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Short communication

Agmatine is not a good candidate as endogenous ligand for imidazoline sites of pancreatic B cells and vascular bed

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Abstract

In order to determine whether agmatine could be a putative endogenous ligand for imidazoline receptors mediating insulin secretion and vasoconstriction, we compared its effects with those of the imidazoline, efaroxan. Agmatine exhibited a much lower potency and efficacy than efaroxan on insulin secretion from rat pancreas perfused with 8.3 mM glucose. On the other hand, in contrast to efaroxan (100 μM), agmatine (3 mM) did not increase arginine-induced insulin release. In addition, agmatine failed to reproduce the vasoconstrictor effect of efaroxan on pancreatic vessels. These results show that agmatine does not behave like efaroxan, an agonist for the imidazoline receptors mediating insulin secretion or vasoconstriction in the pancreas.

Keywords: Agmatine; Efaroxan; Imidazoline receptor; Insulin secretion; Vessel; Pancreas, rat

1. Introduction

Imidazoline receptors have been divided into two main subtypes, I_1 and I_2, on the basis of their ligand selectivity: imidazoline I_1 receptors can be labeled by [^{3}H]clonidine, whereas imidazoline I_2 receptors are preferentially labeled by [^{3}H]idazoxan (Michel and Ernsberger, 1992). The identity of the endogenous ligand(s) for imidazoline receptors still remains under investigation. Recently, Li et al. (1994) reported that agmatine, the decarboxylation product of arginine, may be an endogenous ligand for imidazoline receptors.

It has long been reported that agmatine stimulates insulin release in rat pancreatic islets (Alberti et al., 1973; Sener et al., 1989). On the other hand, several studies have demonstrated that imidazoline compounds stimulate insulin secretion (Schulz and Hasselblatt, 1989; Chan et al., 1991; Jonas et al., 1992; Berdeu et al., 1994). In addition, we have shown that imidazolines had the capacity not only to stimulate insulin secretion but also to exert a vasoconstrictor effect in the isolated perfused rat pancreas (Berdeu et al., 1994). These effects clearly involved different imidazoline receptors: an I_1 type on B cells and an I_2 type on vessels, respectively (Berdeu et al., 1995).

The aim of the present work was to determine whether agmatine could be an endogenous ligand for the different imidazoline sites mediating not only insulin secretion but also vasoconstriction in the isolated perfused rat pancreas. For this purpose, we compared the effects of agmatine and efaroxan, an agonist for pancreatic imidazoline receptors mediating insulin release and vasoconstriction (Berdeu et al., 1994).

2. Materials and methods

The pancreas was isolated from male Wistar rats (Loubatières et al., 1969). The pancreases were perfused through their own arterial system with a Krebs-Ringer bicarbonate buffer containing 2 g/l pure bovine serum albumin (fraction V) and gassed with (95%) O_2 and CO_2 (5%); the pH was about 7.4. The preparation was maintained at 37.5°C. The pancreases were perfused at a constant pressure (ranging between 40 and 45 cm H_2O) selected to give a flow rate of 2.5 ml/min during the 30-min stabilization period. Any change in pancreatic vascular bed resistance was detected by measuring pancreatic effluent output. The flow rate was measured for 1 min for

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each sample; samples were immediately frozen for insulin radioimmunoassay (Berdeu et al., 1994).

For the kinetics of insulin output and flow rate, the results are expressed as changes in relation to the value at time 45 (or 40) min taken as 100%. The data are expressed as means ± S.E.M. In order to establish the concentration-response curves for test substances, we used: (1) for insulin secretion, the mean insulin output over the 20 min of substance infusion calculated as follows: area under the curve/20; (2) for flow rate, the mean of the values at 20 min corresponding to the maximum drop in flow rate recorded with the imidazoline, efaroxan.

Agmatine was obtained from Sigma and efaroxan hydrochloride from Research Biochemicals.

3. Results

The initial experiments served to compare the effects of agmatine and efaroxan on the pancreas perfused with a slightly stimulating glucose concentration (8.3 mM) for insulin secretion. Agmatine was tested in the concentration range of 10 μM–10 mM. At concentrations 10 and 30 μM, agmatine did not significantly affect glucose-induced insulin release. In the range of 0.1–3 mM, it evoked a biphasic insulin response in a concentration-dependent manner (Fig. 1A, upper panel). Compared with the imidazoline agonist, efaroxan, which has been reported to stimulate insulin secretion (Berdeu et al., 1994), agmatine elicited a lower maximal response (about 2.5-fold lower) and was effective at much higher concentrations (Fig. 1B, upper panel). Concerning the pancreatic vascular bed, in contrast to efaroxan, agmatine did not significantly affect the flow rate over the concentration range used (0.01–10 mM) (Fig. 1A and B, lower panels).

Agmatine is the decarboxylation product of arginine. So, in order to elucidate the mechanism of the insulin response to agmatine, we tested for a possible interaction between agmatine or efaroxan and arginine to induce insulin secretion. In these experiments, insulin secretion was stimulated by a high arginine concentration (10 mM) in the presence of a non-stimulating glucose concentration (5 mM). Under these conditions, agmatine at the high concentration of 3 mM (a concentration inducing the maximal secretory effect in the presence of 8.3 mM glucose) failed to increase arginine-induced insulin release (Fig. 2, upper panel). In contrast, efaroxan (100 μM) still provoked an immediate and biphasic insulin response.

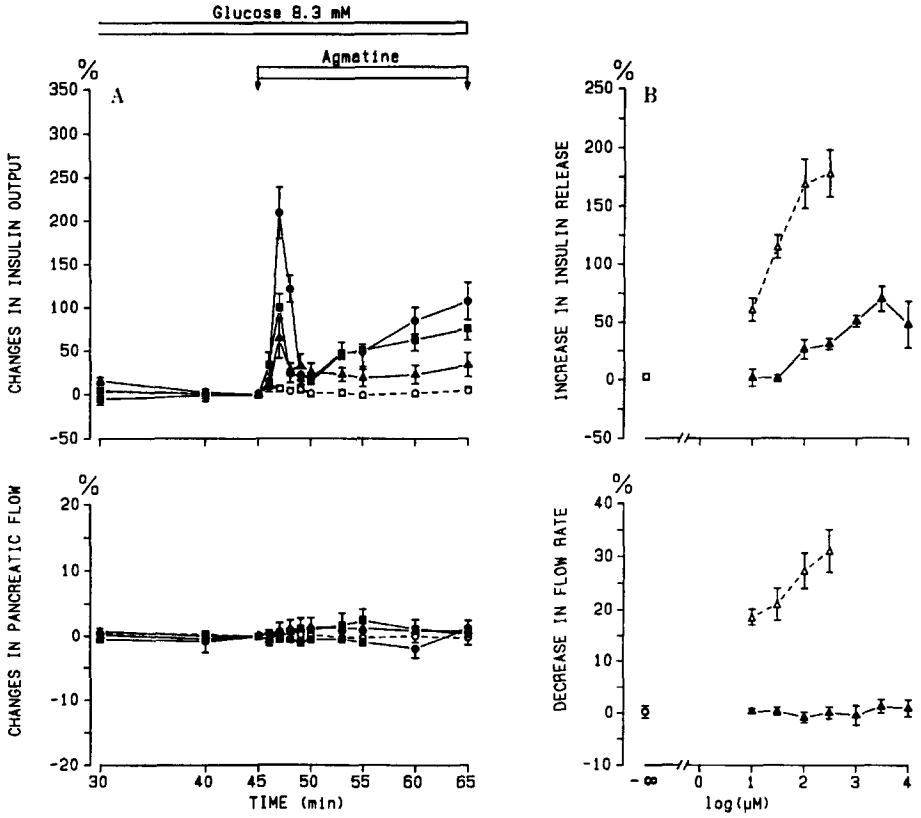

Fig. 1. Effects of agmatine on insulin release and vascular flow rate from the isolated rat pancreas perfused with 8.3 mM glucose. (A) Agmatine: (▲) 100 μM, (■) 1 mM and (●) 3 mM and controls (○). The insulin output rate (ng/min) at 45 min for each set of experiments was: 18.4 ± 4.5; 16.3 ± 2.2; 20.6 ± 5.3; 17.9 ± 3.4, respectively. (B) Comparison of concentration-response curves of agmatine (▲) and efaroxan (△). Each point represents the mean of 4–7 experiments and vertical lines indicate S.E.M.

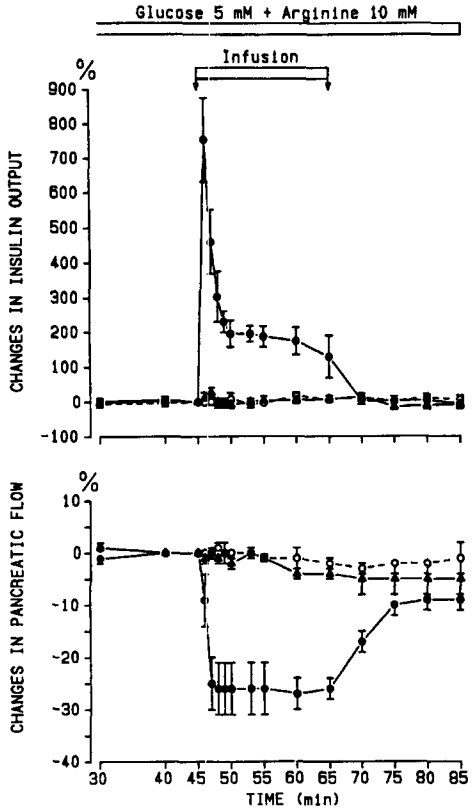

Fig. 2. Compared effects of agmatine and efaroxan on insulin release and vascular flow rate from the isolated rat pancreas perfused with 5 mM glucose and 10 mM arginine. (▲) agmatine 3 mM, (●) efaroxan 100 μM and controls (○). The insulin output rate (ng/min) at 45 min for each set of experiments was: 63.9 ± 10.4; 62.9 ± 5.2; 59.9 ± 6.5, respectively. Each point represents the mean of 4 experiments and vertical lines indicate S.E.M.

Efaroxan elicited an immediate and sustained decrease of the pancreatic flow rate, whereas agmatine remained ineffective (Fig. 2, lower panel).

4. Discussion

The present study showed that, in contrast to the imidazoline efaroxan, the proposed endogenous imidazoline receptor ligand, agmatine, stimulates, only weakly or not at all, insulin secretion and fails to induce vasoconstrictor activity in the isolated perfused rat pancreas.

In pancreas perfused with a moderate stimulatory glucose concentration (8.3 mM), agmatine exhibited much lower efficacy and potency than efaroxan; it induced a 2.5-fold lower maximal response and started to be effective at 100 μM, whereas efaroxan reached its maximal effect at this concentration. It must be noted that agmatine is the decarboxylation product of arginine and is a polyamine. In this context, agmatine was shown to be taken up by pancreatic islet cells and to elicit an insulin secretory response comparable to that evoked by arginine (Sener et al., 1989). Since endogenously formed polyamines may play a role in the insulin secretory response to arginine, the authors proposed that the insulinotropic action of agmatine results from an intracellular metabolic action such as polyamine. In our study, agmatine, at a high concentration (3 mM), inducing the maximal secretory effect in the presence of 8.3 mM glucose, failed to enhance the insulin secretion induced by a high, stimulatory, arginine concentration (10 mM). In contrast, the imidazoline, efaroxan, remained effective on insulin secretion in the presence of arginine. The failure of agmatine to increase, as efaroxan did, arginine-induced insulin secretion thus suggests a different mechanism of action for the two compounds. The weak insulin secretory effect of agmatine recorded in the presence of 8.3 mM glucose can then not be ascribed to an interaction with imidazoline receptors. On the other hand, our study showed that agmatine totally failed to mimic the vasoconstrictor action of efaroxan mediated through imidazoline sites in the pancreatic vascular bed (Berdeu et al., 1994).

Discrepancies have been reported concerning the effects of agmatine as an endogenous ligand on imidazoline receptors. For example, agmatine has been reported to activate, at micromolar concentrations, imidazoline receptors mediating either release of catecholamines from bovine adrenal chromaffin cells (Li et al., 1994) or inhibition of noradrenaline release in sympathetic nerves in the rabbit aorta (Molderings and Göthert, 1995). In contrast, this amine has been shown to act as an inverse agonist rather than as a true agonist at imidazoline sites mediating gastrointestinal secretion responses in rats, since it exerts effects opposite to those of specific imidazoline agonists (Glavin et al., 1995). On the other hand we, as well as others, have found agmatine devoid of activity at imidazoline sites mediating contraction of the rat thoracic aorta and of the rat gastric fundus (Pinthong et al., 1995).

In conclusion, agmatine is not able to mimic the insulin secretory and vasoconstrictor actions of the imidazoline receptor agonist efaroxan. In this context, our results are not consistent with a role of agmatine as an endogenous ligand at imidazoline sites of either pancreatic B cells or vessels.

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