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Effects of the β-carbolines, harmane and pinoline, on insulin secretion from isolated human islets of Langerhans

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Abstract

It is well known that certain imidazoline compounds can stimulate insulin secretion and this has been attributed to the activation of imidazoline I_3 binding sites in the pancreatic β-cell. Recently, it has been proposed that β-carbolines may be endogenous ligands having activity at imidazoline sites and we have, therefore, studied the effects of β-carbolines on insulin secretion. The β-carbolines harmane, norharmane and pinoline increased insulin secretion two- to threefold from isolated human islets of Langerhans. The effects of harmane and pinoline were dose-dependent (EC_{50}: 5 and 25 μM, respectively) and these agents also blocked the inhibitory effects of the potassium channel agonist, diazoxide, on glucose-induced insulin release. Stimulation of insulin secretion by harmane was glucose-dependent but, unlike the imidazoline I_3 receptor agonist efaroxan, it increased the rate of insulin release beyond that elicited by 20 mM glucose (20 mM glucose alone: 253 ± 34% vs. basal; 20 mM glucose plus 100 μM harmane: 327 ± 15%; $P < 0.01$). Stimulation of insulin secretion by harmane was attenuated by the imidazoline I_3 receptor antagonist KU14R (2 (2-ethyl 2,3-dihydro-2-benzofuranyl)-2-imidazole) and was reduced when islets were treated with efaroxan for 18 h, prior to the addition of harmane. The results reveal that β-carbolines can potentiate the rate of insulin secretion from human islets and suggest that these agents may be useful prototypes for the development of novel insulin secretagogues.

Keywords: Efaroxan; β-cell; Insulin secretion; Imidazoline-binding site; KU14R; β-carboline; Clonidine-displacing substance

1. Introduction

It is now well established that the pancreatic β-cell is equipped with a class of imidazoline binding sites involved in the control of insulin secretion (reviewed by Efendic et al., 2002; Eglen et al., 1998; Morgan and Chan, 2001; Morgan et al., 1999; Rustenbeck et al., 1999). These sites appear to belong to the larger family of imidazoline binding proteins that are expressed both in the central nervous system and in peripheral tissues (Bousquet, 2000, 2001; Bousquet et al., 2000; Eglen et al., 1998; Ernsberger et al., 1997; Head et al., 1998; Khan et al., 1999; Molderings and Gothert, 1999; Parini et al., 1996; Regunathan and Reis, 1996). However, the β-cell imidazoline binding sites exhibit a number of differences from those described elsewhere and, in recognition of this, they have been designated "I_3" to differentiate them from the more ubiquitous imidazoline "I_1" and "I_2" sites (Chan et al., 1994; Eglen et al., 1998; Morgan and Chan, 2001).

Binding of agonists to the imidazoline I_3 site in β-cells leads to an increase in insulin secretion and compounds acting at this site have attracted attention as potential therapeutic agents for use in the management of type 2 diabetes (Chan and Morgan, 1990; Chan et al., 2001; Efanov et al., 2001, 2002; Efendic et al., 2002; Mest et al., 2001; Morgan and Chan, 2001). In addition, attention has also been drawn to the exciting possibility that an endogenous ligand may exist which interacts with the β-cell imidazoline I_3 site and is involved in the physiological control of insulin secretion (Chan et al., 1997). Strong evidence for the existence of such a molecule has been provided from experiments in which extracts of rat brain were found to stimulate insulin secretion (Chan, 1998; Chan et al., 1997). Clearly, identification of the

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active compound would have major implications for drug design.

Despite extensive work, the identity of the putative endogenous imidazoline binding site ligand has proved difficult to establish (Atlas, 1995; Parker et al., 1999a,b, 2000; Reis et al., 1995; Singh et al., 1995). The active principle has become known as "clonidine displacing substance" because of its capacity to compete with [^{3}H]-clonidine in radioligand binding assays (Atlas, 1995) and a considerable weight of evidence has accumulated to suggest that clonidine displacing substance is a genuine physiological entity. Indeed, multiple clonidine displacing substance-like molecules may exist and their presence has been inferred in various tissue samples and body fluids (Dontenwill et al., 1993; Meeley et al., 1992; Piletz et al., 1995; Parker et al., 1999a,b, 2000; Reis et al., 1995, 1998; Singh et al., 1995; Wang et al., 1997).

The first structure to be proposed for clonidine displacing substance came from Li et al. (1994) who suggested that the activity may reside in a decarboxylated derivative of arginine, agmatine. These authors described the presence of agmatine in extracts of mammalian brain (Li et al., 1994, 1995; Raasch et al., 1995) and it was subsequently confirmed that agmatine behaves as a clonidine displacing substance in some, though not all, assay systems (Berdeu et al., 1996; Chan et al., 1995; Gao et al., 1995; Herman, 1997; Li et al., 1994, 1995; Pinthong et al., 1995; Raasch et al., 2002). The same group also established that pathways for synthesis and degradation of agmatine are present in certain brain regions (Li et al., 1994, 1995) and has provided firm evidence that agmatine may be a physiologically relevant molecule. However, it has also become clear that, although agmatine is present in pancreatic islet cells (Stickle et al., 1996), it does not fulfil the criteria expected of a ligand active at the imidazoline binding sites involved in control of insulin secretion (Chan et al., 1995; Berdeu et al., 1996; Sener et al., 1989). Thus, an additional compound(s) must account for the insulin-secreting activity found in crude clonidine displacing substance preparations.

We have been conducting studies aimed to characterise the active components of crude clonidine displacing substance preparations more fully and have developed a fractionation method which provides improved resolution of the constituents (Parker et al., 1999a,b, 2000). Using radioligand binding studies to identify potentially active compounds, it was suggested that at least part of the clonidine displacing substance activity may be due to the presence of one or more agents having a β-carboline structure (Husbands et al., 2001; Ruiz-Durantez et al., 2001; Musgrave and Badoer, 2000). Until recently, β-carbolines have not been considered as potential endogenous imidazoline binding site ligands but work by Musgrave and Badoer (2000) has revealed that microinjection of one β-carboline, harmane, into the rostral ventrolateral medulla of the rat leads to a hypotensive response. This is consistent with the possibility that harmane (or a derivative) may exert "clonidine displacing substance-activity" in the brain and suggests that harmane may be active at imidazoline binding sites located in the rostral ventrolateral medulla.

Despite this evidence from rat studies, it is still not clear whether β-carbolines are active at imidazoline sites expressed in human tissues nor whether they can interact functionally with similar sites located outside the central nervous system. In order to clarify these issues, we have examined the effects of two β-carbolines, harmane and pinoline, on insulin secretion from isolated rat and human islets of Langerhans. We have investigated whether these agents can stimulate insulin secretion and have attempted to establish whether the secretory response could reflect their interaction with a β-cell imidazoline binding site.

2. Methods

2.1. Materials

Collagenase (type XI), flurbiprofen, indomethacin, diazoxide, dimethylsulphoxide (DMSO), harmane, pinoline and norharmane were purchased from Sigma (Dorset, UK). Efaroxan hydrochloride was generously provided by RBI (Natick, MA, USA). KU14R (2 (2-ethyl 2,3-dihydro-2-benzofuranyl)-2-imidazole) was synthesised in the Dept. of Chemistry, University of Keele, UK (Clews et al., 2001) and is available from Tocris (Bristol, UK). [^{125}I]-insulin was from Biogenesis and anti-bovine insulin antiserum (for radioimmunoassay, RIA) was from ICN Biomedicals. Crystalline human insulin was a gift from Eli Lilly (Indianapolis, USA). All other reagents were of analytical reagent grade.

2.2. Isolation of islets of Langerhans

Human islets were isolated from heart-beating cadaver organ donors at the Diabetes UK Core Islet Transplant Laboratory in Leicester, UK. Islets were transported to the laboratory in CMRL-1066 containing 20% foetal bovine serum and, on arrival, were harvested (by centrifugation) and resuspended in culture medium RPMI-1640 containing 10% (v/v) foetal calf serum. They were cultured for 1–4 days prior to use in secretion experiments.

2.3. Insulin secretion experiments

Groups of three isolated islets were handpicked under a binocular microscope and incubated in a 500-μl buffer solution (Gey and Gey, 1936) supplemented with 1 mg/ml bovine serum albumin (Type V) in an atmosphere of O_2/CO_2 (95:5) at 37 °C. Test reagents were dissolved in DMSO and added to the incubation medium from concentrated stock solutions. Controls received DMSO alone. After incubation, samples of the medium were removed for

measurement of insulin content by radioimmunoassay, using crystalline human insulin as standard.

2.4. Statistics

Data are presented as means ± standard error of the mean. Since the absolute rates of insulin secretion were variable between different batches of human islets, the data were normalised and insulin secretion expressed relative to an appropriate control (defined as 100%) within each experiment. Data were pooled from two to four separate islet preparations in each case. The statistical significance of differences between means was assessed by analysis of variance (ANOVA) with Tukey's test of least significant difference.

3. Results

3.1. Effects of harmane on insulin secretion in the absence or presence of diazoxide

Initially, we examined whether β-carbolines have the ability to stimulate insulin secretion from isolated human islets of Langerhans and observed (Fig. 1) that three such molecules, harmane, norharmane and pinoline, all caused a significant, two- to threefold, increase in insulin secretion from islets incubated with 6 mM glucose. The magnitude of this increase was similar to that elicited by efaroxan, a well-characterised ligand for the β-cell imidazoline I_3 site (Fig. 1). It is known that agents active at the pancreatic β-cell imidazoline I_3 site have two important characteristics in that they can stimulate insulin secretion directly in a dose-dependent manner and also reverse the inhibition of glucose-induced insulin secretion caused by diazoxide (Morgan et al., 1999). Thus, we next studied the dose–response relationship for stimulation of insulin secretion by harmane (Fig. 2) and discovered that it is a potent stimulator of insulin secretion. A significant increase in secretion was consistently elicited by 1 μM of the compound and the EC_{50} was approximately 5 μM (Fig. 2). Pinoline also caused a dose-dependent increase in insulin secretion, although this agent was somewhat less potent than harmane (EC_{50} ~ 25 μM; not shown). In parallel studies, the effects of harmane on diazoxide-induced inhibition of glucose-induced insulin secretion were also investigated (Fig. 3). Like efaroxan and crude clonidine displacing substance (Chan and Morgan, 1990; Chan et al., 1997), harmane dose-dependently antagonised the ability of diazoxide to

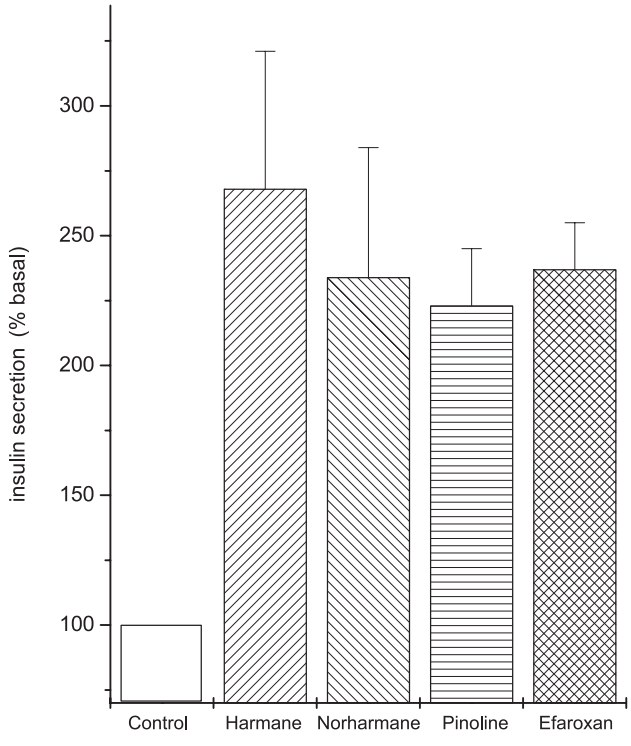

Fig. 1. Stimulation of insulin secretion from isolated human islets by β-carbolines and efaroxan. Human islets were exposed to harmane, norharmane, pinoline or efaroxan (all 100 μM) in the presence of 6 mM glucose for 1 h. Insulin secretion was measured after this time. All compounds caused a significant ($P < 0.01$) increase in insulin release. Data are presented as mean values ± S.E.M. relative to 6 mM glucose alone (100%; $n = 10$).

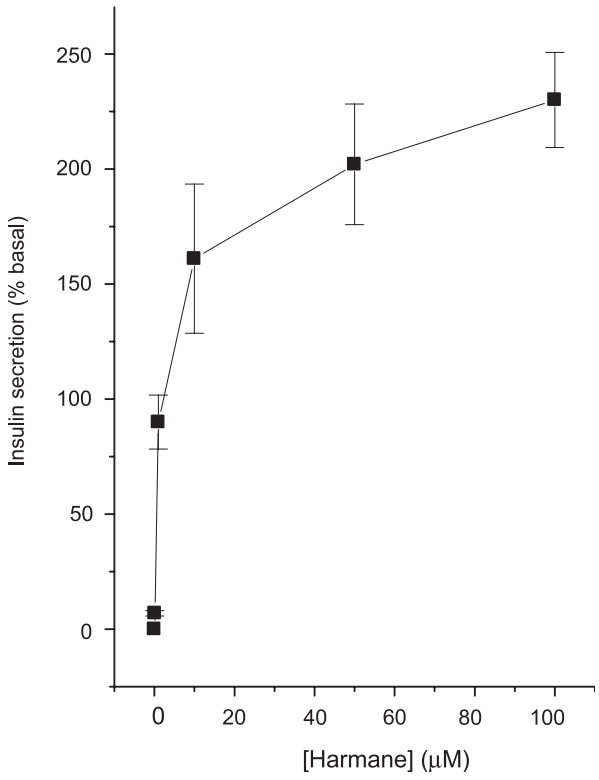

Fig. 2. Dose–response curve for stimulation of insulin secretion by harmane. Isolated human islets were incubated with 6 mM glucose and increasing concentrations of harmane for 1 h. Insulin secretion was measured at the end of the incubation period. Data are presented as mean values ± S.E.M. relative to 6 mM glucose alone (100%; $n = 8$). Harmane significantly augmented insulin secretion at all concentrations above 0.1 μM ($P < 0.01$).

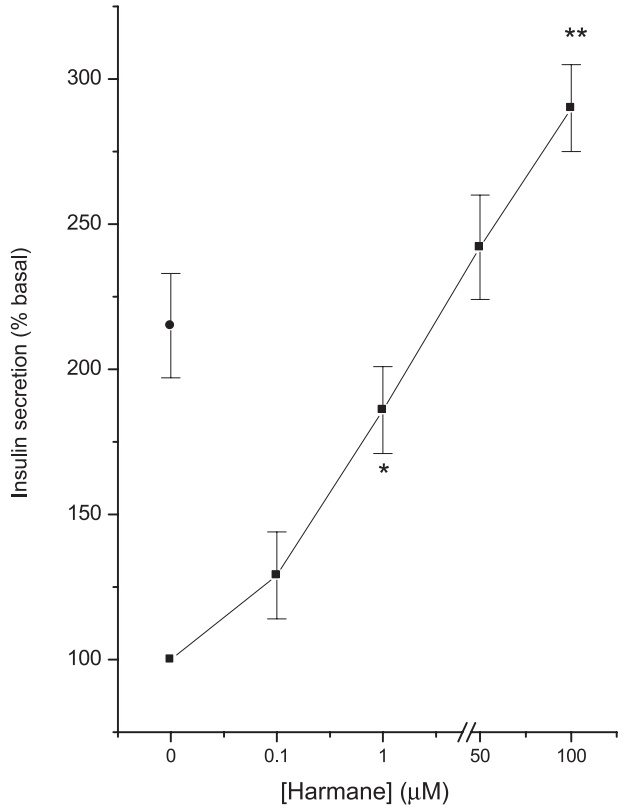

Fig. 3. Antagonism by harmane of the inhibition of glucose-induced insulin secretion caused by diazoxide. Isolated human islets were incubated with 20 mM glucose alone (circle) or 20 mM glucose plus 200 μM diazoxide (squares) and increasing concentrations of harmane for 1 h. Insulin secretion was measured at the end of the incubation period. Data are presented as mean values ± S.E.M. relative to 20 mM glucose plus diazoxide (100%; $n=12$). *$P<0.01$ relative to 20 mM glucose plus diazoxide in the absence of harmane. **$P<0.01$ relative to 20 mM glucose alone (circle).

inhibit insulin secretion. The rate of insulin secretion measured in the presence of 20 mM glucose, 200 μM diazoxide and 100 μM harmane was significantly greater than that elicited by 20 mM glucose alone. This result contrasts with the effects of efaroxan (which does not increase the rate of secretion beyond that elicited by 20 mM glucose alone; Chan and Morgan, 1990) and implies that harmane and efaroxan may influence insulin secretion by different mechanisms.

3.2. Glucose-dependency of the insulin secretory response to harmane

The effects of harmane on insulin secretion in the presence of increasing glucose concentrations were determined to establish whether the response was glucose-dependent and to investigate whether harmane could further increase insulin secretion in the presence of 20 mM glucose when islets were incubated in the absence of diazoxide. The results revealed that 100 μM harmane failed to enhance the rate of insulin secretion from human islets incubated in the absence of glucose (Fig. 4). By contrast, when the glucose concentration was raised to 6 or 20 mM, harmane then significantly enhanced the secretory response (Fig. 4).

3.3. Effects of harmane in islets desensitised to the imidazoline I_3 receptor agonist efaroxan

Exposure of rat islets to the imidazoline I_3 site agonist efaroxan for 18–24 h leads to a state of selective desensitisation such that the subsequent addition of efaroxan then fails to increase insulin secretion (Chan et al., 2001). In the present study, we observed that a similar response also occurs in human islets (Fig. 5A). Treatment of human islets with efaroxan following a period of culture under control conditions resulted in a significant increase in insulin secretion (Fig. 5A). By contrast, islets that had been exposed to the agent during an 18-h culture period, then washed and re-treated with efaroxan, failed to increase insulin secretion beyond the control level. The secretory response to 20 mM glucose was not affected by the presence of efaroxan during islet culture. Significantly, however,

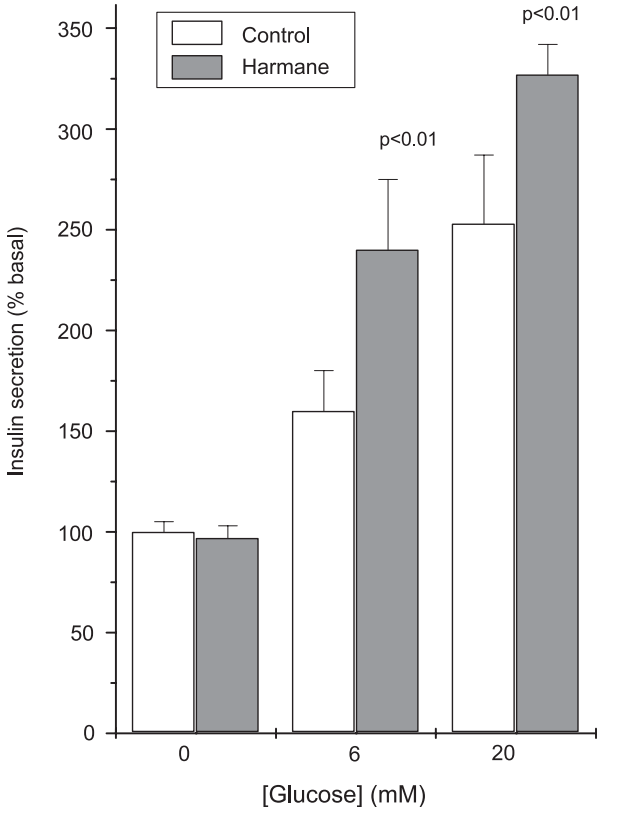

Fig. 4. Glucose dependence of the stimulatory effects of harmane on insulin secretion. Isolated human islets were incubated with increasing concentrations of glucose in the absence (white) or presence (grey) of 100 μM harmane. Insulin secretion was measured after incubation for 1 h. Data are presented as mean values ± S.E.M. relative to insulin secretion in the absence of glucose and harmane (100%; $n=10$). *$P<0.01$ relative to the equivalent glucose concentration in the absence of harmane.

islets that had been desensitised by exposure to efaroxan also became unresponsive to harmane or pinoline (Fig. 5A).

Similar results were obtained when islets were treated with 20 mM glucose in the presence of 200 μM diazoxide after the culture period (Fig. 5B). The attenuated insulin secretory response seen under these conditions was relieved by harmane, pinoline or efaroxan in control islets but none of these agents was effective in islets that had been exposed to efaroxan during the 18-h pre-culture period.

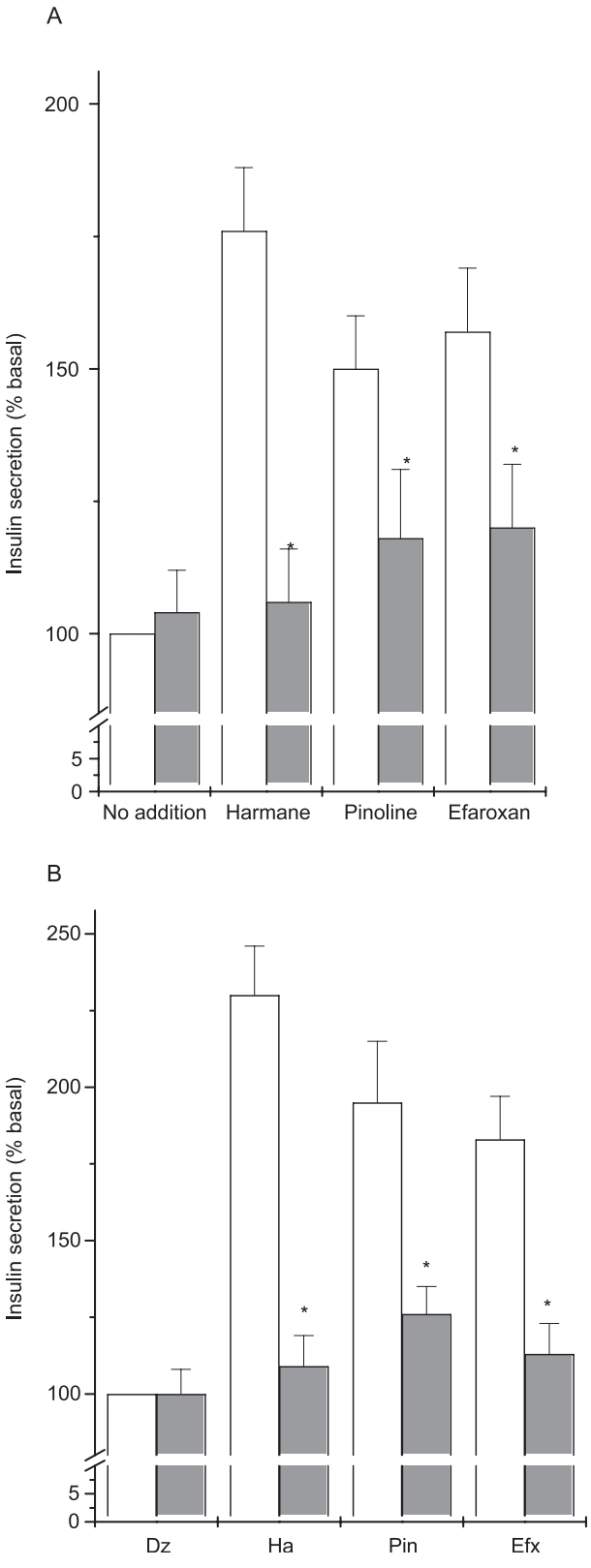

Fig. 5. Effects of β-carbolines on insulin secretion from islets that had been desensitised by exposure to efaroxan. Groups of isolated human islets were cultured for 18 h in either the absence (open bars) or presence (grey bars) of 100 μM efaroxan. (Panel A) The islets were washed then incubated with 6 mM glucose alone (no addition) or 6 mM glucose plus either harmane, pinoline or efaroxan (each 100 μM). Insulin secretion was measured after 1 h. (Panel B) The islets were washed then exposed to 20 mM glucose plus 200 μM diazoxide in the presence of either harmane (Ha), pinoline (Pin) or efaroxan (Efx; each at 100 μM). Insulin secretion was measured after 1 h. Data are presented as mean values ± S.E.M. relative to the secretion rate measured in the presence of 6 mM glucose alone (Panel A) or 20 mM glucose plus 200 μM diazoxide (Panel B). *$P < 0.01$ relative to islets cultured under control conditions.

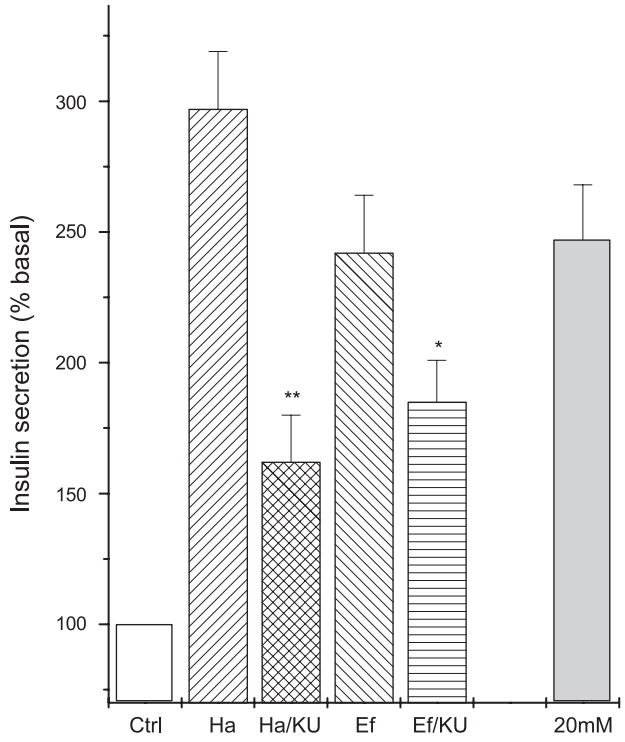

Fig. 6. Effects of KU14R on insulin secretory responses to efaroxan or harmane. Isolated human islets were incubated with 6 mM glucose alone (Ctrl), 6 mM glucose plus harmane (Ha; 100 μM) or 6 mM glucose plus efaroxan (Ef; 100 μM) in the absence or presence of 100 μM KU14R (KU). Insulin secretion was measured after 1 h. The secretory response to 20 mM glucose alone is also shown (grey). Data are presented as mean values ± S.E.M. relative to 6 mM glucose alone (100%; $n = 10$). *$P < 0.01$; **$P < 0.001$ relative to the equivalent condition in the absence of KU14R.

3.4. Effect of the imidazoline I_3 receptor antagonist, KU14R, on insulin secretion mediated by harmane

To further investigate the relationship between the ability of efaroxan and harmane to stimulate insulin secretion, isolated human islets were treated with the imidazoline I_3

receptor antagonist, KU14R (Clews et al., 2001). This agent is a structural analogue of efaroxan and effectively reduces the secretory activity of efaroxan in rat and human islets (Chan et al., 1997, 2001). This effect is illustrated in Fig. 6 where inclusion of KU14R together with efaroxan caused a significant reduction in the extent of insulin secretion from human islets exposed to the imidazoline agonist. KU14R also attenuated the insulin secretory response to harmane in human islets (Fig. 6).

4. Discussion

The recent demonstration that a β-carboline, harmane, can elicit hypotension when injected into rat brain (Musgrave and Badoer, 2000) has fuelled speculation that this, or a closely related molecule may, despite being structurally unrelated to imidazolines, represent one of the long-sought endogenous ligands for the class of ''imidazoline'' binding sites. In the present work, we have examined this proposition in the pancreatic β-cell, which expresses a pharmacologically atypical imidazoline site, termed I_3, involved in the regulation of insulin secretion (Chan et al., 1994; Efendic et al., 2002; Eglen et al., 1998; Rustenbeck, 2002). Although the β-cell site differs from the imidazoline I_1 and I_2 sites expressed in other tissues, its functional pharmacology has been defined in detail (reviewed by Eglen et al., 1998; Morgan et al., 1999; Morgan and Chan, 2001; Rustenbeck, 2002). Moreover, a crude preparation of the putative endogenous ligand, clonidine displacing substance, has been found to stimulate insulin secretion in a manner that would be consistent with the possibility that it contains an active principle having agonist activity at the imidazoline I_3 site (Chan et al., 1997). Thus, the β-cell represents an appropriate experimental system in which to study whether the effects of harmane could be mediated by interaction with intracellular effector pathways regulated by imidazoline binding sites.

Initially, we observed that several β-carbolines were able to stimulate insulin secretion from isolated human pancreatic islets. To the best of our knowledge, this represents the first demonstration that such molecules can act as insulin secretagogues in human islets. The responses were dose-dependent and, in the case of harmane, could be elicited when islets were exposed to as little as 1 μM of the drug (EC_{50} ~ 5 μM). This potency is higher than that of many ''imidazoline'' derivatives that, typically, require concentrations in excess of 10 μM to stimulate insulin secretion.

In order to assess whether the secretagogue activity of harmane in human islet cells could reflect its interaction with the imidazoline I_3 binding site, its effects were examined under conditions similar to those used previously to characterise the responses to efaroxan and crude clonidine displacing substance. Efaroxan is arguably the best-characterised imidazoline I_3 agonist, and, importantly (and in marked contrast to certain other widely used reagents, such as RX871024 (1-phenyl-2-(imidazolin-2-yl) benzimidazole; Efendic et al., 2002; Mourtada et al., 1998, 1999; Zaitsev et al., 1996, 1999)), it exerts few additional effects that might be attributed to activation of non-imidazoline-mediated pathways.

In common with efaroxan and crude clonidine displacing substance, harmane was observed to stimulate insulin secretion in the presence of 6 mM glucose and to antagonise the inhibition of glucose-induced insulin secretion mediated by exposure of human islets to the ATP-sensitive potassium channel agonist, diazoxide. This latter feature is a characteristic of agents acting at the imidazoline I_3 site and the results could imply that harmane stimulates insulin secretion by a mechanism similar to that used by efaroxan (and clonidine displacing substance). In support of this, the imidazoline I_3 receptor antagonist, KU14R, significantly attenuated the secretory response to both harmane and efaroxan. In addition, islets that had been desensitised by culture with efaroxan for 18 h failed to respond to efaroxan, harmane or pinoline upon subsequent re-exposure. Thus, there was a clear concordance between the pharmacology of the secretory response to the β-carboline and that elicited by efaroxan (and crude clonidine displacing substance) in human islets.

However, one important difference was noted between the actions of harmane and efaroxan in the β-cell, in that harmane consistently increased insulin secretion above the level induced by 20 mM glucose alone. This effect occurred both in experiments where diazoxide was present (Fig. 3) and when it was absent (Fig. 4). Since we have not been able to define any conditions under which efaroxan increases insulin secretion beyond the level elicited by 20 mM glucose alone (in either rat or human islets), this implies that certain aspects of the mechanisms of action of harmane and efaroxan in β-cells may be different. In support of this, preliminary studies have indicated that harmane promotes the development of trains of uniform Ca oscillations in β-cells by a mechanism that may involve ryanodine receptors (PE Squires, CE Hills and NG Morgan; in preparation). Thus, it is possible that harmane may interact with both imidazoline I_3 sites and ryanodine receptors to regulate insulin secretion from β-cells.

In summary, therefore, the present results reveal that certain β-carbolines can potently stimulate insulin secretion in a glucose-dependent manner and that this response displays certain features that are consistent with the involvement of imidazoline I_3 binding sites. However, other intracellular actions of harmane may also be involved. Thus, β-carbolines may represent a fertile group of compounds for consideration as novel, glucose-dependent, insulin secretagogues.

Acknowledgements

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References

Atlas, D., 1995. Molecular and physiological properties of clonidine-displacing substance. Ann. N.Y. Acad. Sci. 763, 314–324.

Berdeu, D., Puech, R., Loubatieres-Mariani, M.M., Bertrand, G., 1996. Agmatine is not a good candidate as endogenous ligand for imidazoline sites of pancreatic B cells and vascular bed. Eur. J. Pharmacol. 308, 301–304.

Bousquet, P., 2000. Identification and characterization of I1 imidazoline receptors: their role in blood pressure regulation. Am. J. Hypertens. 13, 84S–88S.

Bousquet, P., 2001. I_1 receptors, cardiovascular function, and metabolism. Am. J. Hypertens. 14, 317S–321S.

Bousquet, P., Dontenwill, M., Greney, H., Feldman, J., 2000. Imidazoline receptors in cardiovascular and metabolic diseases. J. Cardiovasc. Pharmacol. 35 (Suppl. 4), S21–S25.

Chan, S.L.F., 1998. Clonidine-displacing substance and its putative role in control of insulin secretion: a minireview. Gen. Pharmacol. 31, 525–529.

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.

Chan, S.L.F., Brown, C.A., Scarpello, K.E., Morgan, N.G., 1994. The imidazoline site involved in control of insulin secretion: characteristics that distinguish it from I1 and I2 sites. Br. J. Pharmacol. 112, 1065–1070.

Chan, S.L.F., Cable, H.C., Morgan, N.G., 1995. Agmatine-induced insulin secretion: evidence that islet imidazoline binding sites are not involved. Pharmacol. Commun. 7, 29–41.

Chan, S.L.F., Atlas, D., James, R.F.L., Morgan, N.G., 1997. The effect of the putative endogenous imidazoline receptor ligand,clonidine-displacing substance, on insulin secretion from rat and human islets of Langerhans. Br. J. Pharmacol. 120, 926–932.

Chan, S.L.F., Mourtada, M., Morgan, N.G., 2001. Characterization of a K_{ATP} channel-independent pathway involved in potentiation of insulin secretion by efaroxan. Diabetes 50, 340–347.

Clews, J., Morgan, N.G., Ramsden, C.A., 2001. Preparation of the I_3 imidazoline receptor antagonist KU14R and related 2,3-dihydrobenzo [*b*] furan derivatives. Synthesis 10, 1546–1550.

Dontenwill, M., Molines, A., Verdun, A., Bricca, G., Laurent, S., Bousquet, P., 1993. A circulating substance cross-reacting with antiimidazoline antibodies. Detection in serum in relation to essential hypertension. J. Clin. Invest. 92, 1068–1072.

Efanov, A.M., Zaitsev, S.V., Mest, H.-J., Raap, A., Appelskog, I.B., Larsson, O., Berggren, P.-O., Efendic, S., 2001. The novel imidazoline compound BL11282 potentiates glucose-induced insulin secretion in pancreatic beta-cells in the absence of modulation of K(ATP) channel activity. Diabetes 50, 797–802.

Efanov, A.M., Appelskog, I.B., Abdel-Halim, S.M., Khan, A., Branstrom, R., Larsson, O., Ostenson, C.-G., Mest, H.-J., Berggren, P.-O., Efendic, S., Zaitsev, S.V., 2002. Insulinotropic activity of the imidazoline derivative RX871024 in the diabetic GK rat. Am. J. Physiol. 282, E117–E124.

Efendic, S., Efanov, A.M., Berggren, P.-O., Zaitsev, S.V., 2002. Two generations of insulinotropic imidazoline compounds. Diabetes 51 (Suppl. 3), S448–S454.

Eglen, R.M., Hudson, A.L., Kendall, D.A., Nutt, D.J., Morgan, N.G., Wilson, V.G., Dillon, M.P., 1998. 'Seeing through a glass darkly': casting light on imidazoline 'I' sites. Trends Pharmacol. Sci. 19, 381–390.

Ernsberger, P., Friedman, J.E., Koletsky, R.J., 1997. The I1-imidazoline receptor: from binding site to therapeutic target in cardiovascular disease. J. Hypertens. 15, S9–S23.

Gao, Y., Gumusel, B., Koves, G., Prasad, A., Hao, Q., Hyman, A., Lippton, H., 1995. Agmatine: a novel endogenous vasodilator substance. Life Sci. 57, 83–86.

Gey, G.O., Gey, M.K., 1936. Maintenance of human normal cells in continuous culture; preliminary report; cultivation of mesoblastic tumors and normal cells and notes on methods of cultivation. Am. J. Cancer 27, 45–76.

Head, G.A., Gundlach, A.L., Musgrave, I.F., 1998. Recent advances in imidazoline receptor research: ligands-localization and isolation-signaling-functional and clinical studies. J. Auton. Nerv. Syst. 72, 74–79.

Herman, Z.S., 1997. Agmatine—a novel endogenous ligand of imidazoline receptors. Pol. J. Pharmacol. 49, 85–88.

Husbands, S.M., Glennon, R.A., Gorgerat, S., Gough, R., Tyacke, R., Crosby, J., Nutt, D.J., Lewis, J.W., Hudson, A.L., 2001. Beta-carboline binding to imidazoline receptors. Drug Alcohol Depend. 64, 203–208.

Khan, Z.P., Freguson, C.N., Jones, R.M., 1999. Alpha-2 and imidazoline receptor agonists. Their pharmacology and therapeutic role. Anaesthesia 54, 146–165.

Li, G., Regunathan, S., Barrow, C.J., Eshraghi, J., Cooper, R., Reis, D.J., 1994. Agmatine: an endogenous clonidine-displacing substance in the brain. Science 263, 966–969.

Li, G., Regunathan, S., Reis, D.J., 1995. Agmatine is synthesized by a mitochondrial arginine decarboxylase in rat brain. Ann. N.Y. Acad. Sci. 763, 325–329.

Meeley, M.P., Hensley, M.L., Ernsberger, P., Felsen, D., Reis, D.J., 1992. Evidence for a bioactive clonidine-displacing substance in peripheral tissues and serum. Biochem. Pharmacol. 44, 733–740.

Mest, H.-J., Raap, A., Schloos, J., Treinies, I., Paal, M., Giese, U., Koivisto, V., 2001. Glucose-induced insulin secretion is potentiated by a new imidazoline compound. Naunyn-Schmiedeberg's Arch. Pharmacol. 364, 47–52.

Molderings, G.J., Gothert, M., 1999. Imidazoline binding sites and receptors in cardiovascular tissue. Gen. Pharmacol. 32, 17–22.

Morgan, N.G., Chan, S.L.F., 2001. Imidazoline binding sites in the endocrine pancreas: can they fulfil their potential as targets for the development of new insulin secretagogues? Curr. Pharm. Des. 7, 1413–1431.

Morgan, N.G., Chan, S.L.F., Mourtada, M., Monks, L.K., Ramsden, C.A., 1999. Imidazolines and pancreatic hormone secretion. Ann. N.Y. Acad. Sci. 881, 217–228.

Mourtada, M., Smith, S.A., Morgan, N.G., 1998. Effector systems involved in the insulin secretory responses to efaroxan and RX871024 in rat islets of Langerhans. Eur. J. Pharmacol. 350, 251–258.

Mourtada, M., Chan, S.L.F., Smith, S.A., Morgan, N.G., 1999. Multiple effector pathways regulate the insulin secretory response to the imidazoline RX871024 in isolated rat pancreatic islets. Br. J. Pharmacol. 127, 1279–1287.

Musgrave, I.F., Badoer, E., 2000. Harmane produces hypotension following microinjection into the RVLM: possible role of I_1-imidazoline receptors. Br. J. Pharmacol. 129, 1057–1059.

Parini, A., Moudanos, C.G., Pizzinat, N., Lanier, S.M., 1996. The elusive family of imidazoline binding sites. Trends Pharmacol. Sci. 17, 13–16.

Parker, C.A., Hudson, A.L., Nutt, D.J., Dillon, M.P., Eglen, R.M., Crosby, J., 1999a. Comparison of crude methanolic CDS extracts from various tissues. Ann. N.Y. Acad. Sci. 881, 92–96.

Parker, C.A., Hudson, A.L., Nutt, D.J., Dillon, M.P., Eglen, R.M., Chan, S.L.F., Morgan, N.G., Crosby, J., 1999b. Extraction of active clonidine-displacing substance from bovine lung and comparison with clonidine-displacing substance extracted from other tissues. Eur. J. Pharmacol. 378, 213–221.

Parker, C.A., Hudson, A.L., Nutt, D.J., Dillon, M.P., Eglen, R.M., Crosby, J., 2000. Isolation of RP-HPLC pure clonidine-displacing substance from NG108-15 cells. Eur. J. Pharmacol. 387, 27–30.

Piletz, J.E., Chikkala, D.N., Ernsberger, P., 1995. Comparison of the properties of agmatine and endogenous clonidine-displacing substance at imidazoline and alpha-2 adrenergic receptors. J. Pharmacol. Exp. Ther. 272, 581–587.

Pinthong, D., Wright, I.K., Hanmer, C., Millns, P., Mason, R., Kendall, D.A., Wilson, V.G., 1995. Agmatine recognizes alpha 2-adrenoceptor binding sites but neither activates nor inhibits alpha 2-adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 351, 10–16.

Raasch, W., Regunathan, S., Li, G., Reis, D.J., 1995. Agmatine, the bacte-

rial amine, is widely distributed in mammalian tissues. Life Sci. 56, 2319–2330.

Raasch, W., Schafer, U., Qadri, F., Dominiak, P., 2002. Agmatine, an endogenous ligand at imidazoline binding sites, does not antagonize the clonidine-mediated blood pressure reaction. Br. J. Pharmacol. 135, 663–672.

Regunathan, S., Reis, D.J., 1996. Imidazoline receptors and their endogenous ligands. Ann. Rev. Pharmacol. Toxicol. 36, 511–544.

Reis, D.J., Li, G., Regunanthan, S., 1995. Endogenous ligands of imidazoline receptors: classic and immunoreactive clonidine-displacing substance and agmatine. Ann. N.Y. Acad. Sci. 763, 295–313.

Reis, D.J., Yang, X.C., Milner, T.A., 1998. Agmatine containing axon terminals in rat hippocampus form synapses on pyramidal cells. Neurosci. Lett. 250, 185–188.

Ruiz-Durantez, E., Ruiz-Ortega, J.A., Pineda, J., Ugedo, L., 2001. Stimulatory effect of harmane and other beta-carbolines on locus coeruleus neurons in anaesthetized rats. Neurosci. Lett. 308, 197–200.

Rustenbeck, I., 2002. Desensitization of insulin secretion. Biochem. Pharmacol. 63, 1921–1935.

Rustenbeck, I., Kopp, M., Ratzka, P., Luepolt, L., Hasselblatt, A., 1999. Imidazolines and the pancreatic B-cell. Actions and binding sites. Ann. N.Y. Acad. Sci. 881, 229–240.

Sener, A., Lebrun, P., Blachier, F., Malaisse, W.J., 1989. Stimulus-secretion coupling of arginine-induced insulin release. Insulinotropic action of agmatine. Biochem. Pharmacol. 38, 327–330.

Singh, G., Hussain, J.F., Mackinnon, A., Brown, C.M., Kendall, D.A., Wilson, V.G., 1995. Evidence for the presence of a non-catecholamine, clonidine-displacing substance in crude, methanolic extracts of bovine brain and lung. Naunyn-Schmiedeberg's Arch. Pharmacol. 351, 17–26.

Stickle, D., Bohrer, A., Berger, R., Morrissey, J., Klahr, S., Turk, J., 1996. Quantitation of the putative neurotransmitter agmatine as the hexafluoroacetylacetonate derivative by stable isotope dilution gas chromatography and negative-ion chemical ionization mass spectrometry. Anal. Biochem. 238, 129–136.

Wang, H., Regunathan, S., McGowen, D., Bramwell, S., Reis, D.J., 1997. An antiserum to idazoxan recognizes an immunoreactive substance in human serum and cerebral spinal fluid which is not agmatine. Neurochem. Int. 30, 85–94.

Zaitsev, S.V., Efanov, A.M., Efanova, I.B., Larsson, O., Ostenson, C.-G., Gold, G., Berggren, P.-O., Efendic, S., 1996. Imidazoline compounds stimulate insulin release by inhibition of K_{ATP} channels and interaction with the exocytotic machinery. Diabetes 45, 1610–1618.

Zaitsev, S.V., Efanov, A.M., Raap, A., Efanova, I.B., Schloos, J., Steckel-Hamann, B., Larsson, O., Ostenson, C.-G., Berggren, P.-O., Mest, H.-J., Efendic, S., 1999. Different modes of action of the imidazoline compound RX871024 in pancreatic beta-cells. Blocking of K+ channels, mobilization of Ca2+ from endoplasmic reticulum, and interaction with exocytotic machinery. Ann. N.Y. Acad. Sci. 881, 241–252.