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Insulin Secretagogues with an Imidazoline Structure Inhibit Arginine-Induced Glucagon Secretion from Isolated Rat Islets of Langerhans

Mirna Mourtada,* Stephen A. Smith,† and Noel G. Morgan*,[1]

**Cellular Pharmacology Group, Department of Biological Sciences, Keele University, Staffs ST5 5BG, United Kingdom; and †SmithKline Beecham Pharmaceuticals, Cold Harbour Road, Harlow, Essex CM19 5AD, United Kingdom*

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It is well documented that imidazoline compounds such as efaroxan and phentolamine act as potent insulin secretagogues both *in vivo* and *in vitro,* an effect which is mediated principally by blockade of ATP-sensitive potassium channels in the pancreatic B-cell. However, little is known about the effects of these drugs on the secretion of other pancreatic hormones and, in the present work, we have investigated the effects of selective imidazoline compounds on glucagon release from isolated rat islets of Langerhans. None of several imidazoline compounds tested (efaroxan, phentolamine, idazoxan, antazoline) affected glucagon secretion from islets incubated with 4mM glucose. However, when the rate of glucagon release was stimulated by L-arginine (20mM) efaroxan caused a rapid, sustained and dose-dependent inhibition of the secretory response (EC_{50} approximately 30μM). This effect was seen under both static incubation and islet perifusion conditions. Antazoline and phentolamine also inhibited arginine-induced glucagon secretion, whereas idazoxan (an imidazoline which does not affect insulin secretion) failed to alter glucagon release. The inhibitory effects of imidazolines on glucagon release were not secondary to changes in insulin secretion. Taken together, the results indicate that pancreatic A-cells express functional imidazoline receptors which can regulate the secretory activity of the cells.

Imidazoline receptors are a class of nonadrenergic binding sites which display high affinity for ligands bearing an imidazoline moiety or a closely related structure [reviewed in 1-3]. At least two principal subtypes have been defined and are designated I_1 and I_2 sites on the basis of their ligand selectivity [4]. [^{3}H] clonidine and its derivatives have high affinity for I_1 sites, whereas I_2 sites preferentially bind [^{3}H] idazoxan [1-5].

In the endocrine pancreas, certain imidazoline compounds act as potentiators of nutrient-induced insulin secretion [reviewed in 5,6]. This effect derives mainly from the inhibition of ATP-sensitive potassium (K_{ATP}) channels in the pancreatic B-cell [5-9] leading to membrane depolarisation, with subsequent opening of voltage-dependent Ca^{2+} channels, Ca^{2+} influx and a rise in cytoplasmic Ca^{2+} concentration $[Ca^{2+}]_i$ [10-12]. This then triggers insulin release. Since these effects can be observed *in vivo* as well as *in vitro* [13,14] it has been suggested that imidazoline drugs may be useful new anti-hyperglycaemic agents [5,6]. Indeed, several compounds have already shown promise when tested in animal models of diabetes and in human subjects [15-17].

The ideal profile for a compound under development as a therapeutic insulin secretagogue would include not only the ability to stimulate insulin secretion but also a concomitant capacity to reduce glucagon release. This is because glucagon levels are often elevated in diabetic patients, a situation which will tend to exacerbate hyperglycaemia. To date, there have been very few studies on the effects of imidazoline-insulin secretagogues on glucagon release and it is not known whether islet A-cells possess functional imidazoline receptors. Evidence has been presented that circulating glucagon levels were reduced upon administration of the imidazoline, midaglizole, to human subjects [18] and a recent preliminary report has described the lowering of glucagon release by another imidazoline, RX871024, in perfused rat pancreas [19]. However, to our knowledge, there are no studies of the direct effects of imidazoline compounds on A-cell function in isolated rat islets. Therefore, we have investigated the effects of several imidazoline compounds on glucagon release from isolated rat islets of Langerhans to examine

[1] Author for correspondence: Fax: 44-1782-583516; Email: n.g.morgan@keele.ac.uk.

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TABLE 1

Effects of Imidazoline Compounds on Glucagon Secretion in the Presence of 4 mM Glucose

Test reagents	Glucagon secretion (pg/islet/h)
	26 ± 3.9
Arginine (20 mM)	150 ± 29*
Efaroxan (100 μM)	24 ± 5.4^{NS}
Idazoxan (100 μM)	27 ± 3.3^{NS}
Phentolamine (100 μM)	30 ± 5.3^{NS}
Antazoline (100 μM)	21 ± 4.8^{NS}

Groups of five isolated rat islets were incubated in the presence of 4mM glucose for 1h at 37°C in the presence of additional test reagents as shown. Samples of medium were removed at the end of the incubation period and their glucagon levels measured by radioimmunoassay. Data are presented as mean values ± s.e. mean for 6 observations from a representative experiment.

* $p < 0.001$ relative to 4mM glucose in the absence of arginine.

^{NS} Not significantly different from 4mM glucose in the absence of test reagents.

whether imidazoline receptors can regulate exocytosis from A-cells.

METHODS

Pancreatic islets were obtained from male Wistar rats by collagenase digestion as described previously [7,14]. Insulin and glucagon release were measured in static incubation or perifusion experiments in which islets were incubated in a bicarbonate buffered solution equilibrated with 5% CO_2: 95% O_2 to pH 7.4 and supplemented with 1mM $CaCl_2$, 1mg/ml bovine serum albumin and 4mM glucose. In static experiments, five size-matched islets were incubated in 0.5ml of the incubation medium containing test reagents for 1 hour at 37°C. After this time, samples of the medium were removed for measurement of glucagon and insulin secretion by radioimmunoassay. In perifusion experiments, group of 100 islets were perifused at 37°C (flow rate: 1ml min^{-1}) for 40 minutes, after which time the experiment was commenced and samples of the effluent medium were collected at 3 minute intervals for measurement of insulin and glucagon secretion.

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

RESULTS

Several imidazoline derivatives which potentiate glucose-induced insulin secretion from rat islets, were investigated for effects on glucagon secretion. When rat islets were incubated in the presence of 4mM glucose, none of the compounds tested (efaroxan, phentolamine, antazoline or idazoxan) had any effect on the rate of glucagon release (Table 1). Treatment of islets with 20mM L-arginine increased both insulin and glucagon secretion (Tables 1 & 2) and, under these conditions, efaroxan, phentolamine and antazoline (100μM) all caused a marked inhibition of glucagon secretion, whereas thay failed to modify arginine-induced insulin release (Table 2). By contrast, idazoxan, did not alter arginine-induced glucagon or insulin release.

The effects of efaroxan on arginine-induced glucagon secretion were dose-dependent (Figure 1). Concentrations of efaroxan below 10μM failed to significantly alter glucagon release but increasing the concentration above this level caused a progressively greater inhibition of the secretion rate (EC_{50} approximately 30μM). The maximal response was observed at 100μM efaroxan when glucagon secretion was suppressed to a level equivalent to that measured in the absence of arginine.

In perifused islets, introduction of 20mM L-arginine into the medium resulted in a prompt increase in glucagon secretion which reached the maximal level within 5 min. Subsequent addition of 100μM efaroxan caused a rapid and marked inhibition of glucagon release (Figure 2) which was evident within 3 minutes and was sustained for the duration of efaroxan infusion. Under the same conditions, efaroxan did not alter L-arginine induced insulin secretion (Figure 3).

DISCUSSION

A considerable amount of work has been devoted to investigating the mechanisms which underlie the stimulatory effects of imidazoline compounds on insulin secretion, and the weight of current evidence suggests that the response is mediated by a subtype of imidazoline receptor associated with K_{ATP} channels [5-12]. This receptor appears to be distinct from the I_1 and I_2 subtypes defined in other tissues and, as such, may represent a target for a new generation of selective antihyperglycaemic drugs [5,6].

TABLE 2

Effects of Imidazoline Compounds on the Stimulation of Glucagon Secretion by Arginine in Isolated Rat Islets of Langerhans

[Arginine] (20 mM)	Test reagents (100 μM)	Insulin secretion (ng/islet/h)	Glucagon secretion (pg/islet/h)
–	–	0.50 ± 0.09	39 ± 12
+	–	1.89 ± 0.13**	259 ± 31**
+	Idazoxan	1.98 ± 0.14^{NS}	295 ± 67^{NS}
+	Efaroxan	1.79 ± 0.09^{NS}	98 ± 17*
+	Antazoline	1.97 ± 0.15^{NS}	65 ± 8*
+	Phentolamine	2.19 ± 0.27^{NS}	113 ± 22*

Groups of five isolated islets were incubated for 1h at 37°C in the presence of 4mM glucose and additional test reagents as shown. After this time, samples of medium were removed for measurement of glucagon and insulin levels by radioimmunoassay. Data are presented as mean values ± s.e. mean for 10-12 observations.

* $p < 0.01$ relative to 20mM arginine.

** $p < 0.001$ relative to 4mM glucose alone.

^{NS} Not significantly different from 20mM arginine in the absence of test reagents.

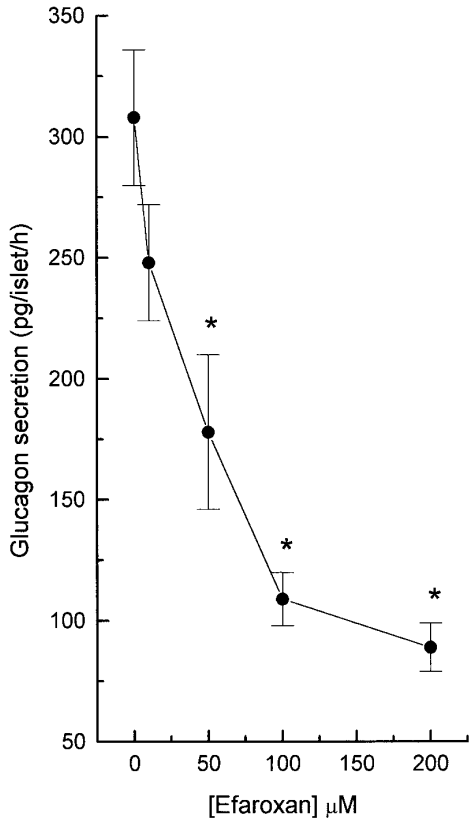

FIG. 1. Effects of increasing concentrations of efaroxan on arginine-induced glucagon release. Group of 5 isolated rat islets were treated with efaroxan at increasing concentrations in the presence of 20mM arginine. Following incubation for 1h samples of the incubation medium were removed and assayed for glucagon secretion. Data represent mean rates of glucagon secretion ± s.e.mean. *$p<0.001$ relative to 20mM arginine alone.

In the present work we provide direct evidence that the imidazoline insulin secretagogues efaroxan, phentolamine and antazoline can also modify the rate of glucagon secretion from pancreatic A-cells. This effect occurred independently of changes in insulin secretion suggesting that it was not mediated by a paracrine mechanism dependent on primary alterations in the secretory activity of the islet B-cells. Indeed, we observed that the insulin secretory effect of 20mM L-arginine was not enhanced by the imidazolines, a finding which is consistent with previous evidence showing that the maximal insulin secretory response to glucose is also unaffected by efaroxan and related compounds, in rat islets [20].

The results suggest that pancreatic A-cells are equipped with an imidazoline receptor involved in the direct regulation of glucagon secretion. The pharmacology of this site requires further study but the evidence presented here suggests that it responds functionally to a similar range of compounds to the equivalent site present in pancreatic B-cells. Thus, efaroxan, phentolamine and antazoline were all able to alter glucagon secretion whereas idazoxan was ineffective. An identical profile is seen for insulin secretion [7-9,20,21-23]. Moreover, the concentrations of efaroxan required for inhibition of glucagon secretion (10-100μM) are precisely equivalent to those necessary to achieve stimulation of insulin secretion [20,23]. On the basis of this evidence, it seems likely that either the same or a closely related subtype of imidazoline receptor is responsible for regulation of the secretory activity of both islet A- and B-cells.

The finding that idazoxan was ineffective as a secretagogue in A-cells accords with the lack of effect of this compound in B-cells [11,20] and suggests that glucagon secretion is not controlled by an I_2-imidazoline receptor, since idazoxan binds to I_2 sites with high affinity whereas efaroxan is only weakly potent as an I_2 ligand [1,2,4,24]. These results also suggest that the inhibition of glucagon secretion does not result from blockade of α_2-adrenoceptors. Islet A-cells are known to express α_2-

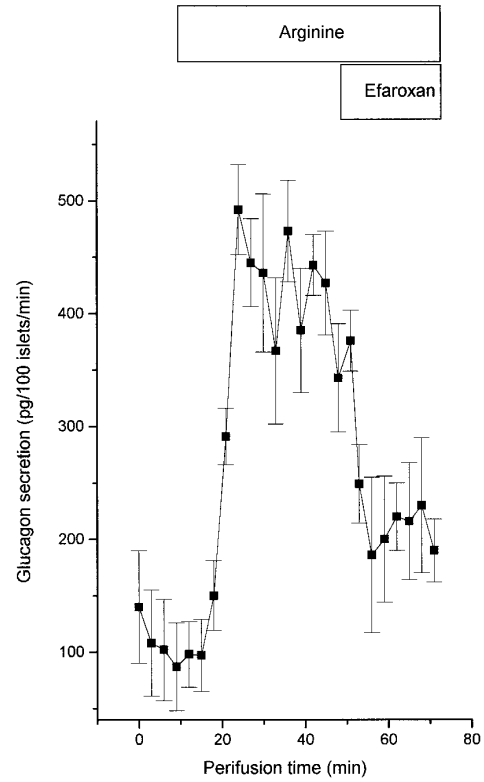

FIG. 2. Effects of efaroxan on arginine-induced glucagon secretion in perifused rat islets. Groups of 100 isolated rat islets were perifused with medium containing 4mM glucose for 40 minutes. After this time (t = 0) samples of medium were collected to establish the basal glucagon secretion rate. 20mM arginine was introduced after 10 minutes and efaroxan (100μM) was infused during the final 30 minutes of the experiment. Samples of the medium were collected and their glucagon content determined by radioimmunoassay. Results represent mean values ± s.e.mean from 3 separate experiments.

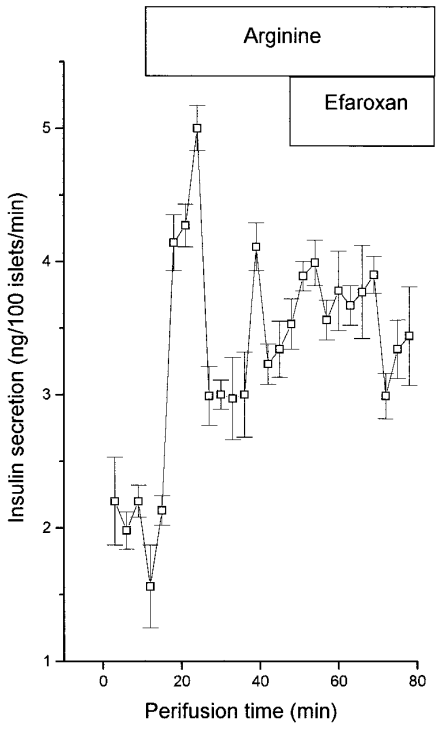

FIG. 3. Effects of efaroxan on arginine-induced insulin secretion in perifused rat islets. Groups of 100 isolated rat islets were perifused with medium containing 4mM glucose for 40 minutes. After this time (t = 0) samples of medium were collected to establish the basal insulin secretion rate. 20mM arginine was introduced after 10 minutes and efaroxan (100μM) was infused during the final period of the experiment. Samples of the medium were collected and their insulin content determined by radioimmunoassay. Results represent mean values ± s.e.mean from 3 separate experiments.

adrenoceptors [25,26] and efaroxan and phentolamine are both potent antagonists at these sites [24]. However, idazoxan is also a potent α_2-antagonist but did not inhibit glucagon secretion confirming that α_2-blockade was not responsible for the inhibitory effects observed here. In support of this, glucagon secretion was inhibited by antazoline, an imidazoline compound which has minimal α_2-antagonist activity [14,23].

In pancreatic B-cells there is good evidence that imidazoline receptors regulate the functional status of K_{ATP} channels, although it is unclear whether the binding site lies close to the channel pore or whether it is located on a separate component of the channel complex. Very recent data suggest that the B-cell receptor may, in addition to controlling K_{ATP} channels, also regulate a more distal component of the exocytotic pathway [27]. If verified, this would suggest that the receptor is separate from the ion-conducting pore of the channel [28]. The present work supports this view since control of glucagon secretion is unlikely to be dependent on K_{ATP} channel activity (although glucagon-secreting cells do express K_{ATP} channels [29], indicating a different site of action for the A-cell imidazoline receptor. Thus, it appears that imidazoline receptors can control the secretion of pancreatic hormones by interacting with at least two sites; one of these is associated with K_{ATP} channels and may be of primary importance in B-cells, whereas the second regulates exocytosis by a different mechanism and may be the principal mechanism operating in A-cells.

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