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The imidazoline site involved in control of insulin secretion: characteristics that distinguish it from I_1- and I_2-sites

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1 The nature of the binding site mediating the insulin secretagogue activity of certain imidazoline compounds remains unclear and the pharmacology of the I_1- and I_2-imidazoline sites, described in many tissues, does not correlate with the observed responses to imidazolines in islets. In the present paper, we describe further results which support the concept that the islet imidazoline site may represent a novel subtype of imidazoline receptor.

2 Culture of rat isolated islets in the presence of imidazoline secretagogues (either efaroxan or phentolamine) resulted in loss of responsiveness on subsequent re-exposure to these agents. However, culture of islets with either idazoxan or UK14,304 (imidazoline ligands that do not stimulate insulin secretion) did not lead to any loss of response when the islets were subsequently exposed to efaroxan. By contrast, islets cultured with UK14,304 (a potent α_2-adrenoceptor agonist), displayed loss of sensitivity to noradrenaline, consistent with down-regulation of α_2-adrenoceptors.

3 In order to characterize the imidazoline site further, radioligand binding studies were performed in membranes from RINm5F insulinoma cells using [^{3}H]-RX821002, an imidazoline insulin secretagogue that does not interact significantly with imidazoline sites in other tissues. [^{3}H]-RX821002 labelled α_2-adrenoceptors with high affinity (2.01 ± 0.7 nM) but also labelled a second, non-adrenoceptor site with much lower affinity.

4 Under conditions of α_2-adrenoceptor blockade (in the presence of adrenaline), efaroxan displaced [^{3}H]-RX821002 binding to the low affinity site, in a dose-dependent manner. Competition studies employing additional imidazoline compounds of varying secretagogue activity revealed that the pharmacological profile of the low affinity site correlates well with that observed in secretion experiments.

5 The results obtained from the down-regulation experiments with isolated islets and from the radioligand binding studies suggest that the low affinity [^{3}H]-RX821002 binding site may represent the functional receptor responsible for the secretagogue activity of imidazoline compounds in the endocrine pancreas and that it has a pharmacological profile distinct from those of I_1- and I_2-sites.

Keywords: Islets of Langerhans; insulin secretion; imidazoline; I-site; RX821002; efaroxan; RINm5F cells; down-regulation

Introduction

Non-adrenoceptor imidazoline binding sites represent a new class of receptors which are thought to mediate the central anti-hypertensive actions of clonidine and related drugs (Michel & Ernsberger, 1992; Ernsberger *et al.*, 1992). In addition, they may also mediate contraction of peripheral smooth muscle, stimulation of insulin release and alterations in renal metabolism induced by imidazoline compounds (Atlas, 1991). Two principal imidazoline receptor subtypes have been defined, based on their affinity for clonidine or idazoxan. I_1-imidazoline sites are labelled preferentially by [^{3}H]-clonidine, or its derivative [^{3}H]-*p*-aminoclonidine, and are pharmacologically distinct from imidazoline recognition sites labelled with [^{3}H]-idazoxan (I_2-sites), in that the latter show 100 fold lower affinity for clonidine and do not recognize imidazoles (Michel & Ernsberger, 1992).

Recent studies have shown that certain compounds which possess an imidazoline moeity within their structure, are able to enhance the rate of insulin secretion, both *in vivo* and *in vitro* (Ahrén & Lundquist, 1985; Schulz & Hasselblatt, 1988; 1989a; Chan *et al.*, 1988; 1991a). $^{86}Rb^+$ efflux experiments and electrophysiological studies have provided evidence that this response is due to a reduction in potassium efflux through ATP-sensitive K^+ channels in the islet β-cell plasma membrane, resulting in membrane depolarization (Chan & Morgan, 1990; Chan *et al.*, 1991a,b; Dunne, 1991; Jonas *et al.*, 1992). In functional secretion experiments, we have shown that, in the endocrine pancreas, the secretory response induced by the imidazoline efaroxan shows stereoselectivity and is subject to down-regulation in the presence of agonist (Chan *et al.*, 1993). These data suggest that an 'imidazoline receptor' is responsible for mediating the effects of imidazoline compounds in islets, although the nature of the binding site is still unclear. I_2-receptors (also known as non-adrenoceptor idazoxan binding sites (NAIBS)) have been described on pancreatic islets (Brown *et al.*, 1993; Lacombe *et al.*, 1993) and on RINm5F insulinoma cells (Remaury & Paris, 1992). However, the emerging pharmacology of the defined I_1- and I_2-sites does not correlate well with the observed responses to imidazolines in islets. Thus, the possibility remains that the islet imidazoline receptor may represent a novel, third type of imidazoline binding site.

To date, it has not proved possible to identify the islet imidazoline receptor using radioligand binding techniques as the imidazoline compounds which are the most effective insulin secretagogues are not readily available in labelled form. The major exception to this, clonidine, labels multiple binding sites in many tissues, making it an unsatisfactory ligand. Therefore, in the present work, we have exploited the finding that the imidazoline α_2-antagonist, RX821002, can stimulate insulin secretion (Chan, 1990; Gross *et al.*, 1991) but does not interact significantly with imidazoline sites in other tissues (Langin *et al.*, 1989). We show that [^{3}H]-RX821002 can label a low affinity site on RINm5F cell membranes, the pharmacology of which is consistent with the possibility that it may represent the putative imidazoline site responsible for regulation of β-cell K^+_{ATP} channel activity. In addition, we present further data from desensitization experiments which support the concept that the imidazoline

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site mediating the secretagogue activity of imidazolines, such as efaroxan and phentolamine, is distinct from the designated I_1- and I_2-sites.

Methods

Insulin secretion experiments

Islets of Langerhans were isolated from the pancreata of male Wistar rats by collagenase digestion (Montague & Taylor, 1968). The isolation medium was a bicarbonate-buffered physiological saline solution (Gey & Gey, 1936), pH 7.4, containing 4 mM glucose and 2 mM $CaCl_2$. For islet incubations, this medium was supplemented with 1 mg ml^{-1} bovine serum albumin. In static incubation experiments, groups of three isolated islets were incubated for 60 min at 37°C with test reagents. After this time, samples of the medium were removed for measurement of their insulin content by radioimmunoassay (RIA).

For desensitization experiments, groups of isolated islets were cultured in RPMI-1640 containing glutamine (2 mM), foetal calf serum (10% v/v), penicillin (400 iu ml^{-1}), streptomycin (200 $\mu g\ ml^{-1}$) and test reagents, in a humidified atmosphere of air:CO_2 (95:5) for 18 h. After this time, islets were washed and selected by hand under a dissecting microscope for use in static incubation experiments.

Radioligand binding experiments

Membranes were prepared from RINm5F cells, cultured in RPMI-1640 supplemented with 10% (v:v) foetal calf serum. Briefly, cell monolayers were washed with phosphate buffered saline and the cells scraped in ice-cold TEM buffer (50 mM Tris-HCl, pH 7.5; 1 mM EDTA; 10 mM $MgCl_2$) supplemented with 50 μM phenylmethylsulphonyfluoride. Following homogenization and centrifugation (40,000 *g*, 20 min, 4°C), the resulting pellet was resuspended in TEM buffer and re-centrifuged. The final crude membrane fraction was resuspended in TEM buffer and aliquots were quickly frozen and stored at − 70°C. Protein content was measured by the bicinchoninic acid method (Smith *et al.*, 1985).

Membranes (50–100 μg protein), [^{3}H]-RX821002 and competing drugs were incubated in a final volume of 100 μl TEM buffer for 60 min at 25°C. The reaction was stopped by adding 3 ml of ice-cold TEM buffer. Bound and free radioligand were separated by filtration through Whatman GF/B filters under vacuum. Filters were washed and their radioactivity measured after addition of scintillant.

For saturation studies, [^{3}H]-RX821002 was used over the range 1–100 nM and non-specific binding was defined in the presence of 100 μM phentolamine. In experiments designed to study specifically binding at the 'imidazoline' site, incubations were performed in the presence of 10 μM adrenaline to block α_2-adrenoceptors. The radioligand binding data were analyzed by iterative non-linear curve fitting using the Fig P Curve Fitter Programme (Biosoft, Cambridge, UK).

Materials

[^{3}H]-RX821002 (specific activity 59 Ci $mmol^{-1}$) was purchased from Amersham International. RX821002 ((2-2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline) and efaroxan were gifts from Reckitt & Colman Products. Diazoxide was obtained from Glaxo Pharmaceuticals. UK14,304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) and prazosin were gifts from Pfizer Research. Phentolamine mesylate was donated by CIBA Labs. Adrenaline and clonidine were from Sigma Chemical Co.

Statistics

Statistical analysis was performed using Student's *t* test for unpaired data and results were considered significant when $P < 0.05$.

Results

Down-regulation of imidazoline-mediated potentiation of insulin-release

It has been shown previously that imidazoline compounds which can stimulate insulin secretion directly, are also able to overcome the inhibitory effects of diazoxide on insulin release (Plant & Henquin, 1990; Chan & Morgan, 1990; Chan *et al.*, 1991a,b). Therefore, the use of diazoxide provides a convenient assay system for determining the effectiveness of imidazolines to interact functionally with islet cells. Figure 1 shows the effects of culturing islets in 100 μM efaroxan (a), phentolamine (b), idazoxan (c) and UK14,304 (d) on the ability of the imidazoine secretagogues efaroxan and phentolamine to antagonize diazoxide-induced inhibition of insulin release. Islets which had been cultured in the presence of either efaroxan (a) or phentolamine (b) were found to be refractory to these compounds upon subsequent re-exposure. Thus, in efaroxan-cultured islets, efaroxan failed to elicit any reversal of the inhibition of glucose-induced insulin release mediated by diazoxide (Figure 1a). Similarly, the capacity of phentolamine to antagonize the inhibitory effects of diazoxide was also impaired in these islets (Figure 1b). Conversely, culture of islets in the presence of either idazoxan (Figure 1c) or UK14,304 (Figure 1d), imidazoline ligands (I_2-site) which do not stimulate insulin release (Chan & Morgan, 1990; Chan *et al.*, 1991b; Brown *et al.*, 1993), did not lead to any loss of response when the islets were subsequently exposed to efaroxan or phentolamine.

In contrast, after islets were cultured in the presence of UK14,304 (a potent α_2-agonist), noradrenaline was unable to inhibit glucose (20 mM)-induced insulin secretion (Figure 1d). This loss of sensitivity to noradrenaline is consistent with down-regulation of α_2-adrenoceptors during exposure to the α_2-agonist. Culture in the absence of UK14,304 did not alter the response of islets to noradrenaline (data not shown; see Hurst & Morgan, 1990). Furthermore, since the ability of efaroxan and phentolamine to antagonize the actions of diazoxide was still evident after exposure to UK14,304, this confirms that the insulin secretagogue activity of these compounds does not involve interaction with α_2-adrenoceptors.

Effects of RX821002 on insulin secretion from rat isolated islets

In insulin secretion experiments, RX821002 (100 μM) significantly stimulated basal insulin release from isolated rat islets (Table 1). Moreover, RX821002 was also able to antagonize the inhibitory effect of diazoxide on glucose-induced insulin secretion (Table 1). These data confirm previous studies suggesting that the mechanism of action of imidazoline compounds involves interaction with a regulatory component associated with ATP-sensitive potassium channels (Plant & Henquin, 1990; Chan & Morgan, 1990; Chan, 1993).

Identification of RX821002 binding sites in RINm5F cell membranes

The binding of [^{3}H]-RX821002 to RINm5F membranes was rapid, with the steady state being reached within 20 min at 25°C (data not shown). When employed at concentrations between 1–10 nM, [^{3}H]-RX821002 labelled a high affinity site which was saturable (Figure 2). However, raising the concentration of [^{3}H]-RX821002 further, revealed the presence of a second binding site of much lower affinity, which remained less than fully saturated even at a radioligand concentration of 100 nM. Analysis of the data by iterative non-linear curve fitting confirmed the presence of at least two sites. The high affinity site bound [^{3}H]-RX821002 with a K_D of 2.01 ± 0.7 nM ($n = 4$) and B_{max} of 34 ± 10.4 fmol mg^{-1} protein, consistent with binding to α_2-adrenoceptors. By contrast, the second site exhibited a much lower affinity with an estimated K_D of

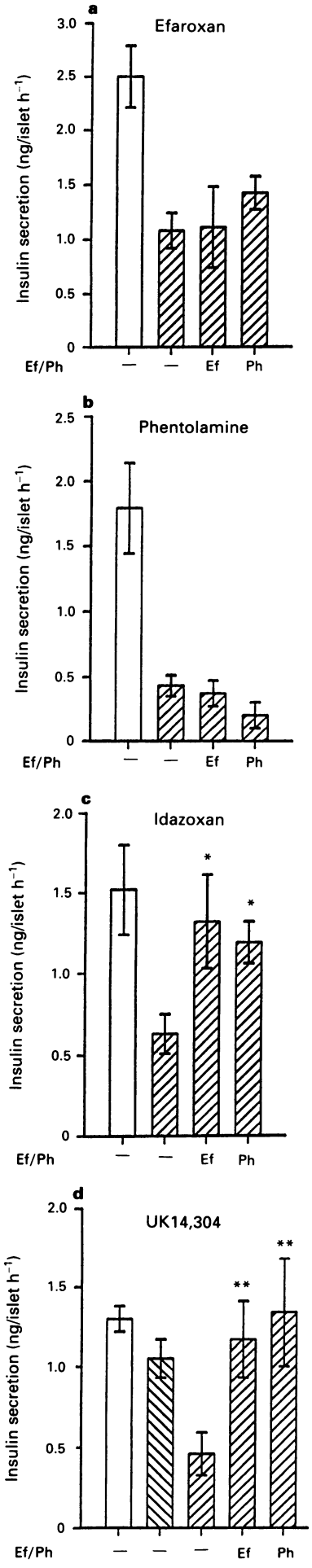

Figure 1 Effects of culture conditions on the ability of imidazoline insulin secretagogues to antagonize the inhibition of insulin secretion induced by diazoxide. Groups of rat isolated islets were cultured for 18 h in medium supplemented with 100 μM efaroxan (a), phentolamine (b), idazoxan (c) or UK14,304 (d). Islets were washed and incubated in the presence of 20 mM glucose. Open columns represent secretion in response to 20 mM glucose alone. Diazoxide (250 μM; [▨]), noradrenaline (10 μM; [▩]), efaroxan (100 μM; Ef) and phentolamine (100 μM; Ph) were included as shown. Insulin secretion was measured after 60 min of incubation at 37°C. Results represent mean values ± s.e.mean for 7–8 observations. $*P<0.01$, $**P<0.001$; relative to incubation with diazoxide in the absence of imidazoline.

Table 1 Effects of RX821002 on basal insulin release and on the inhibitory response to diazoxide in rat islets

Glucose (mM)	*RX821002* (100 μM)	*Diazoxide* (125 μM)	*Insulin secretion* (ng/islet h^{-1})
4	–	–	0.55 ± 0.04
4	+	–	0.81 ± 0.10*
20	–	–	2.11 ± 0.08
20	–	+	0.61 ± 0.07
20	+	+	1.52 ± 0.11**

Groups of three isolated islets were incubated for 60 min at 37°C, in the presence of test reagents as shown. After this time, samples of medium were removed for measurement of insulin content by radioimmunoassay. Data are presented as mean values ± s.e.mean for 12–18 observations. $*P<0.0125$; $**P<0.001$; relative to incubation in the absence of RX821002.

145 ± 22 nM ($n = 4$) and B_{max} of 364 ± 22 fmol mg^{-1} protein. It was difficult to determine the K_D of the low affinity site with any degree of accuracy since the site remained less than fully saturated under all conditions tested and the value obtained from non-linear curve fitting represents a minimum estimate.

Pharmacology of the low affinity [^{3}H]-RX821002 binding site

Further characterization of the low affinity [^{3}H]-RX821002 binding site in competition binding assays employing imidazoline compounds of varying secretagogue activity was difficult, since the K_D of the site is very low and the proportion of non-specific binding increased significantly at high concentrations of radioligand. However, experiments utilizing 50 nM [^{3}H]-RX821002 and performed in the presence of 10 μM adrenaline, revealed a dose-dependent displacement of [^{3}H]-RX821002 binding to the low affinity site by efaroxan (Figure 3). Under these conditions, approximately 45% of total non-adrenoceptor [^{3}H]-RX821002 binding was displaced by 50 μM efaroxan. Figure 4 shows the displacement of [^{3}H]-RX821002 binding by a variety of compounds acting at α-adrenoceptors. The imidazoline ligands efaroxan, RX821-002, phentolamine, and clonidine (10 μM) significantly displaced the binding of the radioligand, whereas idazoxan and UK14,304 (10 μM) had no significant effect on the binding of [^{3}H]-RX821002 at this low affinity site. This excludes the possibility that displacement was from residual α_2-adrenoceptors since all of these compounds are potent α_2-ligands in the endocrine pancreas. Prazosin also failed to alter RX821-002 binding indicating that α_1-adrenoceptors were not responsible for the low affinity binding of the radioligand. Significantly, this pharmacological profile correlates well with the capacity of imidazoline compounds to stimulate insulin release since efaroxan, RX821002, phentolamine and clonidine (under conditions of α_2-blockade) are all secretagogues

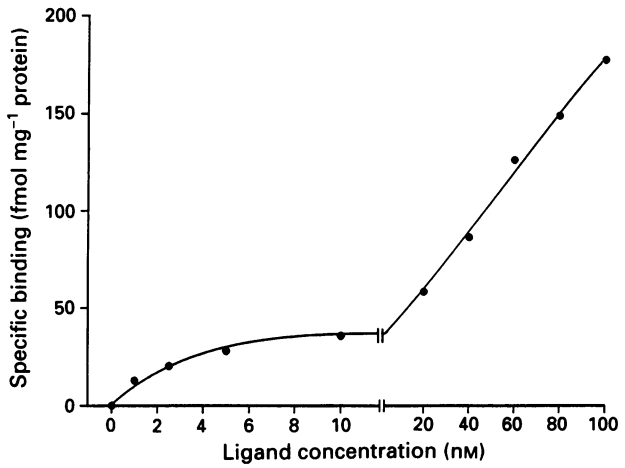

Figure 2 RINm5F membranes were incubated with [^{3}H]-RX821002, over the concentration range 1.0–100 nM, for 60 min at 25°C. Non-specific binding was determined in the presence of 100 μM phentolamine. Shown is a representative experiment with each point determined in triplicate. Binding data were analyzed by iterative non-linear curve fitting using the Fig P Curve Fitter Programme (Biosoft, Cambridge, U.K.).

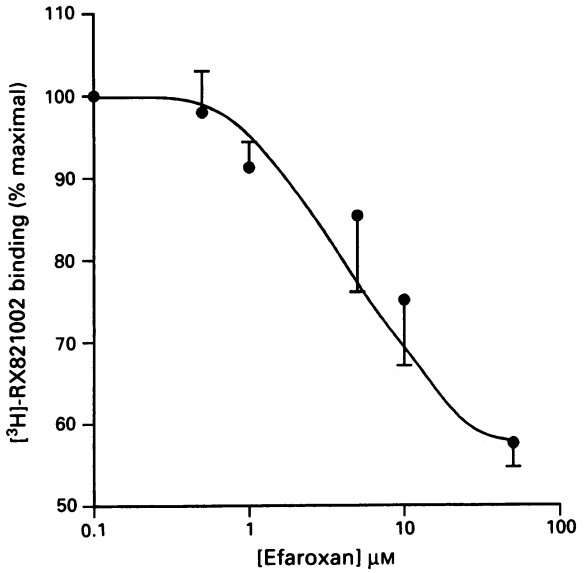

Figure 3 Displacement of [^{3}H]-RX821002 binding to the low affinity binding site in RINm5F membranes by efaroxan. RINm5F membranes were incubated with [^{3}H]-RX821002 (50 nM) in the presence and absence of efaroxan (0.1–100 μM), for 60 min at 25°C. Adrenaline (10 μM) was included to block α_2-adrenoceptors. Each point represents the mean ± s.e.mean for 12 observations.

whereas idazoxan and UK14,304 do not augment insulin secretion (Chan *et al.*, 1988; Schulz & Hasselblatt, 1989b; Chan & Morgan, 1990; Brown *et al.*, 1993). Use of a higher concentration of idazoxan (50 μM) in competition experiments did result in some displacement (20%) of [^{3}H]-RX821002 binding (not shown) suggesting that idazoxan may interact with the site when present at very high concentrations.

Discussion

The ability of several imidazoine compounds to increase insulin release both *in vitro* and *in vivo* has been ascribed to their blockade of ATP-sensitive potassium channels in islet

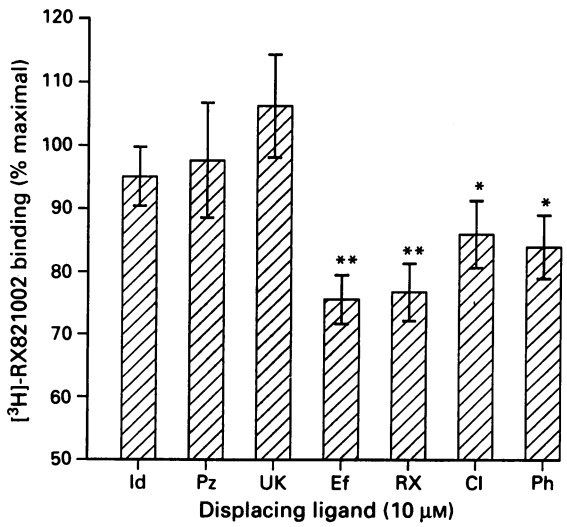

Figure 4 Effects of compounds acting on α-adrenoceptors on the binding of [^{3}H]-RX821002 to the low affinity binding site in RINm5F membranes. Competition binding studies were performed on RINm5F membranes with a single concentration (50 nM) of [^{3}H]-RX821002. Competing agents were present at a concentration of 10 μM; idazoxan (Id); prazosin (Pz); UK14,304 (UK); efaroxan (Ef); RX821002 (RX); clonidine (Cl); and phentolamine (Ph). Adrenaline (10 μM) was included in all tubes to block α_2-adrenoceptors. The data are expressed as percentages of the control binding measured in the absence of competitor. Each point represents the mean ± s.e.mean for 12–18 observations. *$P<0.01$; **$P<0.001$, versus the control.

β-cells (Plant & Henquin, 1990; Chan, 1993). As yet, the nature of the binding site mediating this effect has not been disclosed. In this study, we have sought to identify a radioligand that maybe suitable for labelling of the novel 'imidazoline' binding site responsible for mediating the secretagogue activity of certain imidazoline compounds.

It might be anticipated that clonidine (or a derivative) would be a suitable ligand for labelling of islet imidazoline receptors since clonidine stimulates insulin secretion under conditions of α_2-adrenoceptor blockade (Schulz & Hasselblatt, 1989b; Lacombe *et al.*, 1993). To investigate this possibility, preliminary studies were performed in which the high specific activity ligand [^{125}I]-iodoclonidine was used to label clonidine binding sites in RINm5F cells. RINm5F is a rat islet derived cell line which expresses a wide range of receptors and has been used extensively to investigate the mechanisms of signal transduction by cell surface receptors that control insulin secretion (Praz *et al.*, 1983; Aminaroff *et al.*, 1988). These cells express imidazoline receptors that regulate K^+_{ATP} channel activity (Chan *et al.*, 1991a; Dunne, 1991) and thus, we have used crude membrane preparations from RINm5F cells in the radioligand binding studies. [^{125}I]-iodoclonidine interacted with both α_2-adrenoceptors and imidazoline sites in RINm5F membranes but in the latter case binding was displaced more potently by idazoxan than by efaroxan (unpublished observations), suggesting that it represented mainly interaction with I_2-sites (Ernsberger *et al.*, 1992). Thus, it was inappropriate to employ [^{125}I]-iodoclonidine in further studies due to its multiple sites of interaction.

Therefore, we next considered the possibility that [^{3}H]-RX821002 might be an appropriate ligand. [^{3}H]-RX821002 is an alpha-2 antagonist of the imidazoline family which has been shown to be highly specific for α_2-adrenoceptors with no significant interaction with I_2-sites (NAIBS), over the range of concentrations used in binding studies (Langin *et al.*, 1989). However, we have shown in a previous study that RX821002 can potentiate insulin secretion from rat isolated islets (Chan, 1990) and similar results have also been reported in perfused rat pancreas (Gross *et al.*, 1991). This

response does not reflect α_2-receptor blockade since it is not reproduced by other equally effective α_2-antagonists that are not imidazolines (Chan, 1993). It seems likely, therefore, that the response to RX821002 is mediated by the putative islet imidazoline receptor site implicated in regulation of β-cell potassium permeability.

Binding experiments revealed that [^{3}H]-RX821002 labelled a low affinity site on RINm5F cell membranes, the pharmacology of which is consistent with the possibility that it may represent the previously elusive 'imidazoline' receptor responsible for regulation of β-cell K^+_{ATP} channel activity. The site is clearly distinct from either α_2-adrenoceptors or I_2-sites since the residual binding of [^{3}H]-RX821002, measured in the presence of adrenaline, was not displaced by idazoxan or UK14,304, which interact with both types of receptor with high affinity (Ernsberger *et al.*, 1992; Brown *et al.*, 1993). Furthermore, the site is unlikely to represent an I_1-imidazoline site since efaroxan is reported to be an I_1-antagonist (Ernsberger *et al.*, 1992) but acts agonistically on insulin secretion and K^+_{ATP} channel blockade (Chan & Morgan, 1990; Chan *et al.*, 1991b). Furthermore, the low potency with which efaroxan stimulates insulin secretion (Chan & Morgan, 1990) and displaces [^{3}H]-RX821002 binding (Figure 3) are not consistent with interaction at an I_1 site (Ernsberger *et al.*, 1992).

A number of previous studies have reported the labelling of pancreatic β-cell α_2-adrenoceptors with [^{3}H]-RX821002 (Remaury & Paris, 1992; Lacombe *et al.*, 1993), although none of these has described an additional lower affinity site. Indeed, we also conducted an earlier study in rat isolated islets which revealed only a single [^{3}H]-RX821002 binding site (Morgan *et al.*, 1989). The important difference in the present work is that the range of concentrations of [^{3}H]-RX821002 employed has been extended considerably to facilitate labelling of any low affinity sites. At concentrations below 10 nM, [^{3}H]-RX821002 labels α_2-adrenoceptors exclusively in RINm5F cells and pancreatic islets, and the parameters for α_2-adrenoceptor binding determined in the present work are in excellent agreement with those reported in RINm5F cells by Remaury & Paris (1992).

In view of the very low affinty of the [^{3}H]-RX821002 site identified in this work, it is surprising that the site could be detected by use of vacuum filtration methods to separate bound from free ligand. It might be anticipated that the rate of dissociation of ligand from receptor would be sufficiently rapid that the ligand would dissociate during filtration and washing. Despite these considerations, however, we were consistently able to detect displaceable binding of [^{3}H]-RX821002 to RINm5F cell membranes over a large number of experiments. It is noteworthy that MacKinnon *et al.* (1993) have also recently characterized a low affinity imidazoline receptor (K_D; 128 nM) in rat kidney membranes using a tritiated ligand and vacuum filtration methods.

Down-regulation of receptors in response to chronic agonist exposure has been widely reported in pathological conditions and during chronic drug treatment. A number of observations have indicated that imidazoline sites are also regulated in this manner (Hamilton *et al.*, 1992; 1993; Olmos *et al.*, 1992; 1993). Indeed, alterations in imidazoline receptor site number and/or regulation have been implicated in the pathogenesis of endogenous depression and hypertension (Olmos *et al.*, 1992; 1993). In the present study, we have confirmed that the imidazoline site responsible for mediating the insulin secretagogue activity in islets can be down-regulated on long-term exposure (18 h) to agonists. However, imidazolines which are unable to stimulate insulin release directly (idazoxan and UK14,304) did not affect the capacity of efaroxan and phentolamine to antagonize the inhibitory actions of diazoxide on secretion in these cultured islets. Thus, these data suggest that efaroxan and phentolamine act as agonists at the islet imidazoline site. Efaroxan has been classified as a potent antagonist at the I_1-site since it can inhibit the rise in arterial blood pressure induced by monoxidine, a highly selective and potent agonist at I_1-sites (Ernsberger *et al.*, 1992). Thus, it would appear that the islet imidazoline site displays pharmacological characteristics that differ from those of I_1-sites. Moreover, idazoxan and UK14,304 have been documented to act at I_2-sites in brain and kidney (Bidet *et al.*, 1990; Hamilton *et al.*, 1992; 1993). Since neither of these compounds is an insulin secretagogue, nor acts as antagonist to the activity of efaroxan (Brown *et al.*, 1993), and was unable to down-regulate the actions of efaroxan and phentolamine in the present study, it can be concluded that the islet imidazoline site represents an imidazoline-preferring site that is also distinct from the I_2-site.

In conclusion, in isolated islets RX821002 can stimulate insulin release by a mechanism which involves interaction with a regulatory component involved in control of ATP-sensitive potassium channel activity. Besides labelling the α_2-adrenoceptors with high affinity, [^{3}H]-RX821002 also labelled with much lower affinity, a class of binding sites with a pharmacological profile similar to that expected of the site involved in stimulation of insulin release by imidazoline receptors. This site displays characteristics which are distinct from those of the designated I_1- and I_2-sites. The radioligand [^{3}H]-RX821002 may be of value for assay of the islet imidazoline receptor during purification.

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