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Short communication

Evidence that the ability of imidazoline compounds to stimulate insulin secretion is not due to interaction with σ receptors

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Abstract

Recent studies have suggested that a variety of ion channels possess a binding site for ligands such as phencyclidine (PCP), dizocilpine and certain σ ligands and that some imidazoline compounds can also bind to this site. We have investigated whether interaction with this binding site could account for the ability of imidazolines to stimulate insulin secretion from rat islets. Neither PCP nor dizocilpine shared the insulin secretory activity of the imidazoline efaroxan in rat islets suggesting that they do not have similar actions in the pancreatic B-cell. Further, we were able to define a new antagonist, KU14R (2 (2-ethyl 2,3-dihydro-2-benzofuranyl)-2-imidazole), which selectively blocks the insulin secretory response to imidazolines. The results suggest that imidazolines do not stimulate insulin secretion by causing physical blockade of the K^+-ATP channel in pancreatic B-cells and show that their effects are not reproduced by PCP or σ receptor ligands.

Keywords: Imidazoline receptor; Efaroxan; σ Receptor; Insulin secretion; Phencyclidine; (+)-MK-801 (dizocilpine)

1. Introduction

It is now well established that a range of compounds bearing an imidazoline moiety can directly stimulate insulin secretion from pancreatic B-cells. This results from blockade of ATP-sensitive K^+ (K^+-ATP) channels by these drugs and is mediated by an atypical binding site which differs pharmacologically from the imidazoline I_1 and I_2 receptors defined in many other tissues (Morgan et al., 1995).

Recently, several groups have drawn attention to the fact that a number of distinct types of ion channel possess a binding site which can be occupied by PCP, various σ receptor ligands and by dizocilpine (MK-801) and that these molecules then cause channel blockade, presumably by physically occluding the pore (Molderings et al., 1996; Olmos et al., 1996). In support of this, it has been shown that phencyclidine can directly block K^+-ATP channels in cardiac tissue (Kokoz et al., 1994) and that dizocilpine inhibits ion flux through 5-HT-regulated channels (Molderings et al., 1996). Evidence has also been presented that some imidazoline drugs can displace the binding of ligands from σ-like binding sites on ion channels indicating that the two types of compound may compete for a common binding site (Molderings et al., 1996; Olmos et al., 1996). Based on such observations, the proposal has been advanced that the capacity of imidazoline compounds to potentiate insulin secretion may reflect their ability to occupy a σ binding site which leads to occlusion of the ion-conducting pore of the B-cell K^+-ATP channel (Molderings et al., 1996; Olmos et al., 1996). This would then reduce the rate of K^+ efflux, promoting membrane depolarisation and insulin secretion.

We have examined this hypothesis by studying the effects of PCP and dizocilpine on insulin secretion and comparing their effects with the well characterised imidazoline insulin secretagogue, efaroxan. We have also defined a new antagonist of the islet imidazoline receptor (KU14R) which appears to occupy the imidazoline binding site without causing a functional insulin secretory response. Taken together, the results argue against the hypothesis that the insulin secretagogue activity of imidazoline compounds derives principally from interaction with

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binding sites associated with the pore of the K^+-ATP channel.

2. Materials and methods

Efaroxan and phencyclidine were purchased from Sigma (Poole, UK). Dizocilpine was from Tocris Cookson (Bristol, UK). KU14R (2 (2-ethyl 2,3-dihydro-2-benzofuranyl)-2-imidazole) was synthesised in house, by a method to be described elsewhere (data not shown here). ^{125}I for labelling of insulin was purchased from DuPont-NEN. All other reagents were of analytical reagent grade.

Islets of Langerhans were prepared from Wistar rats by collagenase digestion as described previously (Brown et al., 1993a,b). The islets were used within 2 h of isolation and were incubated in groups of 3 for secretion experiments. Insulin levels were measured by standard radioimmunoassay procedures.

3. Results

Imidazoline insulin secretagogues have the ability to overcome the inhibitory effects of the K^+-ATP channel agonist diazoxide on glucose-induced insulin secretion and this response represents a convenient assay of their functional activity in the endocrine pancreas (Brown et al., 1993a,b). As shown in Table 1, incubation of rat islets with 100 μM efaroxan significantly antagonised the inhibitory effect of diazoxide in the presence of 20 mM glucose. By contrast, two compounds which display high affinity for σ-like binding sites associated with ion channels, phencyclidine and dizocilpine (whose binding affinities are several orders of magnitude higher than efaroxan at these sites (Molderings et al., 1996)), caused, at best, only a partial reversal of the diazoxide effect at a concentration of 100 μM (Table 1). These drugs did not significantly alter glucose-induced insulin secretion in the absence of diazoxide (Table 1).

A number of other ligands which bind to σ sites with

Table 1
Effects of PCP and dizocilpine on insulin secretion

[Glucose] (mM)	Diazoxide (200 μM)	Drug (100 μM)	Insulin secretion (ng/islet per h)
4	−	−	1.72 ± 0.17 (16)
20	−	−	4.00 ± 0.18 (18)
20	−	PCP	3.23 ± 0.28 (13)
20	−	MK-801	4.08 ± 0.30 (13)
20	+	−	1.23 ± 0.13 (17)
20	+	PCP	1.86 ± 0.22 (7)
20	+	MK-801	1.79 ± 0.25 (14)
20	+	Ef	3.22 ± 0.17 (18) [a]

Groups of 3 isolated islets were incubated at 37°C for 1 h under the conditions shown, in the absence or presence of 200 μM diazoxide, and test reagents: phencyclidine (PCP), dizocilpine (MK-801) and efaroxan (Ef), all at 100 μM. Data are expressed as means ± S.E.M., for number of observations shown in parentheses.
[a] $P < 0.001$ relative to 20 mM glucose plus diazoxide.

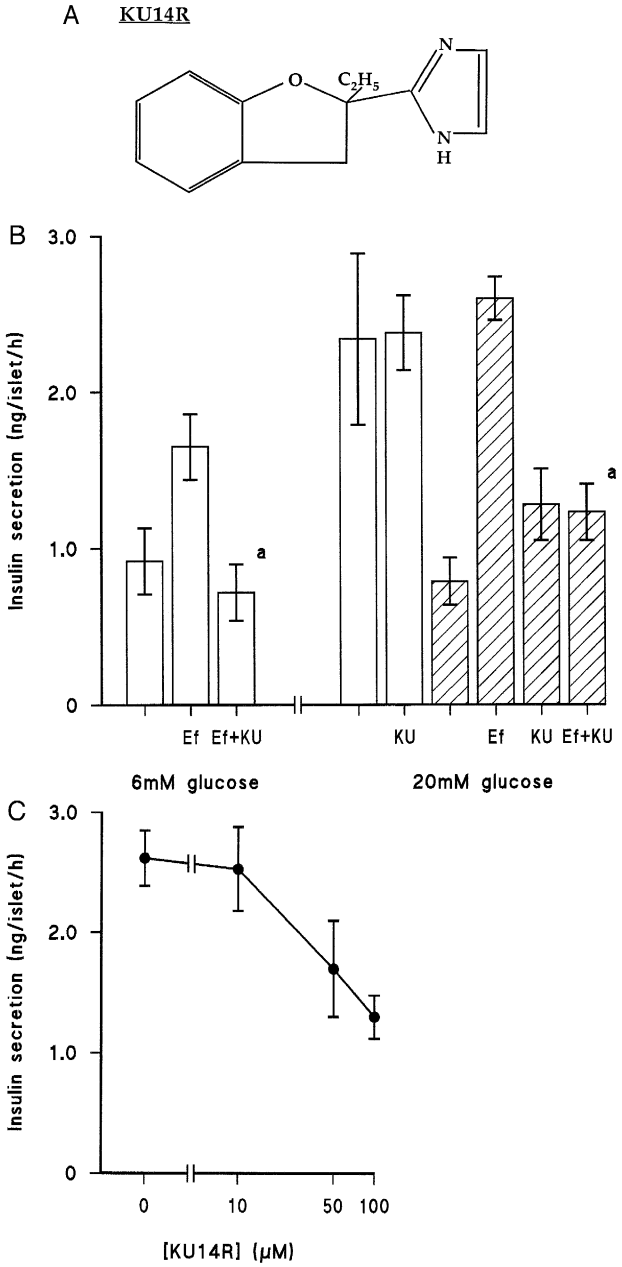

Fig. 1. Effects of KU14R on insulin release. (A) Structure of KU14R, 2(2-ethyl 2,3-dihydro-2-benzofuranyl)-2-imidazole. (B) Groups of 3 isolated islets were incubated at 37°C for 1 h in the presence of either 6 mM or 20 mM glucose. Diazoxide (200 μM, hatched bars), efaroxan (100 μM, Ef) and KU14R (100 μM, KU) were included as shown. Data are expressed as means ± S.E.M. for 12 replicates in each case. [a] $P < 0.01$ relative to equivalent response mediated by efaroxan in the absence of KU14R. (C) Isolated rat islets were incubated in the presence of 20 mM glucose, 200 μM diazoxide and 100 μM efaroxan. Increasing concentrations of KU14R were also included and insulin release monitored after incubated for 1 h at 37°C. The secretory response in the presence of 20 mM glucose plus diazoxide (in the absence of efaroxan) averaged 0.80 ± 0.06 ng/islet/h ($n = 12$).

high affinity were also tested in similar experiments but the more potent agents (including haloperidol, ifenprodil and ketamine) could not be used in functional assays as they were found to directly inhibit glucose-induced insulin secretion in control experiments.

In a further series of studies, we attempted to define agents which can interact antagonistically at the islet imidazoline binding site. To date, only one compound has been reported to act as an imidazoline antagonist in isolated rat islets (RX801080; Brown et al., 1993a) but this is no longer available from the manufacturer. We observed, however, that a newly synthesised analogue of efaroxan in which the imidazoline ring was converted to the imidazole (KU14R; Fig. 1A) behaved as a functional antagonist in insulin secretion experiments (Fig. 1B and C). KU14R did not affect insulin secretion at any glucose concentration tested when added to islets alone but it significantly antagonised the insulin secretory response to efaroxan (Fig. 1B and C) and other imidazoline drugs (unpublished observations). The effect of KU14R was dose-dependent (Fig. 1C) and the compound also reversed the blockade of islet K^+-ATP channels mediated by efaroxan in patch-clamp studies (M.J. Dunne, personal communication).

4. Discussion

It has become clear recently that a variety of ion channels possess a binding site for the drugs PCP and dizocilpine (and certain σ ligands) and that occupation of this site can lead to channel blockade (Molderings et al., 1996; Olmos et al., 1996), presumably by direct occlusion of the ion-conducting pore. Some imidazoline drugs can also bind to these sites with low affinity (Molderings et al., 1996) and it has been suggested there may be a correlation between the potency with which imidazolines displace [^{3}H]dizocilpine binding from sites in rat brain and their potency to stimulate insulin secretion from the rat insulinoma cell line, RIN-5AH (Olmos et al., 1996). On the basis of this correlation, it has been proposed that the functional effects of imidazoline compounds in the pancreatic B-cell may result from K^+-ATP channel blockade occurring as a consequence of occupation of a PCP or σ binding site (Molderings et al., 1996; Olmos et al., 1996). Such a generalised mechanism of channel blockade would provide a convenient explanation for the ability of imidazoline compounds to inhibit several types of ion channel. For example, imidazoline-mediated closures of calcium-activated K^+ channels (Coupry et al., 1996; Zaitsev et al., 1996), nicotinic cholinergic channels (Musgrave et al., 1995) and 5-HT-regulated ion channels (Molderings et al., 1996) have all been described.

In the present work, we demonstrate that neither phencyclidine (which blocks K^+-ATP channels; Kokoz et al. (1994)) nor dizocilpine exhibit significant functional activity in an assay system previously validated for assessment of the action of imidazoline insulin secretagogues (Table 1; Brown et al., 1993a,b). Moreover, these drugs did not cause any direct potentiation of glucose-induced insulin secretion. This lack of effect is unlikely to reflect a failure of the compounds to enter pancreatic B-cells since both are relatively lipophilic and have been shown to directly inhibit ion fluxes in intact cells (Kokoz et al., 1994; Molderings et al., 1996).

The results obtained indicate that despite their high affinity for a binding site present in several types of ion channel, occupation of these sites by phencyclidine and dizocilpine did not culminate in an increase in insulin secretion equivalent to that observed with the imidazoline compound, efaroxan. One interpretation of these data would be that, in common with several other ion channels, the islet K^+-ATP channel possesses a binding site to which PCP and dizocilpine can bind but that occupation of this site in intact cells does not reduce the ion flow sufficiently to overcome completely the activating effect of diazoxide on the channel.

This conclusion differs from that reached by Olmos et al. (1996) based on comparisons between binding data from rat brain and imidazoline insulin secretagogue potency in RIN-5AH cells. However, it should be noted that the potency series for stimulation of insulin secretion by imidazolines in RIN-5AH cells is rather different from that found in normal rat islets. For example, RX821002 is an effective insulin secretagogue in rat islets (similar potency to efaroxan) whereas idazoxan is ineffective (Brown et al., 1993b) and may even be antagonistic (Berdeu et al., 1995). In RIN-5AH cells idazoxan is reported to be an insulin secretagogue and RX821002 requires sub-millimolar concentrations for activity (Olmos et al., 1996) suggesting that control of insulin secretion by imidazolines in these cells may be altered relative to normal pancreatic B-cells.

In support of the concept that imidazoline responses are not mediated by physical occlusion of the K^+-ATP channel, we have demonstrated that an analogue of efaroxan in which the imidazoline ring has been converted to the imidazole (KU14R) acts as a functional antagonist in rat islets (Fig. 1). KU14R did not affect insulin secretion when added to rat islets in the presence of glucose concentrations between 4 and 20 mM but it dose-dependently reversed the effects of efaroxan. Thus, KU14R appears to occupy the efaroxan binding site without promoting a functional response. If the binding site were located within the pore of the K^+-ATP channel such that binding of ligands leads to channel blockade by their physical presence, then any molecule occupying the site would lead to restricted ion flow and should behave as an agonist (i.e., it should stimulate insulin secretion). The finding that KU14R acts antagonistically to efaroxan provides strong support for the concept that the islet imidazoline receptor controls channel activity from a more remote location. Previous work has shown that the imidazoline RX801080 also acts as an efaroxan antagonist in intact rat islets (Brown et al.,

1993a) and that the imidazole, cimetidine, can block responses to efaroxan in perfused pancreas (Berdeu et al., 1995). Thus, functional antagonism of the islet imidazoline receptor is not a unique characteristic restricted to a single molecule having unusual structural features.

The results presented herein do not exclude the possibility that the islet K-ATP channel may possess a PCP/σ receptor site which causes occlusion of the ion-conducting pore upon binding of imidazoline ligands. However, the data suggest that the ability of imidazoline compounds to stimulate insulin secretion derives primarily from interaction with a second, distinct, receptor site(s).

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