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Short communication

Dual action of clonidine on insulin release: suppression, but stimulation when α_2-adrenoceptors are blocked

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Summary. As shown previously clonidine reduces glucose-stimulated insulin release and this effect is mediated by inhibitory postsynaptic α_2-adrenoceptors.

The present experiments demonstrate that clonidine has the additional property to also stimulate insulin release. This became evident when the α_2-adrenoceptors of isolated islets were blocked by benextramine, and thus protected from being stimulated by clonidine.

In the presence of benextramine, clonidine no longer reduced, but on the contrary enhanced, the release of insulin in response to glucose. In control experiments benextramine by itself failed to affect insulin release from isolated islets.

These results show that the imidazoline derivative clonidine shares the property of other imidazoline compounds to enhance the insulin response to glucose. All of these agents may stimulate insulin by binding to "imidazoline-preferring" sites, that clearly differ from α-adrenoceptors.

Key words: Insulin release – Clonidine – Imidazolines – Benextramine

Introduction

Clonidine is known to suppress insulin release from the pancreatic B-cell by acting on inhibitory α_2-adrenoceptors. Thus, in man a single high dose of clonidine reduced plasma insulin and raised blood glucose levels (Metz et al. 1978; Lal et al. 1981). A similar response was obtained in various animal experiments (Iwata 1969; Humphreys and Reid 1979; Gorewit 1980). Clonidine inhibited insulin release not only in the whole animal but also on isolated islets (Andersson and Nygren 1983; Ismail et al. 1983; Garcia-Morales et al. 1984; Laychock 1987) or B-cell suspensions (Nilsson et al. 1987). This effect on the isolated tissue has been shown to depend on the concentration of clonidine present (Leclercq-Meyer et al. 1980; Langer et al. 1983). The adrenoceptors involved in this inhibition are of the α_2-subtype (Nakadate et al. 1980; Nakaki et al. 1980).

We have recently shown that imidazoline derivatives such as phentolamine increase the insulin response to glucose (Schulz and Hasselblatt 1989). Since clonidine is also an imidazoline in structure, we have now tested whether it shares this property of chemically related imidazoline compounds. We therefore measured the effect of clonidine on glucose-induced insulin release from isolated mouse islets following blockade of the α_2-adrenoceptors by benextramine.

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Material and methods

Chemicals. Collagenase (Boehringer, Mannheim, FRG); bovine serum albumin (fraction V) (Miles, Frankfurt, FRG), bovine serum γ-globulin (fraction II) (Serva, Heidelberg, FRG); HEPES (N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)), clonidine hydrochloride (Sigma, Chem. Corp., St. Louis, MO, USA); benextramine tetrahydrochloride monohydrate (Aldrich, Steinheim, FRG); ^{125}J-labelled porcine insulin (Behringwerke, Frankfurt, FRG); crystalline rat insulin (Novo, Bagsvaerd, Denmark); insulin antibody from own sources. All other reagents were analytical grade from Merck, Darmstadt, FRG.

Experimental procedures. Isolation and perifusion of mouse pancreatic islets and the analysis of insulin released into the perifusion fluid by radioimmunoassay have been described in detail (Schulz and Hasselblatt 1988).

Statistical analysis. Results are given as mean ± SEM for independent experiments. Significance was tested by the two-tailed U-test of Wilcoxon (1945) and of Mann and Whitney (1947). $P < 0.05$ was considered significant. The amount of insulin released by each group in response to 30 mmol/l D-glucose was compared.

Results

When isolated pancreatic islets from mice were perifused with glucose, clonidine strongly inhibited insulin release when present in concentrations in the range from 3.80 nmol/l to 0.38 μmol/l (Fig. 1). In another group of experiments islets were pretreated with benextramine (13.70 μmol/l) which was perifused for the 40 min preceding the glucose stimulus. This was done to irreversibly block the α_2-adrenoceptors in the tissue prior to its being exposed to clonidine. Following the α-adrenoceptor blockade clonidine at a concentration that was otherwise clearly inhibitory (0.38 μmol/l) failed to significantly reduce the output of insulin.

Thus, benextramine successfully prevented clonidine from stimulating the inhibitory α_2-adrenoceptors.

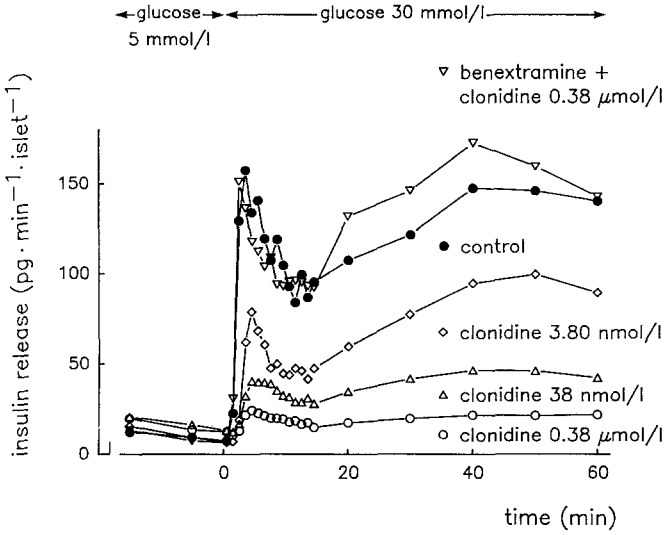

Fig. 1. Effects of increasing concentrations of clonidine on glucose-induced insulin release from pancreatic islets of mice. Islets were perifused either with glucose alone (●) or from time zero onwards in addition with clonidine (3.80 nmol/l; ◇, 38 nmol/l; △, 0.38 μmol/l: ○). In all experiments the glucose concentration was elevated from 5 to 30 mmol/l at time zero. In one group (▽) islets were perifused with benextramine (13.70 μmol/l) alone for 40 min in the presence of 5 mmol/l glucose. At zero time the glucose concentration was raised to 30 mmol/l and clonidine (0.38 μmol/l) was added. Means of results from 4 separate experiments are given. SEM values were omitted for clarity. The total amount of insulin released within the 60 min in response to glucose (30 mmol/l) was reduced significantly by clonidine (3.80 nmol/l, 38 nmol/l or 0.38 μmol/l) ($p < 0.05$; U-test). Insulin released from islets that had been pretreated with benextramine and subsequently exposed to clonidine (0.38 μmol/l) did not differ significantly from that of controls. Cumulative amounts of insulin released during 60 min in the presence of 30 mmol/l glucose: Controls: 8154 ± 701 pg/islet; clonidine 3.80 nmol/l: 4480 ± 1007 pg/islet; clonidine 38 nmol/l: 2558 ± 786 pg/islet; clonidine 0.38 μmol/l: 1276 ± 328 pg/islet; benextramine pretreated, clonidine 0.38 μmol/l: 8946 ± 605 pg/islet

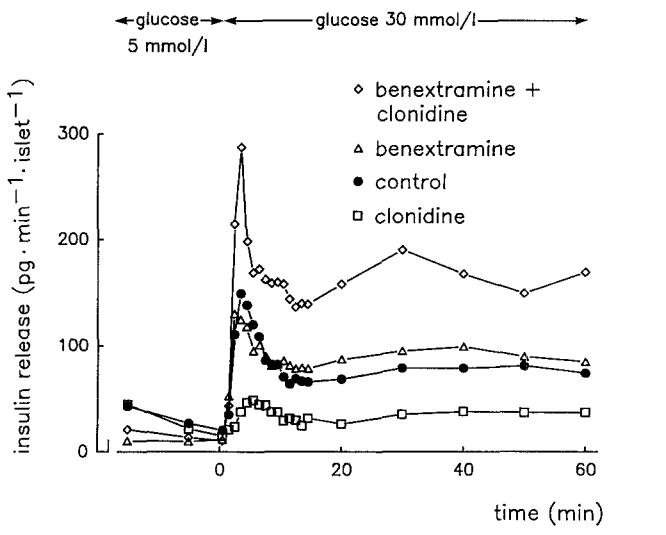

Fig. 2. Effects of benextramine and clonidine when added alone or combined on the insulin response of isolated mouse pancreatic islets to glucose. Means of results from 4 (◇ and □) resp. 5 (● and △) perifusion experiments are given. SEM values were omitted for clarity. At time zero the glucose concentration was elevated from 5 to 30 mmol/l. The media contained clonidine (3.80 μmol/l) alone (□), benextramine (13.70 μmol/l) alone (△) or in the control series neither of these substances (●). In a further group of experiments the islets were first exposed to benextramine (13.70 μmol/l) alone for 40 min in the presence of 5 mmol/l glucose. At time zero glucose was raised to 30 mmol/l and clonidine (3.80 μmol/l) was added (◇). The total amount of insulin released within the 60 min in response to glucose (30 mmol/l) was significantly reduced by clonidine ($p < 0.05$; U-test). Significant amounts of additional insulin were released from islets pretreated with benextramine and subsequently (from time zero to the 60th min) exposed to clonidine ($p < 0.05$; U-test). The amount of insulin released in the presence of benextramine alone did not differ from that of corresponding controls. Cumulative amounts of insulin released during 60 min in the presence of 30 mmol/l glucose: Controls: 5015 ± 670 pg/islet; clonidine 3.80 μmol/l: 2343 ± 382 pg/islet; benextramine 13.70 μmol/l: 5850 ± 715 pg/islet; benextramine pretreated, clonidine 3.80 μmol/l 10672 ± 2451 pg/islet

In a second series of experiments (Fig. 2) islets were similarily pretreated with benextramine (13.70 μmol/l) and subsequently exposed to a tenfold higher concentration of clonidine (3.80 μmol/l). It was at these high concentrations that clonidine significantly elevated the insulin response to glucose.

The same concentration of clonidine (3.80 μmol/l) when given alone, reduced the insulin response to 30 mmol/l glucose. Benextramine (13.70 μmol/l) by itself did not change insulin release to glucose.

Discussion

Several imidazoline derivatives have been found to stimulate insulin release (Schulz and Hasselblatt 1989). Among these are α-adrenoceptor antagonists but also agents of different properties like the antihistaminic antazoline. Moreover α-adrenoceptor blocking drugs lacking the imidazoline structure failed to similarily elevate the insulin response. We therefore concluded that the stimulatory effect did not result from α-adrenoceptor blockade but was an independent property of imidazoline compounds.

The results presented here demonstrate that even the imidazoline derivative clonidine shares the property of other imidazolines to increase insulin release. As this effect is normally masked by the more prominent α_2-adrenoceptor-mediated inhibition, an irreversible blockade of α_2-adrenoceptors was necessary to reveal this stimulatory property of clonidine. Such blockade was achieved by exposing islets to the irreversibly α_2-blocking agent benextramine (Melchiorre 1981). As benextramine itself failed to affect insulin release, the insulin response of the islets in these experiments was not normally inhibited by endogenous noradrenaline liberated from remaining nerve tissue.

There have been previous indications that clonidine at high concentrations might have some stimulatory effect on insulin release. Thus, rising concentrations of clonidine were found to progressively reduce insulin release until maximal suppression was achieved at 1 μmol/l. At still higher concentrations the inhibitory effect declined (Langer et al. 1983).

Our results demonstrate that nanomolar concentrations of clonidine produce the well known dose-dependent suppression of insulin release, while micromolar concentrations are necessary to potentiate insulin release after blockade of inhibitory α_2-adrenoceptors.

Imidazoline compounds of widely differing profiles of activity share the common property to stimulate insulin re-

lease in the presence of glucose. This includes an agent stimulating α_2-receptors like clonidine as well as α-adrenoceptor blockers like phentolamine or tolazoline and the antihistaminic antazoline which has no prominent effects on α_2-adrenoceptors (Schulz and Hasselblatt 1989).

Binding studies have revealed imidazoline-preferring sites in various tissues. Using a radiolabelled clonidine analogue Ernsberger et al. (1987) demonstrated two distinct populations of binding sites in the ventrolateral medulla of bovine brain. The label apparently binds to α_2-adrenoceptors and to additional imidazoline-binding sites. Such sites have also been detected in kidney membranes (Coupry et al. 1987; Michel et al. 1989) and in the rabbit forebrain (Hamilton et al. 1988).

It thus appears that members of the otherwise heterogenous imidazoline "family" all bind to some "imidazoline-preferring" sites that have been found in different tissues. Possible effects resulting from such binding have not yet been defined. In the islet tissue stimulation of glucose-induced insulin release could be one of those.

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