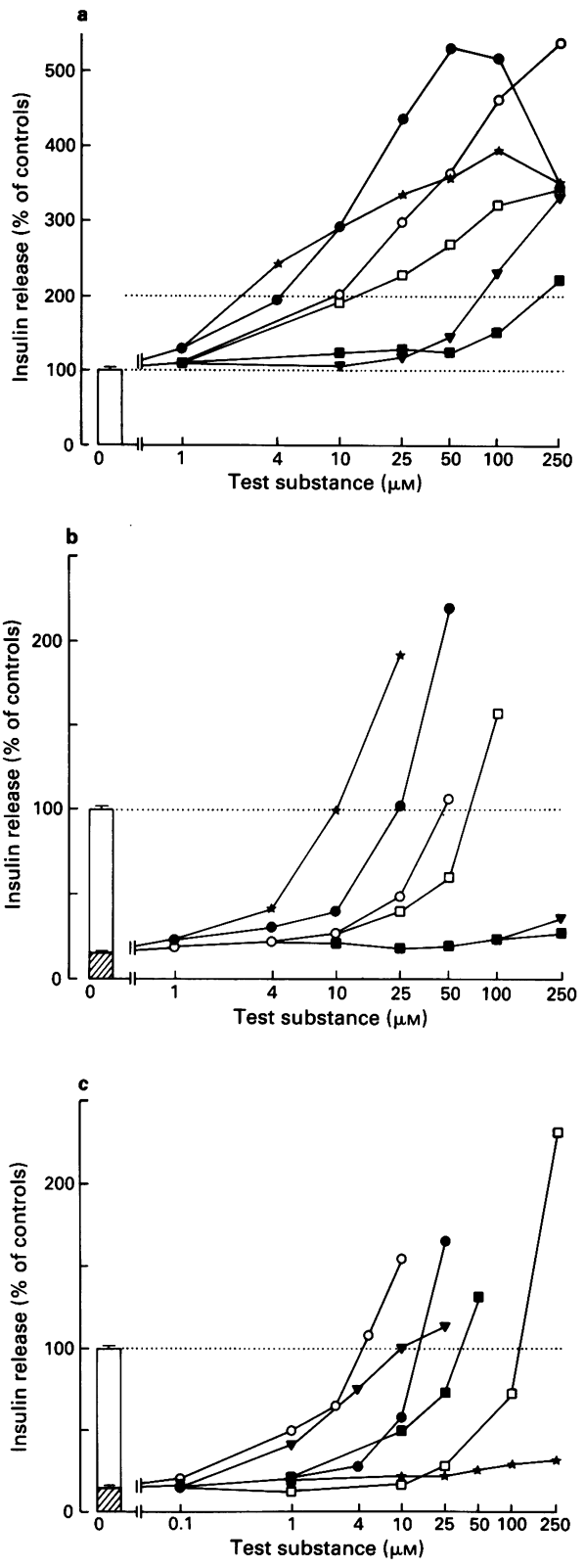

Figure 4 Concentration-dependency of the effects of four imidazolines (antazoline ●; phentolamine ○; tolazoline ■; alinidine □), of tolbutamide (*) and of yohimbine (▼) on insulin release by incubated mouse islets. The incubation medium contained (a) 15 mM glucose alone; (b) 15 mM glucose and 40 μM diazoxide; (c) 15 mM glucose and 100 nM clonidine. Results are expressed as a percentage of insulin release in 15 mM glucose alone (open columns). In (b) and (c), hatched columns show insulin release in the presence of 15 mM glucose and either diazoxide or clonidine. Dotted lines are drawn at the levels of 100 and 200% of control insulin release measured in the presence of 15 mM glucose alone. Values are means for 15–20 batches of islets from 3–4 experiments. S.e.means were omitted for the sake of clarity.

Table 2 Comparison of the efficacy of various imidazolines, of yohimbine and of tolbutamide on insulin release by mouse islets incubated under different conditions

Test substance	*Glucose* 15 mM *alone*	*Experimental conditions* + *Diazoxide* 40 μM	+ *Clonidine* 100 nM
Antazoline	4.3 ± 1.2	23.5 ± 2.9	14.4 ± 2.8
Phentolamine	9.1 ± 5.3	46.0 ± 3.3	4.4 ± 0.3
Tolazoline	190 ± 70	>>250	31.8 ± 8.1
Alinidine	11.3 ± 5.0	68.7 ± 10.3	117 ± 23
Tolbutamide	2.9 ± 1.1	9.8 ± 3.2	>>250
Yohimbine	76 ± 21	>>250	10.0 ± 4.6

The table gives the concentration of test substance (in μM) that doubles control insulin release in the presence of 15 mM glucose alone, or that reverses the inhibition produced by the indicated concentrations of diazoxide or clonidine. This concentration was estimated for each experiment. Values are means ± s.e.mean for 3–4 experiments.

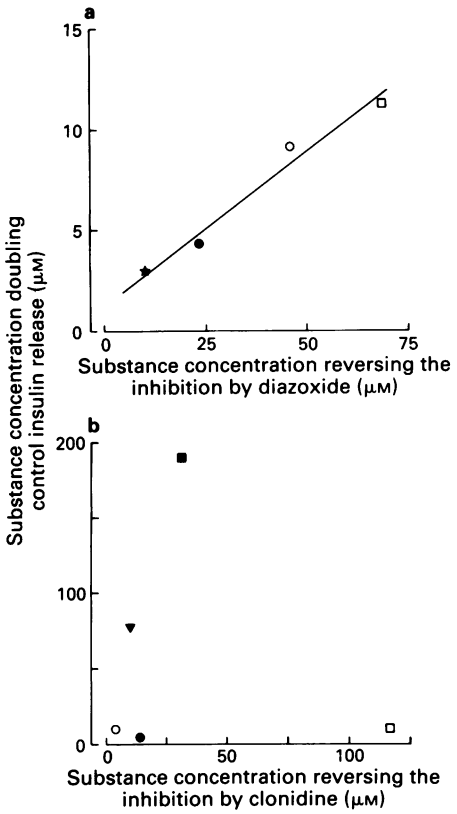

Figure 5 Correlation between the concentrations of test substance doubling control insulin release (in the presence of 15 mM glucose alone) and the concentration reversing the inhibition by (a) 40 μM diazoxide or (b) 100 nM clonidine. The drugs tested here are: antazoline (●); phentolamine (○); tolazoline (■); alinidine (□); tolbutamide (*); yohimbine (▼). In (a) the regression analysis gave a coefficient of correlation of 0.98.

Discussion

All six imidazolines tested in this study were found to affect control insulin release stimulated by glucose alone. Two of them, tramazoline and compound ST 91, inhibited release, whereas the other four increased it in a concentration-dependent manner. This effect of phentolamine has long been established (see Introduction), and those of antazoline and tolazoline have been reported for a single concentration of the drugs (Schulz & Hasselblatt, 1989).

The six imidazolines were also found to act on α_2-adrenoceptors in β-cells. Since the inhibition of insulin release caused by tramazoline and ST 91 was prevented by yohimbine, one may conclude that they behaved as α_2-agonists, as in other tissues (Malta *et al.*, 1981). On the other hand, the other four imidazolines behaved as α_2-antagonists since they relieved the inhibition of insulin release caused by clonidine. This was expected for phentolamine and tolazoline (Schulz & Hasselblatt, 1989; Reynolds, 1989), but less so for antazoline which is usually classified as a histamine receptor antagonist (anti-H_1) (Reynolds, 1989). In another study with mouse islets (Schulz & Hasselblatt, 1989) antazoline was claimed not to antagonize the effects of clonidine, probably because clonidine was used at a 10 fold higher concentration when the effects of antazoline were tested than when the effects of phentolamine were tested. Alinidine is an antiarrythmic agent with weak α_2-antagonist activity (Heinzow *et al.*, 1982). It was clearly the weakest of the antagonists tested here.

The six imidazolines also inhibited ATP-sensitive K^+ channels in β-cells, as demonstrated by the decrease in ^{86}Rb efflux that they produced in islets perifused with a medium containing a low glucose concentration, by their ability to antagonize the acceleration of ^{86}Rb efflux brought about by diazoxide and, for some of them, by the decrease in ATP-sensitive K^+ current. The observation that qualitatively similar effects were produced by agonists and antagonists of α_2-adrenoceptors reinforces our previous conclusion that the action of these substances on ATP-sensitive K^+ channels is independent of the type of action they exert on the α_2-adrenoceptor (Plant & Henquin, 1990; Plant *et al.*, 1991). Quantitative comparisons of the potency of the different drugs on ATP-sensitive K^+ channels are not possible on the basis of the present data. They would not be easily obtained because the slow action of these substances makes it difficult to differentiate small effects from run-down of the current. It is, however, clear that tolazoline was much less effective than phentolamine, antazoline and alinidine. Tolazoline was also found to be less potent than phentolamine and antazoline in inhibiting ATP-sensitive K^+ channels in RINm5F, an insulin-secreting cell line derived from a tumour (Dunne, 1991). In this preparation, the channels were more rapidly, more strongly and more reversibly blocked by phentolamine or antazoline than in the normal β-cells that we used. This difference could be due to the use of distinct experimental models and configurations of the patch-clamp technique. It might also suggest that the site of action of these imidazolines is located at the inner face of the plasma membrane, where it is much more easily reached when open-cell patches are used (Dunne, 1991) than when intact membranes are studied with the whole cell configuration employed here.

Four imidazolines tested here were thus found to inhibit both ATP-sensitive K^+ channels and α_2-adrenoceptors in β-cells, and to potentiate glucose-induced insulin release. The latter property correlated strongly with the ability of the drugs to relieve the inhibition by diazoxide, and not at all with their ability to reverse the inhibition by clonidine. We, therefore, conclude that the increase in insulin release brought about by these agents under control conditions is due to blockade of ATP-sensitive K^+ channels. This conclusion can probably be extended to efaroxan, another imidazoline derivative, which increased control insulin release

and reversed the inhibitory effect of diazoxide within a similar range of concentrations, higher than that required to antagonize the inhibitory effect of noradrenaline (Chan & Morgan, 1990).

The observation that the maximal increase in insulin release produced by antazoline and phentolamine is larger than that produced by tolbutamide suggests, however, that the effect of the two imidazolines is not solely due to blockade of ATP-sensitive K^+ channels. One can exclude the possibility that this difference is due to the simultaneous blockade of α_2-adrenoceptors because yohimbine did not increase insulin release induced by tolbutamide or glibenclamide in the presence of 10–15 mM glucose (Garrino & Henquin, 1990; unpublished observations). Unlike tolbutamide (Ashford, 1990), phentolamine (Plant & Henquin, 1990) and antazoline (this study) also inhibit voltage-dependent K^+ channels in β-cells. This might contribute to their larger maximal effect on insulin release.

The number of tested compounds is too low to draw meaningful conclusions as to structure-activity relationships. We simply note that the three imidazolines (antazoline, phentolamine and tolazoline) were more potent on α_2-adrenoceptors than on ATP-sensitive K^+ channels, whereas the reverse was true for alinidine, which is an imidazolidine. From the present and previous studies (Plant & Henquin, 1990; Plant *et al.*, 1991; Dunne, 1991; Chan *et al.*, 1991a,b), it is clear that imidazolines can be added to the already long list of pharmacological agents able to inhibit ATP-sensitive K^+ channels in β-cells (Ashford, 1990; Henquin, 1990). We previously suggested that an imidazoline-binding site might be involved in the control of ATP-sensitive K^+ channels in B-cells (Plant *et al.*, 1991). It appears, however, that such a site is clearly distinct from the known brain or kidney imidazoline receptors which have a high affinity for idazoxan and tolazoline (Michel & Insel, 1989; Atlas, 1991), two agents that are weakly active on K^+ channels (this study, Chan *et al.*, 1991a).

In conclusion, the ability of several imidazoline antagonists of α_2-adrenoceptors to increase insulin release *in vitro* can be ascribed to their blockade of ATP-sensitive K^+ channels in β-cells rather than to their interaction with the adrenoceptor. A similar mechanism might also account for, or at least contribute to, the changes in plasma insulin levels that this class of compounds may cause. It is, however, premature to exclude formally the possibility that their *in vivo* effects also involve attenuation of inhibitory sympathetic signals in β-cells.

This work was supported by grant 3.4607.90 from the FRSM, Brussels, by grant SPPS-AC 89/95-135 from the Ministry of Scientific Policy, Brussels, and by the Deutsche Forschungsgemeinschaft SFB 246 (Projekt C4). J-C.J. is 'Aspirant' and J.C.H. is 'Directeur de Recherches' of the FNRS, Brussels. We are grateful to M. Nenquin for editorial help.

References

AHREN, B. & LUNDQUIST, I. (1985). Effects of α-adrenoceptor blockade by phentolamine on basal and stimulated insulin secretion in the mouse. *Acta Physiol. Scand.*, **125**, 211–217.

ASHCROFT, F.M., ASHCROFT, S.J.H. & HARRISON, D.E. (1988). Properties of single potassium channels modulated by glucose in rat pancreatic β-cells. *J. Physiol.*, **400**, 501–527.

ASHFORD, M.L. (1990). Potassium channels and modulation of insulin secretion. In *Potassium Channels: Structure, Classification, Function and Therapeutic Potential*. ed. Cook, N.S. pp. 300–325. Chichester: Ellis Horwood.

ATLAS, D. (1991). Clonidine-displacing substance (CDS) and its putative imidazoline receptor. New leads for further divergence of α_2-adrenergic receptor activity. *Biochem. Pharmacol.*, **41**, 1541–1549.

BROADSTONE, V.L., PFEIFER, M.A., BAJAJ, V., STAGNER, J.I. & SAMOLS, E. (1987). Alpha-adrenergic blockade improves glucose-potentiated insulin secretion in non-insulin-dependent diabetes mellitus. *Diabetes*, **36**, 932–937.

BUSE, M.G., JOHNSON, A.H., KUPERMINC, D. & BUSE, J. (1970). Effect of α-adrenergic blockade on insulin secretion in man. *Metabolism*, **19**, 219–225.

CERASI, E., EFENDIC, S. & LUFT, R. (1969). Role of adrenergic receptors in glucose-induced insulin secretion in man. *Lancet*, **ii**, 301–302.

CHAN, S.L.F., DUNNE, M.J., STILLINGS, M.R. & MORGAN, N.G. (1991a). The α_2-adrenoceptor antagonist efaroxan modulates K^+ ATP channels in insulin-secreting cells. *Eur. J. Pharmacol.*, **204**, 41–48.

CHAN, S.L.F., STILLINGS, M.R. & MORGAN, N.G. (1991b). Mechanisms involved in stimulation of insulin secretion by the hypoglycaemic alpha-adrenergic antagonist, DG 5128. *Biochem. Biophys. Res. Commun.*, **176**, 1545–1551.

CHAN, S.L.F. & MORGAN, N.G. (1990). Stimulation of insulin secretion by efaroxan may involve interaction with potassium channels. *Eur. J. Pharmacol.*, **176**, 97–101.

DUNNE, M.J. (1991). Block of ATP-regulated potassium channels by phentolamine and other α-adrenoceptor antagonists (in RINm5F cells). *Br. J. Pharmacol.*, **103**, 1847–1850.

EFENDIC, S., CERASI, E. & LUFT, R. (1975). Effect of phentolamine and preperfusion with glucose on insulin release from the isolated perfused pancreas from fasted and fed rats. *Diabetologia*, **11**, 407–410.

GARRINO, M.G. & HENQUIN, J.C. (1988). Highly potent and stereoselective effects of the benzoic acid derivative AZ-DF 265 on pancreatic β-cells. *Br. J. Pharmacol.*, **93**, 61–68.

GARRINO, M.G. & HENQUIN, J.C. (1990). β-cell adrenoceptors and sulphonylurea-induced insulin release. *Diabetologia*, **33**, 145–147.

GARRINO, M.G., PLANT, T.D. & HENQUIN, J.C. (1989). Effects of putative activators of K^+ channels in mouse pancreatic β-cells. *Br. J. Pharmacol.*, **98**, 957–965.

GAUTIER, J.F., SCHEEN, A.J., JAMINET, C. & LEFEBVRE, P.J. (1991). SL 84 0418, a new potent alpha 2 antagonist, increases insulin secretion after intravenous glucose in normal men. *Diabetologia*, **34** (Suppl. 2), A15.

HEINZOW, B.C.J., ANGUS, J.A. & KORNER, P.I. (1982). Effects of alinidine (ST 567) on baroreceptor heart rate reflexe, and its interactions with clonidine on the baroreflex and on the sympathetic terminals of the isolated atrium. *Eur. J. Pharmacol.*, **84**, 177–187.

HENQUIN, J.C. (1978). D-Glucose inhibits potassium efflux from pancreatic islet cells. *Nature*, **271**, 271–273.

HENQUIN, J.C. (1990). Established, unsuspected and novel pharmacological insulin secretagogues. In *New Antidiabetic Drugs*. ed. Bailey, C.J. & Flatt, P.R. pp. 93–106. London: Smith-Gordon.

HENQUIN, J.C. & MEISSNER, H.P. (1982). Opposite effects of tolbutamide and diazoxide on $^{86}Rb^+$ fluxes and membrane potential in pancreatic β-cells. *Biochem. Pharmacol.*, **31**, 1407–1415.

KAWAZU, S., SUZUKI, M., NEGISHI, K., WATANABE, T. & ISHII, J. (1987). Studies of midaglizole (DG-5128). A new type of oral hypoglycaemic drug in healthy subjects. *Diabetes*, **36**, 216–220.

LUNDQUIST, I. (1972). Interaction of amines and aminergic blocking agents with blood glucose regulation. II. α-Adrenergic blockade. *Eur. J. Pharmacol.*, **18**, 225–235.

MALTA, E., RAPER, C. & TAWA, P.E. (1981). Pre- and postjunctional effects of clonidine- and oxymetazoline-like compounds in guinea-pig ileal preparations. *Br. J. Pharmacol.*, **73**, 355–362.

MICHEL, M.C. & INSEL, P.A. (1989). Are there multiple imidazoline binding sites? *Trends Pharmacol Sci.*, **10**, 342–344.

MISBIN, R.I., EDGAR, P.J. & LOCKWOOD, W.H. (1970). Adrenergic regulation of insulin secretion during fasting in normal subjects. *Diabetes*, **19**, 688–693.

MISLER, S., FALKE, L.C., GILLIS, K. & MCDANIEL, M.L. (1986). A metabolite-regulated potassium channel in rat pancreatic β-cells. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 7119–7123.

NAKAKI, T., NAKADATE, T. & KATO, R. (1980). α_2-Adrenoceptors modulating insulin release from isolated pancreatic islets. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **313**, 151–153.

ORTIZ-ALONSO, F.J., HERMAN, W.H., GERTZ, B.J., WILLIAMS, V.C., SMITH, M.J. & HALTER, J.B. (1991). Effect of an oral α_2-adrenergic blocker (MK 912) on pancreatic islet function in non-insulin dependent diabetes mellitus. *Metabolism*, **40**, 1160–1167.

PLANT, T.D. (1988). Properties and calcium-dependent inactivation of calcium currents in cultured mouse pancreatic β-cells. *J. Physiol.*, **404**, 731–747.

PLANT, T.D. & HENQUIN, J.C. (1990). Phentolamine and yohimbine inhibit ATP-sensitive K^+ channels in mouse pancreatic β-cells. *Br. J. Pharmacol.*, **101**, 115–120.

PLANT, T.D., JONAS, J.-C. & HENQUIN, J.C. (1991). Clonidine inhibits ATP-sensitive K^+ channels in mouse pancreatic β-cells. *Br. J. Pharmacol.*, **104**, 385–390.

REYNOLDS, J.E.F. (ed) (1989). *Martindale – The Extra Pharmacopoeia.* pp. 1–1896. London: The Pharmaceutical Press.

ROBERTSON, R.P., HALTER, J.B. & PORTE, D. (1976). A role for alpha-adrenergic receptors in abnormal insulin secretion in diabetes mellitus. *J. Clin. Invest.*, **57**, 791–795.

RORSMAN, P. & TRUBE, G. (1985). Glucose-dependent K^+-channels in pancreatic β-cells are regulated by intracellular ATP. *Pflügers Arch.*, **405**, 305–309.

SCHULZ, A. & HASSELBLATT, A. (1988). Phentolamine, a deceptive tool to investigate sympathetic nervous control of insulin release. *Naunyn Schmiedebergs Arch. Pharmacol.*, **337**, 637–643.

SCHULZ, A. & HASSELBLATT, A. (1989). An insulin-releasing property of imidazoline derivatives is not limited to compounds that block α-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **340**, 321–327.

SMITH, M. & FURMAN, B.L. (1988). Augmentation of glucose-induced insulin secretion by pertussis vaccine, phentolamine and benextramine: involvement of mechanisms additional to prevention of the inhibitory actions of catecholamines in rats. *Acta Endocrinol.*, **118**, 89–95.

SMITH, P.A., BOKVIST, K., ARKHAMMAR, P., BERGGREN, P.O. & RORSMAN, P. (1990). Delayed rectifying and calcium activated K^+-channels and their significance for action potential repolarization in mouse pancreatic β-cells. *J. Gen. Physiol.*, **95**, 1041–1059.

TRUBE, G., RORSMAN, P. & OHNO-SHOSAKU, T. (1986). Opposite effects of tolbutamide and diazoxide on the ATP-dependent K^+ channel in mouse pancreatic β-cells. *Pflügers Arch.*, **407**, 493–499.

(Received January 23, 1992
Revised April 6, 1992
Accepted April 22, 1992)