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Evidence for two different imidazoline sites on pancreatic B cells and vascular bed in rat

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Abstract

The relative potencies of imidazoline compounds to induce insulin secretion and vascular resistance were compared in the isolated perfused rat pancreas. On insulin secretion, only the two imidazolines, antazoline and efaroxan, induced a concentration-dependent response, antazoline being 10 times more potent than efaroxan. In contrast, idazoxan, a blocker of imidazoline I_1 sites, at concentrations up to 30 μM, antagonized the insulin response to 10 μM efaroxan ($IC_{50} \cong 14 \pm 2$ μM) without affecting that to 3 μM tolbutamide. On pancreatic vessels, not only antazoline and efaroxan but also idazoxan induced a concentration-dependent vasoconstriction; the rank order of agonist potency was antazoline > efaroxan > idazoxan. In addition, cimetidine, an imidazole known to bind imidazoline I_1 sites, ineffective per se, partially reversed the insulin stimulatory effect of efaroxan without affecting its vasoconstrictor effect. This study demonstrates that the insulin secretory and vasoconstrictor actions of imidazolines involve different imidazoline sites in rat pancreas. The results provide evidence for an I_1 type mediating insulin secretion on B cells and an I_2 type mediating vasoconstriction in vessels.

Keywords: Imidazoline; Imidazoline site; Insulin secretion; Vessel; Pancreas; Efaroxan; Idazoxan; (Rat)

1. Introduction

It is now widely recognized that some imidazolines and derivatives, in addition to having high affinity for α_2-adrenoceptors, also bind to non-adrenergic imidazoline sites in various tissues and species (Michel and Insel, 1989; Hamilton, 1992). The imidazoline sites form a heterogeneous population; they are divided into two major subtypes, I_1 and I_2, principally on the basis of the ligand used for their labelling: imidazolines I_1 sites are labelled by [^{3}H]clonidine or [^{3}H]*p*-aminoclonidine, whereas imidazoline I_2 sites are preferentially labelled by [^{3}H]idazoxan (Ernsberger, 1992). Although idazoxan exhibits a lower affinity at the imidazoline I_1 site, it has been reported to antagonize the effect of clonidine-like drugs (Ernsberger et al., 1990; Tibiriça et al., 1991; Sannajust and Head, 1994). In contrast, at the imidazoline I_2 site which shows a low or no affinity for clonidine and its derivatives, idazoxan seems to behave as an agonist (Bidet et al., 1990; Göthert and Molderings, 1991; Olmos et al., 1994). Furthermore, the imidazoline I_1 binding site can be distinguished from the imidazoline I_2 site by its ability to recognize imidazole compounds (Wikberg and Uhlén, 1990; Reis et al., 1992).

It has long been reported that phentolamine, an α-adrenergic blocker with an imidazoline structure, stimulates insulin secretion in vivo (Cerasi et al., 1969; Efendic et al., 1975; Ahrén and Lundquist, 1985). Since insulin release is inhibited by α_2-adrenoceptor activation (Nakaki et al., 1980), it was concluded that the insulin secretory effect of phentolamine resulted from the blockade of an inhibitory adrenergic tone on pancreatic B cells. However, more recent studies show that phentolamine (Schulz and Hasselblatt, 1988) and other α_2-adrenoceptor antagonists with an imidazoline structure, such as efaroxan (Chan and Morgan, 1990) and

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midaglizole (Kameda et al., 1982), are able to directly increase insulin release in vitro. It appears that the effect of these compounds cannot be related to their α_2-adrenoceptor antagonist action but rather to their imidazoline structure. Indeed, there is no correlation between the concentrations required to block α_2-adrenoceptors and those to stimulate insulin release. In addition other imidazolines devoid of α_2-adrenergic activity exhibit an insulin secretory effect also (Schulz and Hasselblatt, 1989; Jonas et al., 1992).

We have recently shown that, in the isolated perfused rat pancreas, some imidazolines and derivatives are able not only to stimulate insulin release but also to induce vasoconstriction; however, the apparent difference in the efficacy of the substances tested suggested that two different imidazoline sites were present on the pancreatic B cell and vascular bed (Berdeu et al., 1994). The nature of the sites mediating the effects of imidazolines in both pancreatic B cell and blood vessels remains unclear. Indeed, although the existence of [^{3}H]idazoxan sites has been reported in the rat insulinoma cell line, RINm5F (Remaury and Paris, 1992), no correlation has been found between the capacity of some imidazoline compounds to stimulate insulin release and their ability to bind this site (Brown et al., 1993a). In addition, no data on binding of either [^{3}H]clonidine or [^{3}H]idazoxan in blood vessels have been reported.

The present work was designed to further characterize the imidazoline sites mediating insulin secretion and vasoconstriction in the isolated perfused pancreas of the rat.

2. Materials and methods

2.1. Isolated perfused pancreas

Our experiments were performed with male Wistar rats fed ad libitum and weighing 320–350 g. The surgical procedure for the isolated perfused rat pancreas has already been described (Loubatières et al., 1969; Bertrand et al., 1986). After total isolation from all neighbouring tissues, the pancreas was perfused through its own arterial system and at a constant pressure with a Krebs-Ringer bicarbonate buffer containing 2 g/l pure bovine serum albumin (fraction V) and glucose 8.3 mM. A mixture of O_2 (95%) and CO_2 (5%) was continuously bubbled through this medium; the pH was about 7.4. The preparation was maintained

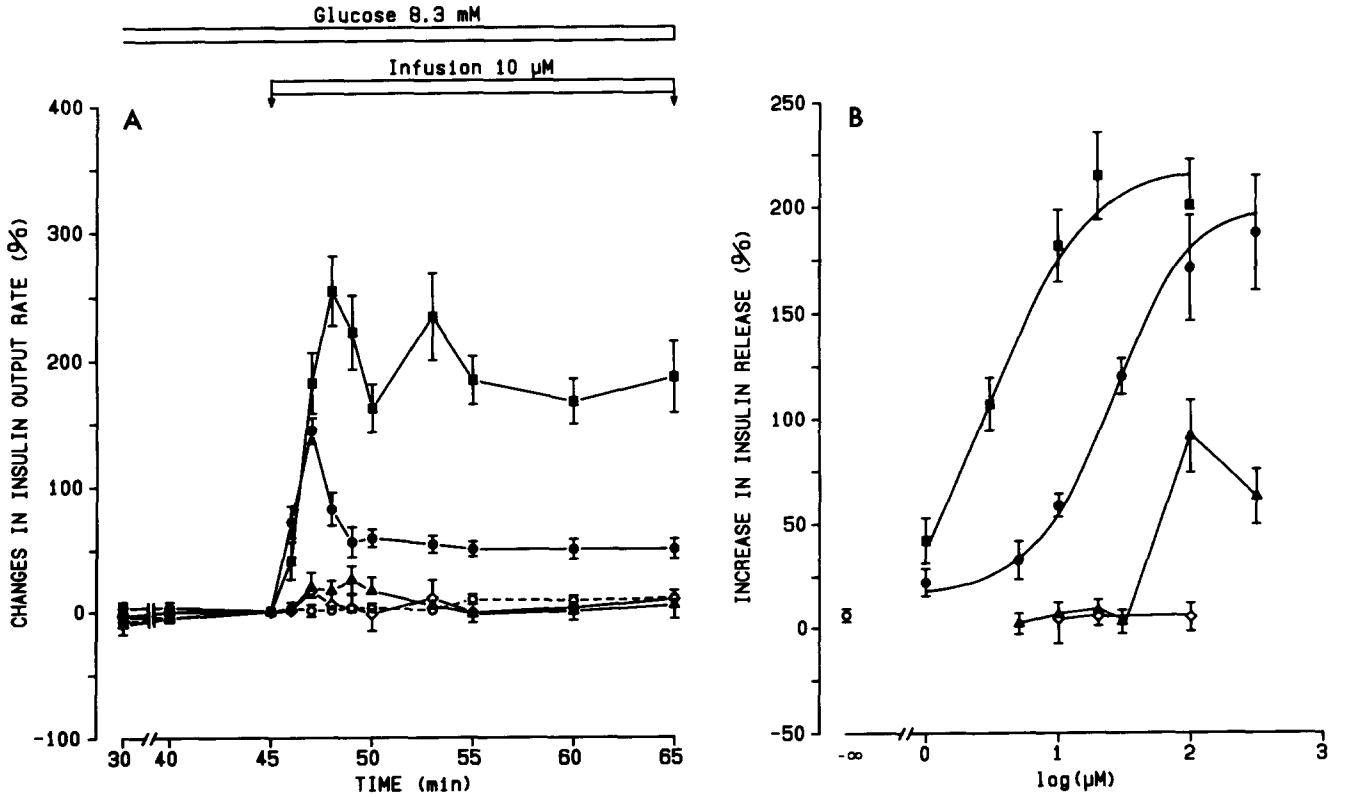

Fig. 1. Comparative effects of imidazolines and derivatives on insulin release in the isolated perfused rat pancreas: antazoline (■); efaroxan (●); idazoxan (▲); cimetidine (◇) and controls (○): (A) effects at 10 µM; (B) concentration-response curves. Each point represents the mean of 4–14 experiments and vertical lines indicate S.E.M.

at 37.5°C. Any change in pancreatic vascular bed resistance was detected by measuring pancreatic effluent output. The pressure (ranging between 40 and 45 cm H_2O) was selected to give a flow rate of 2.5 ml/min during the stabilization period.

2.2. Experimental protocol

In all experiments, a 30-min adaptation period was allowed before the first sample was collected. The test substances were infused for 20 min from time 45 min in the absence or from 55 min in the presence of blockers. The blockers were added from 40 min, 15 min before the agonist infusion. The flow rate was measured for 1 min for each sample; samples were immediately frozen for insulin radioimmunoassay.

2.3. Insulin assay

Insulin was assayed by the method of Herbert et al. (1965), using the antibody supplied by Miles Laboratories (Paris, France). Pure rat insulin (Novo, Copenhagen, Denmark) was used as the reference standard, the biological activity of which was 22.3 μU/ng. The intra- and inter-assay variations were respectively 9 and 13%.

2.4. Expression of data and statistical analysis

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 obtain 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. The values obtained were plotted as a function of the logarithm of test substance concentrations. For each compound we carried out a linear regression analysis from the values and a comparison of potency was performed by the parallel method (Armitage, 1980).

The data were submitted to analysis of variance followed by Student's t-test or by Newman-Keuls test for multiple comparisons (Zar, 1974).

2.5. Drugs

Efaroxan hydrochloride and idazoxan hydrochloride were obtained from Research Biochemicals. Cimetidine, yohimbine hydrochloride, antazoline and tolbutamide were from Sigma.

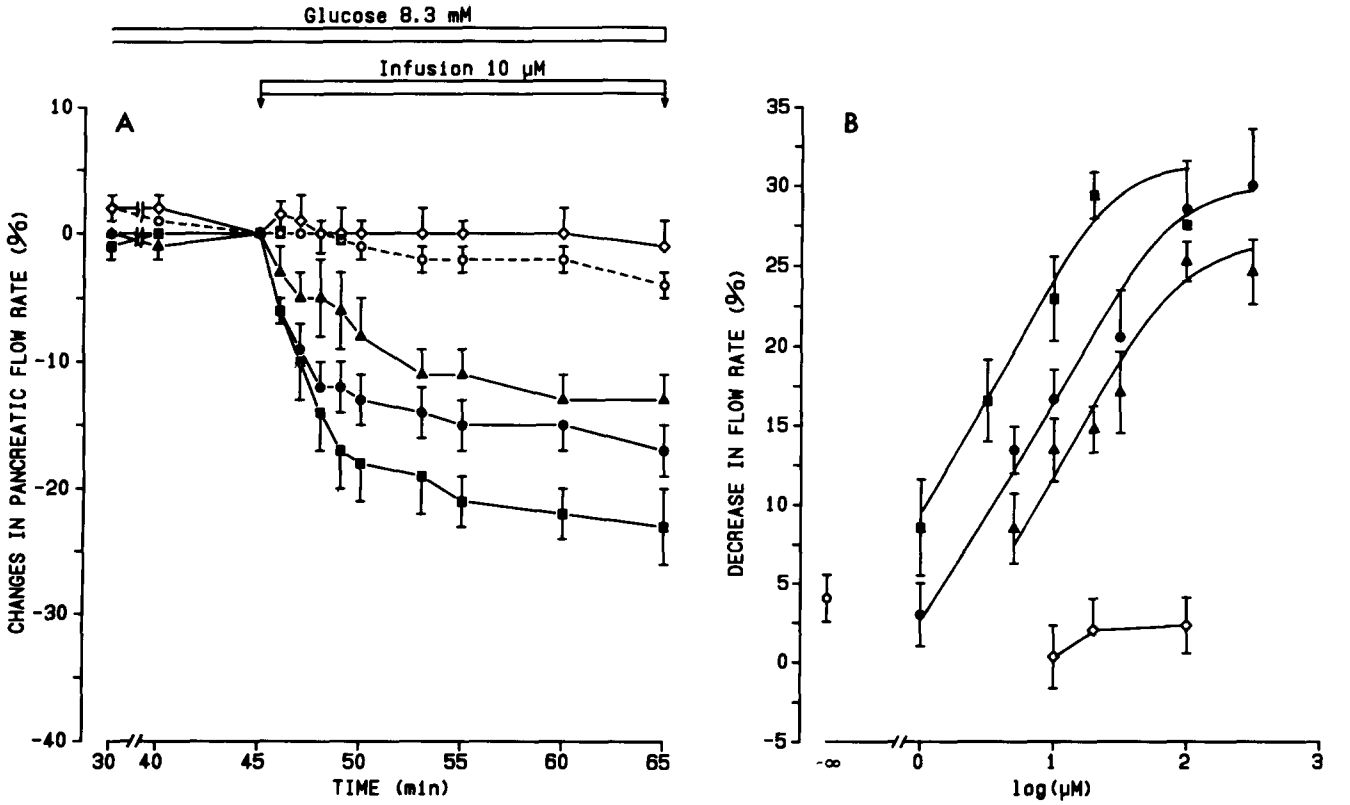

Fig. 2. Effects of imidazolines and derivatives on vascular flow rate in the isolated perfused rat pancreas: antazoline (■); efaroxan (●); idazoxan (▲); cimetidine (◇) and controls (○): (A) effects at 10 μM; (B) concentration-response curves. Each point represents the mean of 4–14 experiments and vertical lines indicate S.E.M.

3. Results

3.1. Comparative effects of imidazolines and derivatives on insulin secretion

In pancreases perfused with the slightly stimulating glucose concentration of 8.3 mM (controls), the rate of insulin secretion was relatively stable and averaged 27.2 ± 3.4 ng/min at 45 min (the reference value).

The test substances were studied in the concentration range 1–300 μM. As shown in Fig. 1A, at 10 μM, the two imidazolines, antazoline and efaroxan, induced a biphasic insulin release. In contrast, at the same concentration the imidazoline, idazoxan, and the imidazole derivative, cimetidine, were ineffective.

Fig. 1B illustrates the concentration-response curves of the substance-induced insulin secretion. The two imidazolines, antazoline (1–10 μM) and efaroxan (1–100 μM) provoked a concentration-dependent insulin response. Antazoline (10 μM) and efaroxan (100 μM) elicited a similar maximal increase in the mean insulin output rate: +181.7 ± 16.5 and +171.4 ± 24.8%, respectively. Antazoline was 10-fold more potent than efaroxan with [6–15] for 95% confidence limits. In contrast, idazoxan was ineffective in the range 10–30 μM; at 100 μM and 300 μM it induced, in a non-concentration-dependent manner, an increase in insulin release (+91.8 ± 17.3 and +62.9 ± 13.1%, respectively) about 2-fold lower than the maximal increases induced by antazoline or efaroxan. This increase was comparable to that obtained with 100 μM yohimbine, a non-imidazoline α_2-adrenoceptor antagonist: +103.2 ± 34.3% (result not shown). On the other hand, the imidazole compound, cimetidine (10–100 μM), was ineffective on insulin secretion.

3.2. Comparative effects of imidazolines and derivatives on pancreatic flow rate

In similar experiments, as shown in Fig. 2A, the three imidazolines, antazoline, efaroxan and idazoxan, but not cimetidine, provoked a progressive and sustained decrease in the pancreatic flow rate at 10 μM.

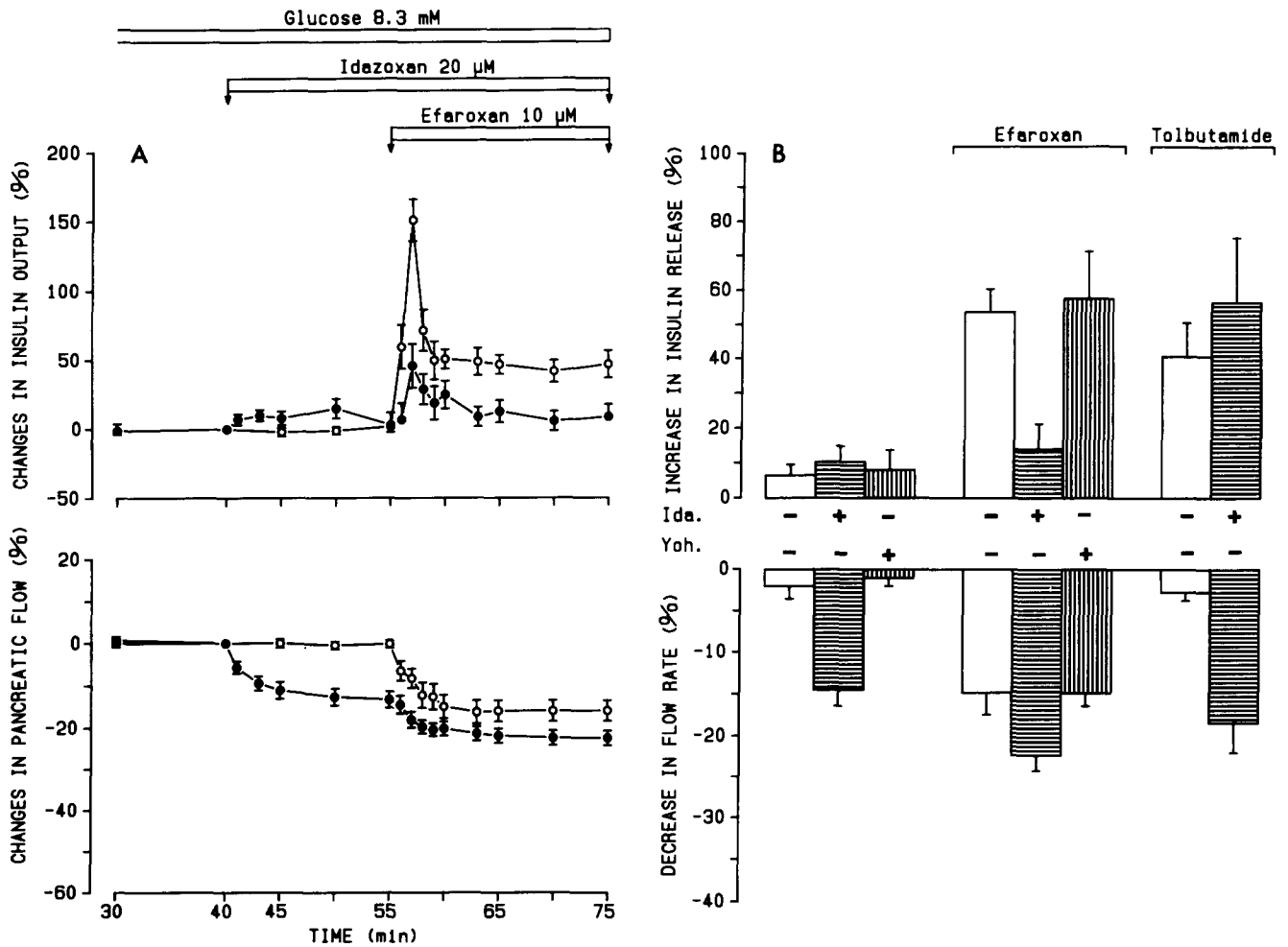

Fig. 3. (A) Effects of idazoxan on the insulin and vascular responses induced by efaroxan: efaroxan alone (○) and in the presence of idazoxan (●). (B) Comparative effects of idazoxan and yohimbine at 20 μM on insulin and vascular responses to efaroxan (10 μM) or tolbutamide (3 μM). Each point represents the mean of 5–12 experiments and vertical lines indicate S.E.M.

The concentration-response curves of the test substance effects on flow rate are shown in Fig. 2B. The three imidazolines were effective in a concentration-dependent manner and there were similar maximal decreases: 29.4 ± 1.5, 28.5 ± 3.1 and 25.2 ± 1.2%, with antazoline (20 μM), efaroxan (100 μM) and idazoxan (100 μM), respectively. Antazoline and efaroxan were 8.1- and 2.2-fold more potent than idazoxan with [4.6–14.1] and [1.3–3.8] for 95% confidence limits, respectively. In contrast, the imidazole derivative, cimetidine (10–100 μM) was ineffective.

3.3. Attempt to block the insulin and vascular responses to efaroxan

Effect of idazoxan

In a first set of experiments, we studied the effects of idazoxan. This imidazoline was used in the range 10–30 μM, since it did not modify insulin release at concentrations up to 100 μM.

Efaroxan was used at 10 μM. Idazoxan was introduced 15 min before efaroxan and was present during the 20 min agonist infusion period.

Fig. 3 shows the results obtained with idazoxan 20 μM on both the insulin and vascular responses to efaroxan. On insulin secretion, idazoxan, ineffective per se during the 15 min pretreatment period (+10.4 ± 4.6 versus +6.4 ± 3.1% in controls), reduced the response to efaroxan (+14.2 ± 7.2 versus +53.8 ± 6.7% with efaroxan alone, $P < 0.001$). On pancreatic flow rate, idazoxan alone at 20 μM elicited an immediate and sustained decrease (−13.3 ± 1.9% at min 55). On superimposition of the infusion of efaroxan, a further decrease in flow rate occurred (−22.5 ± 1.8 versus −16.1 ± 2.4% with efaroxan alone at min 75, $P < 0.05$).

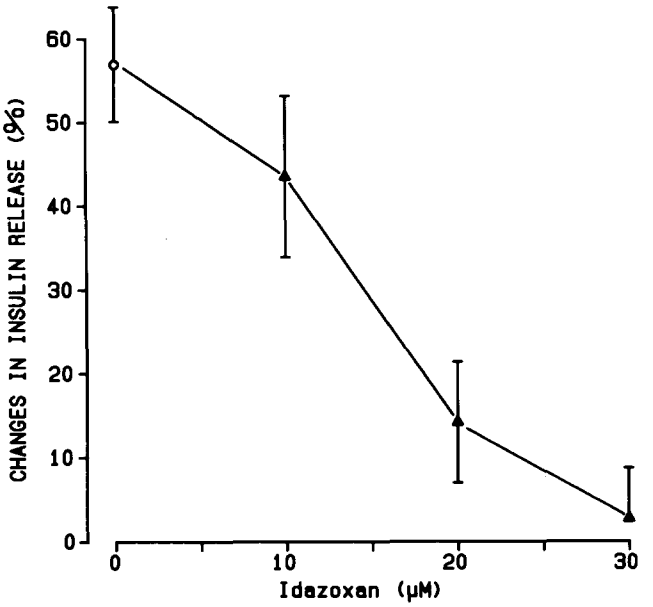

Fig. 4. Effect of idazoxan on the insulin response induced by efaroxan (10 μM). Idazoxan was added from 40 min, 15 min before efaroxan infusion. Each point represents the mean of 6–12 experiments and vertical lines indicate S.E.M.

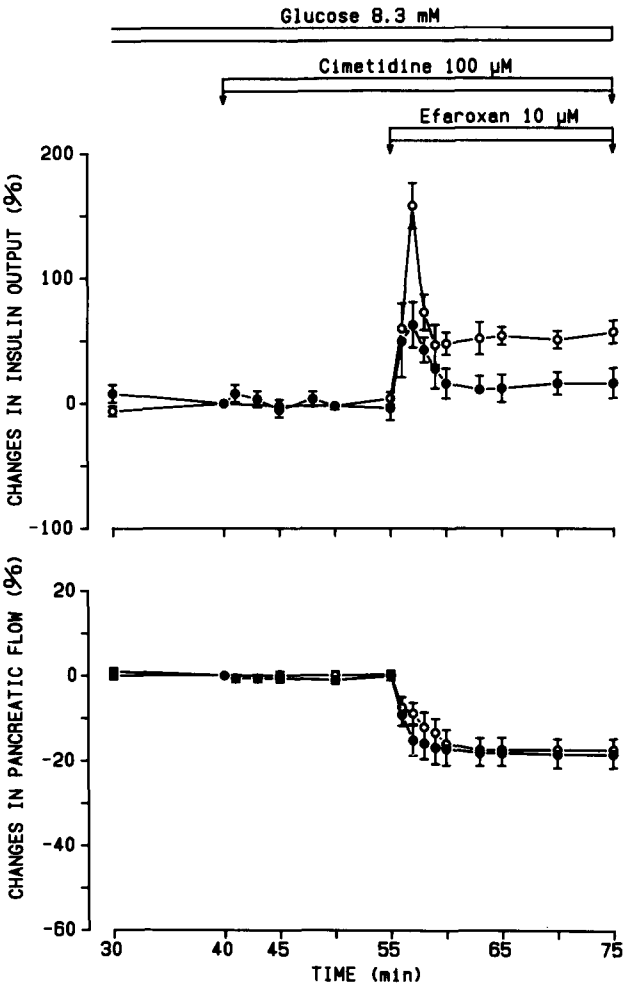

Fig. 5. Effect of cimetidine on the insulin and vascular responses induced by efaroxan: efaroxan alone (○) and in the presence of cimetidine (●). Each point represents the mean of 6 experiments and vertical lines indicate S.E.M.

On the other hand, at the same concentration of 20 μM the non-imidazoline α_2-adrenoceptor antagonist, yohimbine, ineffective per se, altered neither the efaroxan-induced insulin release nor the decrease in flow rate.

In order to establish whether idazoxan selectively blocked the insulin response to efaroxan, we studied its effect on the tolbutamide response. Tolbutamide (3 μM) elicited an increase of insulin release comparable to that obtained with efaroxan 10 μM. Idazoxan did not affect this response.

As shown in Fig. 4, idazoxan (10–30 μM) inhibited in a concentration-dependent manner the insulin response to 10 μM efaroxan (median inhibitory concentration (IC_{50}) ≅ 14 ± 2 μM).

Effect of cimetidine

In a second set of experiments, we studied the effect of cimetidine (100 μM) which was ineffective on both insulin release and the pancreatic vascular bed. As shown in Fig. 5, pretreatment with cimetidine at 100 μM attenuated the insulin response to efaroxan; the mean change in insulin output rate was $+20.7 \pm 10.4$ versus $+58.4 \pm 7.8\%$ with efaroxan alone ($P < 0.01$). In contrast, the imidazole derivative did not affect the vasoconstriction induced by efaroxan: -18.5 ± 3.1 versus $-17.4 \pm 2.6\%$ at min 75.

4. Discussion

This study provides further evidence for the involvement of two different imidazoline sites mediating insulin secretion and vasoconstriction in rat pancreas. Indeed, the present results show a clear difference in the relative efficacies of the imidazolines tested to induce these effects: thus, only antazoline and efaroxan behaved as full agonists on both effects, whereas idazoxan exhibited an opposite behaviour.

It is now established that imidazoline compounds can bind specific sites. Binding studies have demonstrated that these sites form a heterogeneous population of at least two subtypes, I_1 and I_2, which preferentially bind [^{3}H]clonidine and [^{3}H]idazoxan, respectively (Ernsberger, 1992; Reis et al., 1992). These sites exhibit different pharmacological properties: the imidazoline I_1 sites recognize idazoxan and imidazole compounds whereas the imidazoline I_2 sites show a 100-fold lower or no affinity for clonidine and do not recognize imidazoles. In addition, it appears from functional studies that idazoxan exhibits opposite pharmacological properties: idazoxan behaves as an agonist at the I_2 type but acts as an antagonist at the I_1 type (Bidet et al., 1990; Ernsberger et al., 1990).

On insulin secretion, only antazoline and efaroxan, but not idazoxan, induced a concentration-dependent insulin response, antazoline being 10 times more potent than efaroxan. However, idazoxan at the higher concentrations of 100 and 300 μM exhibited an insulin secretory effect but in a non-concentration-dependent manner and to a lesser extent than efaroxan and antazoline. Similarly, it must be reported that at the high concentration of 100 μM, yohimbine, a non-imidazoline α_2-adrenoceptor antagonist known to have no affinity for imidazoline sites (Hamilton et al., 1988; Yakubu et al., 1990), induced a low insulin response similarly to idazoxan. This stimulatory effect can be attributed to a partial inhibition of ATP-sensitive K^+ channels of pancreatic B cells as previously reported for these agents at such a high concentration (Plant and Henquin, 1990; Chan et al., 1991a). In contrast, at concentrations up to 30 μM, idazoxan, ineffective per se, inhibited the insulin response to efaroxan in a concentration-dependent manner. The competitivity of idazoxan for the efaroxan effect on insulin secretion suggests an action on a common site. In addition, the effect of idazoxan seems to result from a specific action on an imidazoline site since its antagonist property is not shared by yohimbine, a selective α_2-adrenoceptor antagonist without affinity for imidazoline sites, as previously reported. On the other hand, there is evidence that the ability of imidazolines, including efaroxan, to enhance insulin secretion results from the inhibition of ATP-sensitive K^+ channels in the B cell membrane (Plant and Henquin, 1990; Dunne, 1991; Chan et al., 1991b; Jonas et al., 1992). In order to ascertain that idazoxan exerts a selective blocking action on imidazoline insulin secretory activity, we tested it on the effect of the non-imidazoline K^+-ATP channel inhibitor, tolbutamide. The fact that idazoxan failed to affect the insulin response to this agent is indicative of a selective antagonist action at a site distinct from the sulfonylurea site. On the other hand, there is evidence that idazoxan can behave as a selective antagonist at imidazoline I_1 sites. Indeed, several studies have shown that this agent blocks the central hypotensive effect (Ernsberger et al., 1990; Tibiriça et al., 1991; Sannajust and Head, 1994) or the increase of renal sodium excretion (Allan et al., 1993) by clonidine and related drugs, both effects mediated by imidazoline I_1 sites. Thus in this context, the insulin-releasing action of the imidazolines appears to be mediated by imidazoline sites of the I_1 type. However, it has to be noted that recently Brown et al. (1993a) showed that idazoxan does not antagonize the ability of efaroxan to directly stimulate insulin secretion or reverse the inhibitory effect of diazoxide in rat islets. This discrepancy can be explained by the different experimental conditions and the high concentration of idazoxan used (100 μM) which was previously found to partially reverse the diazoxide-induced inhibition of insulin secretion and to block ATP-sensitive K^+ channels (Chan et al., 1991a). On the other hand, the same group has recently reported that another imidazoline, RX801080, acts as an antagonist on the actions of efaroxan but also blocks the effect of glibenclamide which is, like tolbutamide, a non-imidazoline K^+-ATP channel inhibitor (Brown et al., 1993b). Nevertheless, in agreement with the present results, these authors concluded that the stimulatory effect of imidazolines on insulin secretion is not due to an interaction with [^{3}H]idazoxan binding sites (I_2 sites), since their insulin secretory action is not correlated to their ability to bind these sites, but with imidazoline sites distinct from the sulphonylurea sites.

On the pancreatic flow rate, both antazoline, efaroxan and idazoxan induced a vasoconstrictor effect; the apparent rank order of agonist potency was

antazoline > efaroxan > idazoxan. It has to be noted that idazoxan exhibited a maximal effect similar to that of the two other imidazolines. Thus, in contrast to what is observed with B cells, idazoxan behaves as an agonist on pancreatic vessels: this finding is supported by the apparent additivity of efaroxan and idazoxan when infused together. Several functional studies have shown that this imidazoline acts as an agonist at [^{3}H]idazoxan sites (I_2 sites) mediating the inhibition of Na^+ uptake in renal proximal tubule cells (Bidet et al., 1990), or mediating the inhibition of norepinephrine release from sympathetic nerve terminals of the pulmonary artery and aorta (Göthert and Molderings, 1991; Molderings et al., 1991) and the glial fibrillary acidic protein expression in cortical astrocytes (Regunathan et al., 1993; Olmos et al., 1994). Thus, in the present study, the behaviour of idazoxan as a full agonist is consistent with the possibility of an imidazoline I_2 site mediating vasoconstriction in rat pancreas. Imidazolines have been shown to increase spontaneous myogenic activity in the rat portal vein (Schwietert et al., 1992) and, as in our study, antazoline appeared to be more potent than idazoxan. Nevertheless, it has to be mentioned that the potency of efaroxan on vessels is in apparent disagreement with the low affinity of this compound for the I_2 sites reported in binding studies (Ernsberger et al., 1992). However, imidazoline I_2 sites have been reported to exhibit a major difference in their ability to bind drugs in various tissues and species and the existence of multiple interrelated binding site subtypes has recently been suggested (Bricca et al., 1993a; Rangel et al., 1993).

Finally, the results obtained with the imidazole, cimetidine, also support the view of different imidazoline sites in rat pancreas: an imidazoline I_1 site on the pancreatic B cell and an imidazoline I_2 site on the vascular bed. Indeed, as previously reported, imidazole compounds including cimetidine have been reported to recognize imidazoline I_1 but not I_2 sites. However, discrepancies concerning the actions of cimetidine appear at the central imidazoline I_1 site involved in the vasodepressor effect of clonidine; thus an antagonist effect (Karppanan, 1981), an agonist effect (Ernsberger et al., 1990) or no action (Bricca et al., 1993b) have been reported for this imidazole drug. The present results show that cimetidine is ineffective per se on both insulin secretion and pancreatic flow rate. However, it can partially reverse the insulin stimulatory effect of efaroxan but does not affect its vasoconstrictor effect. In addition, the relatively low potency of cimetidine to block the efaroxan effect, compared to that of idazoxan, could be related to the rank order of affinity reported for the I_1 site: idazoxan > cimetidine (see Reis et al., 1992).

In conclusion, the insulin secretory and vasoconstrictor actions of imidazolines clearly involve different imidazoline sites in rat pancreas. This study provides evidence for an I_1 type on the B cell and an I_2 type on the vascular bed. In this context, this difference in sites may be useful in the development of new antidiabetic drugs exhibiting a selectivity for the I_1 site of B cells but devoid of I_2 agonist activity on vessels.

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