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Short communication

The imidazoline I_1 receptor agonist, moxonidine, inhibits insulin secretion from isolated rat islets of Langerhans

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Abstract

In order to study the pharmacology of the putative imidazoline receptor involved in stimulation of insulin secretion, the potent and selective imidazoline I_1 receptor agonist, moxonidine, was employed. Surprisingly, this agent caused a rapid and complete inhibition of glucose-induced insulin secretion in isolated rat islets of Langerhans. This response was reversible upon removal of the compound but was only partially attenuated under conditions of complete α_2 blockade, suggesting that it did not derive entirely from the weak α_2-adrenoceptor agonist activity of moxonidine. Furthermore, the response could not be attributed to activation of imidazoline I_1 receptors since it was not reproduced by a second potent imidazoline I_1 receptor agonist, cimetidine, and could not be alleviated by the imidazoline I_1 receptor antagonist efaroxan. The results confirm that the imidazoline receptor involved in control of insulin secretion differs from the I_1 subclass and suggest that moxonidine inhibits insulin secretion by a mechanism unrelated to imidazoline I_1 receptor agonism.

Keywords: Islets of Langerhans; Endocrine pancreas; Imidazoline receptor; Imidazoline I_1 site; Moxonidine; Efaroxan; Cimetidine; Diazoxide; Insulin secretion

1. Introduction

Many tissues express binding sites which possess a high affinity for ligands bearing an imidazoline ring (or a closely related structure) and these have been termed 'imidazoline binding sites' or 'imidazoline receptors' (Michel and Ernsberger, 1992; Ernsberger et al., 1992). They comprise a family of molecules with distinct pharmacological characteristics but functional analysis has not yet substantiated the pharmacological classification and it remains an important objective to define the functional roles of the various imidazoline receptor subtypes. This has been achieved to some extent for imidazoline I_1 receptors in the central nervous system, which are implicated in the regulation of blood pressure (Michel and Ernsberger, 1992; Ernsberger et al., 1992), but the role(s) of imidazoline I_2 sites is still largely undefined. In this context, there is one tissue, the endocrine pancreas, in which the functional characterisation of imidazoline responses has proceeded more rapidly than the pharmacological analysis. This is because certain imidazoline ligands act as potentiators of insulin secretion (both in vivo and in vitro) (Ahrén and Lundquist, 1985; Chan, 1993; Schulz and Hasselblatt, 1989) and have attracted attention as possible new anti-hyperglycaemic drugs. Their capacity to stimulate insulin release derives from an interaction with ATP-sensitive potassium channels (Chan et al., 1991; Dunne, 1991; Jonas et al., 1992) which results in a reduction in the potassium permeability of the β-cell plasma membrane, gating of voltage sensitive Ca^{2+} channels and stimulation of Ca^{2+} influx. This, then, enhances insulin secretion.

The pharmacology of the imidazoline receptors involved in control of insulin secretion appears atypical in that the order of potency of imidazoline ligands acting as insulin secretagogues does not correlate with the order of potency for binding of ligands to either imidazoline I_1 or I_2 sites in other tissues (Chan, 1993; Chan et al., 1994). Thus, it has been proposed that the islet receptor may be a novel subtype of imidazoline receptor, although it possesses characteristics which resemble those of the imidazoline I_1 receptor, in that

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certain ligands (e.g. efaroxan, clonidine) interact with both binding sites (Ernsberger et al., 1992; Chan, 1993). In order to clarify this relationship further, we have studied the effects of a highly selective imidazoline I_1 receptor agonist, moxonidine, on insulin secretion from isolated rat islets of Langerhans. Moxonidine is among the most potent imidazoline I_1 ligands currently available and has been undergoing clinical evaluation as a novel anti-hypertensive drug (Ernsberger et al., 1993). Moreover, recent evidence suggests that it can stimulate insulin secretion from the clonal pancreatic β-cell line, RIN-5AH (Olmos et al., 1994). Thus, it is important to establish whether this response also occurs in normal islets and whether it can be attributed to activation of an imidazoline I_1 receptor in the endocrine pancreas.

2. Materials and methods

2.1. Materials

Collagenase (type XI), noradrenaline, yohimbine, cimetidine and diazoxide were purchased from Sigma Chemical Co. (Dorset, UK). Moxonidine was a gift from Beirsdorf-Lilly (Hamburg, Germany). Phenoxybenzamine was donated by Ciba-Geigy (UK). All other reagents were of analytical reagent grade. Drugs were prepared as stock solutions in either water or dimethyl sulphoxide (DMSO). The final DMSO concentration did not exceed 1% and did not alter insulin secretion.

2.2. Methods

Islets of Langerhans were isolated from Wistar rats of either sex by collagenase digestion of excised pancreata. The islets were selected by hand under a binocular dissecting microscope and were used within 2 h of isolation. They were incubated either under static conditions or in a perifusion system. For static incubation experiments, groups of 3 individual islets were selected and incubated in 0.5 ml of a bicarbonate-buffered physiological saline solution gassed with O_2/CO_2 (95:5) to pH 7.4, containing bovine serum albumin (1 mg/ml) and test reagents. Following incubation at 37°C for 1 h, samples of the incubation medium were removed for measurement of insulin secretion by radioimmunoassay.

In perifusion experiments, groups of 80 islets were placed in male-female luer connectors plugged with glass wool and perifused at 37°C (flow rate of 1 ml/min) with bicarbonate-buffered physiological saline solution supplemented with bovine serum albumin (1 mg/ml). Test reagents were introduced by infusion at a flow rate of 10 μl/min. Samples of effluent medium were collected at timed intervals and assayed for insulin by radioimmunoassay.

Statistical analysis of results was performed by one- or two-way analysis of variance with Tukey's multiple comparison test.

3. Results

In initial experiments, the effect of moxonidine on glucose-induced insulin secretion was investigated using perifused rat islets (Fig. 1a). The islets were al-

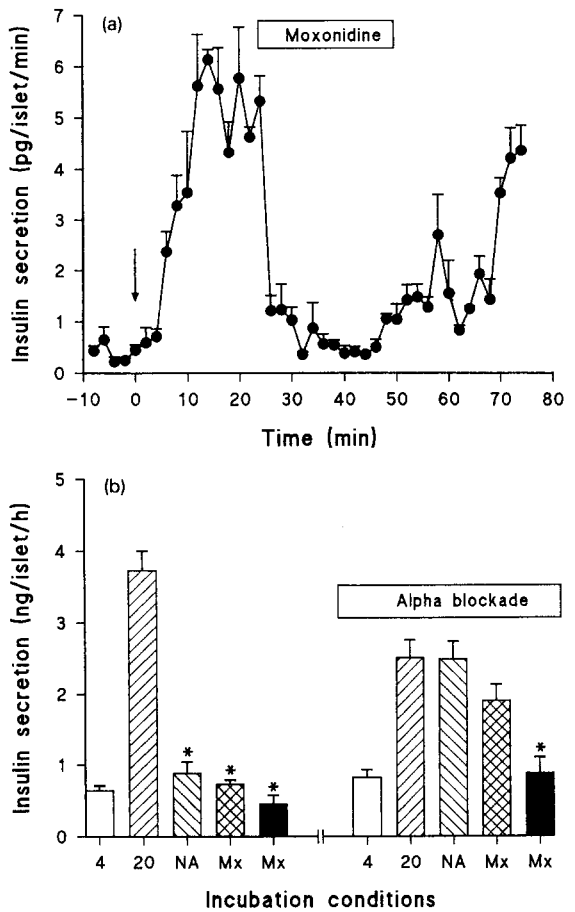

Fig. 1. Effects of moxonidine on insulin secretion from isolated rat islets of Langerhans. Panel a: Groups of isolated rat islets were perifused with medium containing 4 mM glucose for 30 min, after which time ($t = 0$) 20 mM glucose was introduced (arrow). Moxonidine (100 μM) was infused from $t = 30$–50 min (bar). Samples of perifusion medium were collected at 2 min intervals and their insulin content determined by radioimmunoassay. Results represent mean values ± S.E.M. from 3 separate experiments. Panel b: Isolated rat islets were preincubated for 30 min either in the absence (left) or presence (right) of 10 μM phenoxybenzamine. The islets were then incubated in the absence of α-adrenoceptor antagonists (left) or in the presence of 10 μM phenoxybenzamine plus 200 μM yohimbine (right). The incubation conditions were: 4 mM glucose (open bar); 20 mM glucose (20; right hatches); 20 mM glucose plus noradrenaline (NA, 10 μM; left hatched bars); 20 mM glucose plus moxonidine (Mx) 10 μM (cross hatched bars) or 100 μM (solid bars). * Significant inhibition relative to 20 mM glucose control ($P < 0.001$).

lowed to stabilise by perifusion with medium containing 4 mM glucose for 30 min, after which time the glucose concentration of the medium was increased to 20 mM. As expected, this resulted in a prompt increase in insulin secretion which was sustained during the period of glucose infusion (Fig. 1a). Introduction of moxonidine (100 μM) during the glucose stimulation resulted in a rapid and complete inhibition of insulin secretion, which returned to the basal rate within 5 min (Fig. 1a). Subsequent removal of moxonidine from the perifusion medium was accompanied by a renewed increase in insulin secretion towards the stimulated rate, suggesting that the inhibitory effect of moxonidine was reversible.

Since moxonidine possesses weak α_2-adrenoceptor agonist activity, the inhibition of insulin secretion observed under perifusion conditions may have resulted from activation of islet α_2-adrenoceptors. However, inclusion of 200 μM yohimbine failed to prevent the inhibitory effect of moxonidine on insulin secretion (not shown) suggesting that an alternative mechanism must be operative. In order to confirm this, islets were exposed to conditions of extreme α_2 blockade by preincubation with the covalent antagonist phenoxybenzamine (10 μM) followed by incubation with both phenoxybenzamine and 200 μM yohimbine. Under these conditions, the ability of the α_2-adrenoceptor agonist noradrenaline to inhibit glucose induced insulin secretion was totally abolished (Fig. 1b) suggesting that the blockade of α_2-adrenoceptors was complete. Despite this, 100 μM moxonidine was still able to inhibit glucose-induced insulin secretion significantly (Fig. 1b) although the effect of a lower concentration (10 μM) was partially antagonised.

In a third series of experiments, the interactions between moxonidine and the imidazoline insulin secretagogue, efaroxan, were investigated. In these studies, the K^+ channel agonist diazoxide was used to inhibit glucose-induced insulin secretion and the well characterised ability of efaroxan to reverse this inhibition, monitored. The experiments were all performed in the presence of 200 μM yohimbine to minimise α_2-adrenoceptor activation. As observed previously, efaroxan effectively antagonised the inhibition of insulin secretion mediated by diazoxide, a response which is believed to reflect the interaction of efaroxan with islet imidazoline receptors. Efaroxan, itself, had no effect on the secretory response of islets incubated in the presence of 20 mM glucose (Chan et al., 1991). However, the addition of moxonidine, in the presence of efaroxan, resulted in marked inhibition of glucose-induced insulin secretion (Table 1) and in complete failure of efaroxan to enhance the insulin secretory rate when diazoxide was also present (Table 1). By contrast, the presence of a different imidazoline I_1 receptor agonist, cimetidine, did not lead to any modulation of the response to efaroxan and, unlike moxonidine, cimetidine did not inhibit glucose-induced insulin secretion directly (Table 1).

4. Discussion

In the pancreatic β-cell, imidazoline ligands, such as efaroxan, induce the closure of ATP-sensitive K^+ channels and, as a result, depolarise the plasma membrane and cause an increase in insulin secretion (Chan et al., 1991; Dunne, 1991; Jonas et al., 1992). This response derives from interaction with components present in the β-cell plasma membrane which remain uncharacterised biochemically, but display a number of the characteristics expected of functional imidazoline receptors. Despite this, the pharmacology of imidazoline responses in islets does not correlate with that

Table 1
Effects of the imidazoline I_1 agonists moxonidine and cimetidine on responses to efaroxan in isolated rat islets

Incubation conditions					Insulin secretion (ng/islet/h)
[Glucose] (mM)	Efaroxan (100 μM)	Diazoxide (250 μM)	Moxonidine (100 μM)	Cimetidine (100 μM)	
4	−	−	−	−	0.96 ± 0.15
20	−	−	−	−	2.68 ± 0.38
20	−	+	−	−	1.28 ± 0.09 [a]
20	+	+	−	−	2.20 ± 0.27 [b]
20	−	−	+	−	0.62 ± 0.06 [a]
20	−	−	−	+	2.70 ± 0.32
20	+	−	+	−	1.05 ± 0.24 [a]
20	+	+	+	−	1.27 ± 0.15 [a]
20	+	+	−	+	2.56 ± 0.44

Groups of isolated rat islets were incubated in the presence of 200 μM yohimbine under the conditions shown for 1 h at 37°C, after which samples of the medium were removed and the amount of insulin secreted was measured by radioimmunoassay. Results are presented as mean rates of insulin secretion ± S.E.M. for 12–18 observations. [a] Significant inhibition relative to 20 mM glucose alone ($P < 0.001$). [b] Significant increase relative to 20 mM glucose + diazoxide ($P < 0.001$).

displayed by either the I_1 or I_2 subtypes of imidazoline receptor defined in other tissues (Chan, 1993; Chan et al., 1994). On this basis, it has been proposed that the islet imidazoline receptor may represent a novel subtype. An important piece of evidence which supports this concept derives from the observation that efaroxan is an antagonist of imidazoline I_1 responses in the brain stem (Ernsberger et al., 1992) but acts as an agonist in the endocrine pancreas (Chan, 1993; Chan et al., 1991; Berdeu et al., 1994). Thus, although efaroxan can interact with both imidazoline I_1 receptors and with the putative islet imidazoline receptor, it exerts opposite functional effects at these two sites.

In order to investigate the relationship between imidazoline I_1 receptors and the islet imidazoline receptor further, we have employed the potent I_1 ligand, moxonidine (Ernsberger et al., 1992). This agent exhibits anti-hypertensive activity in vivo and is suggested to exert its effects by virtue of an interaction with imidazoline I_1 receptors localised within the central nervous system (Ernsberger et al., 1993). Moxonidine offers considerable advantages over other imidazoline anti-hypertensive drugs such as clonidine, in that it displays minimal α_2-adrenoceptor agonist activity at therapeutic doses (Ernsberger et al., 1993).

In view of its weak α_2-adrenoceptor agonist activity, it was surprising to find that moxonidine induced a rapid and complete suppression of glucose-induced insulin secretion from isolated rat islets. This response was unaffected by inclusion of 200 μM yohimbine, and only partially attenuated when yohimbine was combined with the covalent α-antagonist phenoxybenzamine. Thus, although part of the response may reflect the α_2-adrenoceptor agonist activity of the drug, it is unlikely that the inhibition was mediated entirely by this mechanism. In support of this, it was confirmed that the extent of α blockade was complete in the present experiments, since the ability of a supra-maximal dose of noradrenaline to inhibit insulin secretion was abolished under conditions when moxonidine still caused inhibition of insulin secretion. Furthermore, we have confirmed that this regimen of α blockade is sufficient to prevent the α_2-adrenoceptor agonist activity of 100 μM clonidine in rat islets (C.A. Brown and N.G. Morgan, unpublished observations) which is significant since clonidine is a more potent α_2-adrenoceptor agonist than moxonidine.

These considerations, therefore, argue against the primary involvement of α_2-adrenoceptors and raise the possibility that the inhibition of insulin secretion induced by moxonidine might be mediated by interaction with islet imidazoline I_1 receptors. However, this also seems unlikely since a second, potent, imidazoline I_1 receptor agonist, cimetidine (Ernsberger et al., 1992), failed to reproduce the inhibitory effect of moxonidine (see Table 1). Furthermore, moxonidine still caused complete inhibition of secretion when islets were also incubated with efaroxan. As efaroxan is a potent imidazoline I_1 receptor antagonist, it would be expected to attenuate any response mediated by I_1 receptors.

Thus, the inhibitory effects of moxonidine in the endocrine pancreas cannot be attributed entirely to either α_2-adrenoceptor or imidazoline I_1 receptor agonist activity. It can also be concluded that the response was not due to direct toxicity of the drug since the inhibitory effect on glucose-induced insulin secretion was reversed when moxonidine was removed from the medium perifusing rat islets (Fig. 1a).

The present data with normal rat islets are at variance with a recent report of the effects of moxonidine on insulin secretion from the transformed clonal β-cell line RIN-5AH (Olmos et al., 1994). In these cells, moxonidine was observed to enhance insulin secretion when used at high concentrations (500 μM and above) and no evidence for inhibitory effects was presented. The reasons for this discrepancy remain to be clarified.

In conclusion: although the possibility that moxonidine may interact with islet imidazoline receptors cannot be excluded completely, the data presented herein demonstrate that its effects in the β-cell cannot be due to interaction with imidazoline I_1 receptors. Overall, therefore, the present results further support the concept that the imidazoline receptor responsible for control of insulin secretion in the endocrine pancreas, is pharmacologically distinct from the currently recognised imidazoline I_1 and I_2 receptor subtypes.

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References

Ahrén, B. and I. Lundquist, 1985, Effects of α-adrenoceptor blockade by phentolamine on basal and stimulated insulin secretion in the mouse, Acta Physiol. Scand. 125, 211.

Berdeu, D., R. Gross, G. Ribes, M.-M. Loubatières-Mariani and G. Bertrand, 1994, Effects of imidazolines and derivatives on insulin secretion and vascular resistance in perfused rat pancreas, Eur. J. Pharmacol. 254, 119.

Chan, S.L.F., 1993, Role of α_2-adrenoceptors and imidazoline-binding sites in the control of insulin secretion, Clin. Sci. 85, 671.

Chan, S.L.F., M.J. Dunne, M.R. Stillings and N.G. Morgan, 1991, The α_2-adrenoceptor antagonist efaroxan modulates K^+_{ATP} channels in insulin-secreting cells, Eur. J. Pharmacol. 204, 41.

Chan, S.L.F., C.A. Brown, K.E. Scarpello and N.G. Morgan, 1994, The imidazoline site involved in control of insulin secretion: characteristics that distinguish it from I_1- and I_2-sites, Br. J. Pharmacol. 112, 1065.

Dunne, M.J., 1991, Block of ATP-regulated K^+ channels by phentolamine and other alpha-adrenoceptor antagonists, Br. J. Pharmacol. 103, 1847.

Ernsberger, P.R., K.L. Westbrooks, O. Christen and S.G. Schäfer, 1992, A second generation of centrally acting antihypertensive agents act on putative imidazoline-I_1 receptors, J. Cardiovasc. Pharmacol. 20, S1.

Ernsberger, P., T.H. Damon, L.M. Graff, S.G. Schäfer and M.O. Christen, 1993, Monoxidine, a centrally acting antihypertensive agent, is a selective ligand for imidazoline-I_1 sites, J. Pharmacol. Exp. Ther. 264, 172.

Jonas, J.-C., T.D. Plant and J.C. Henquin, 1992, Imidazoline antagonists of α_2-adrenoceptors increase insulin release in vitro by inhibiting ATP-sensitive K^+ channels in pancreatic β-cells, Br. J. Pharmacol. 107, 8.

Michel, M.C and P. Ernsberger, 1992, Keeping an eye on the I site: imidazoline-preferring receptors, Trends Pharmacol. Sci. 13, 369.

Olmos, G., R.N. Kulkarni, M. Haque and J. MacDermot, 1994, Imidazolines stimulate release of insulin from RIN-5AH cells independently from imidazoline I_1 and I_2 receptors, Eur. J. Pharmacol. 262, 41.

Schulz, A. and A. Hasselblatt, 1989, Phentolamine, a deceptive tool to investigate sympathetic nervous control of insulin release, Naunyn-Schmied. Arch. Pharmacol. 337, 637.