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Effects of Imidazoline Derivative RX871024 on Insulin, Glucagon, and Somatostatin Secretion from Isolated Perfused Rat Pancreas

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The effects of the imidazoline compound RX871024 on arginine-induced insulin, glucagon, and somatostatin secretion in the isolated perfused rat pancreas have been investigated. Arginine induced biphasic insulin, glucagon, and somatostatin release when infused for 20 min at 20 mM concentration and 3.3 mM glucose in the medium. RX871024, at 10 μM, did not influence basal hormone secretion but enhanced arginine-stimulated insulin and somatostatin release. In contrast, glucagon secretion was markedly inhibited by 10 μM imidazoline. RX871024 (1 μM) did not significantly affect arginine-induced insulin and somatostatin secretion but had an inhibitory effect on the second phase of glucagon release. In conclusion, RX871024 exerts a complex effect on the endocrine pancreas challenged by arginine, comprising stimulation of insulin and somatostatin release and inhibition of glucagon release. These effects on hormone release probably constitute the main mechanism of the antidiabetogenic action of the imidazolines.

Key Words: **imidazoline; perfused rat pancreas; insulin; glucagon; somatostatin.**

Hormone release from the endocrine pancreas is regulated in a complex manner by various nutrients, hormones, ions and drugs (1). Of compounds that are potential candidates in the treatment of diabetes, imidazolines have attracted a lot of interest (2–8).

The imidazoline compound RX871024 stimulates insulin release by at least two mechanisms (2). The first mechanism involves blocking of the ATP-dependent K^+ channel, K_{ATP} channel, leading to an increase in cytosolic free Ca^{2+} concentration, $[Ca^{2+}]_i$, and corresponding insulin release. According to the second mechanism, the imidazoline directly affects the exocytotic machinery without changing $[Ca^{2+}]_i$, an effect involving protein kinases A and C (2). Since the compound influenced also a distal process in the insulin secretory machinery we presently investigated the effect of RX871024 on the release of glucagon and somatostatin, two pancreatic hormones that together with insulin play an essential role in regulation of blood glucose (9–11). The study was performed in the isolated perfused rat pancreas using arginine to stimulate insulin, glucagon and somatostatin release (12).[1]

MATERIALS AND METHODS

Materials. RX871024 was from Reckitt and Colman (UK). RPMI 1640 medium and fetal calf serum were obtained from GIBCO BRL (UK). Bovine serum albumin, BSA, was purchased from Sigma (USA). All other reagents of analytical grade were from MERCK (Germany).

Perfusion of isolated pancreas. Non-diabetic Wistar rats (male and female, 180–230 g, aged 8 to 12 weeks) were obtained from B&K Universal (Sweden). The animals were anaesthetized with an i.p. injection of phenobarbital (100 mg/kg body wt). The pancreas was dissected free from the adjacent tissues (13). The perfusion medium, consisting of Krebs–Ringer bicarbonate buffer (containing in mM: 115 NaCl, 4.7 KCl, 2.56 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 20 $NaHCO_3$, 16 Hepes, 3.3 glucose and pH 7.4) supplemented with 1 mg/ml BSA. This medium was passed to the pancreas through a cannula inserted in the abdominal aorta. Pancreas was first perfused with basal medium for 20 min and then stimulated with 20 mM arginine, with or without RX871024. All the perfusion samples were collected into

Abbreviations used: BSA, bovine serum albumin; K_{ATP} channels, ATP-dependent K^+ channels, $[Ca^{2+}]_i$, cytoplasmic free Ca^{2+} concentration.

[1] The material included in this paper was presented in abstract form at the 32nd Annual Meeting of the European Association for the Study of Diabetes, Vienna, 1–5 September 1996.

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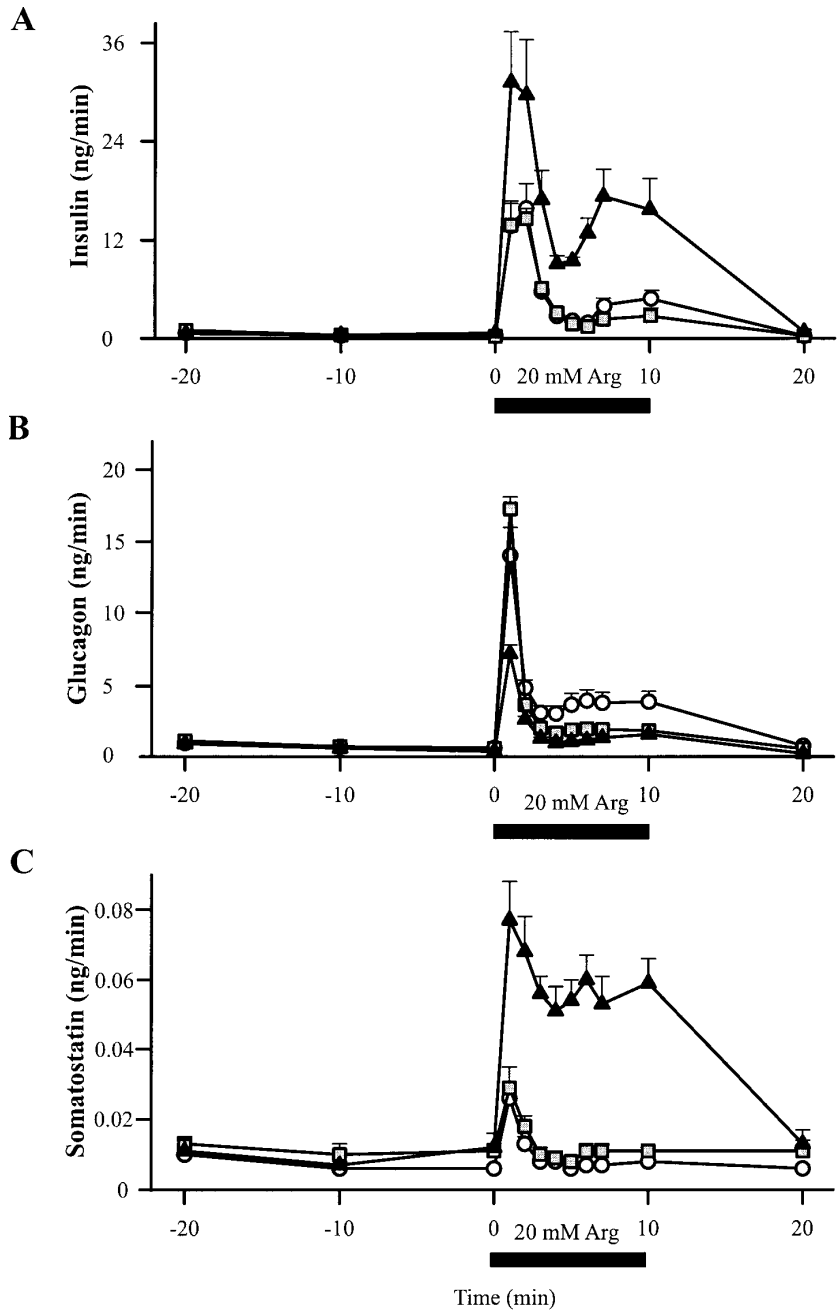

FIG. 1. (A–C) Effects of RX871024 on arginine-induced insulin (A), glucagon (B) and somatostatin (C) release from perfused pancreas of Wistar rats. The pancreas was equilibrated for 20 min in the absence (open circles) and presence of 1 μM (half-tone squares) or 10 μM (closed triangles) RX871024 before administration of 20 mM arginine. Each value represents the mean ± SEM of six (A) and four (B, C) experiments.

ice-chilled tubes, stored at −20°C and analyzed in radioimmunoassays for insulin, glucagon and somatostatin.

Presentation of results. Data analysis was carried out using the program Sigma Plot for Windows (version 1.02, SPSS Inc., USA) and the program Statistica for Windows (version 5.0, StatSoft Inc., USA). Integrated hormonal responses were calculated as areas under the curve. All results are expressed as means ± SEM for indicated number of experiments. The statistical significance of differences between means was assessed by analysis of variance for multiple comparisons with *P* values corrected by Bonferroni method.

RESULTS

In the presence of 3.3 mM glucose, 20 mM arginine stimulated secretion of insulin, glucagon and somatostatin from perfused rat pancreas with the typical biphasic pattern for all three hormones (Fig. 1). The imidazoline compound RX871024, at a concentration of 10 μM, did not show any effect on basal hormone secretion at 3.3 mM glucose. However, 10 μM

TABLE 1

Effect of Imidazoline Compound RX871024 on Arginine-Induced Hormonal Release from Perfused Pancreas of Wistar Rat

	Arginine (n=6)	Arginine + 1 μM RX871024 (n=4)	Arginine + 10 μM RX871024 (n=4)
Insulin			
0–4 min	40.4 ± 6.2	40.5 ± 5.6	96.8 ± 17.7*
5–20 min	51.7 ± 5.2	28.9 ± 5.2	171.9 ± 35.2***
0–20 min	92.1 ± 8.8	69.4 ± 8.6	268.7 ± 51.3***
Glucagon			
0–4 min	30.4 ± 3.3	30.1 ± 1.7	14.3 ± 0.9
5–20 min	52.9 ± 9.9	22.8 ± 4.5*	16.9 ± 1.3**
0–20 min	83.3 ± 12.7	52.9 ± 5.9**	31.2 ± 2.0***
Somatostatin			
0–4 min	0.065 ± 0.007	0.078 ± 0.014	0.298 ± 0.037***
5–20 min	0.108 ± 0.006	0.140 ± 0.027	0.676 ± 0.069***
0–20 min	0.173 ± 0.013	0.218 ± 0.038	0.974 ± 0.095***

Note. Data presented as areas under the curves for insulin (ng), glucagon (ng), and somatostatin (ng) release during the respective periods.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs respective controls (without RX871024).

RX871024 significantly enhanced both the first (0–4 min) and the second (5–20 min) phases of arginine-stimulated insulin and somatostatin secretion, while both phases of arginine-induced glucagon release were significantly inhibited (Fig. 1 and Table 1).

The suppression of the second phase of arginine-induced glucagon release was observed even in the presence of 1 μM RX871024 (Table 1), while there was no statistically significant effect on arginine-induced insulin and somatostatin release at this low concentration of the imidazoline.

DISCUSSION

Arginine stimulates insulin release by depolarizing the β-cell, due to its own transport across the membrane in a positively charged form (14, 15), which results in opening of voltage-gated L-type Ca^{2+}-channels and a subsequent increase in $[Ca^{2+}]_i$ and thereby exocytosis of insulin. The imidazoline compound RX871024 significantly enhanced both phases of arginine-stimulated insulin secretion in a perfused pancreas in the presence of 3.3 mM glucose. The stimulatory effect of RX871024 on arginine-induced insulin secretion can be explained partially by the blockade of the K_{ATP} channel (2), which may facilitate arginine-induced β-cell membrane depolarization (15). In addition, this imidazoline compound has been found to directly stimulate the exocytotic machinery when the islets were challenged by glucose (2).

A key role for K_{ATP} channels and voltage gated L-type Ca^{2+} channels was suggested also for regulation of somatostatin secretion from pancreatic δ-cells (16, 17). Therefore, the above discussed mechanisms may as well be involved in enhanced arginine-induced somatostatin secretion in the presence of RX871024.

The mechanisms behind inhibition of glucagon release by RX871024 are not clear. They may involve both a paracrine effect on the α-cell by increased release of insulin and/or somatostatin and as well a direct inhibitory effect of RX871024 on glucagon exocytosis. Indeed, the imidazoline compound at a low dose inhibited glucagon secretion without significant influence on insulin and somatostatin release. We have previously demonstrated that also the sulfonylurea drug glibenclamide inhibited arginine-induced glucagon release while insulin and somatostatin responses were enhanced (18). It is not clear whether inhibition of glucagon secretion is due to a direct effect of glibenclamide on α-cells or solely reflects a secondary paracrine action of insulin and somatostatin.

In addition to RX871024 other imidazolines have been shown to stimulate insulin release *in vitro* (6). Imidazoline compound midaglizole also exerted strong antidiabetogenic effects in patients with noninsulin-dependent diabetes mellitus, NIDDM (19). The latter finding was attributed not only to stimulation of insulin release but also to improved insulin sensitivity (20). Thereby the imidazoline compounds may improve the two main defects underlying glucose intolerance in NIDDM patients.

The novel finding in the present study is that the imidazoline compound RX871024 stimulates not only insulin release but also somatostatin release and suppresses glucagon release. Since patients with NIDDM exhibit both exaggerated glucagon and decreased somatostatin release (9–11, 21), it is possible that the presently described effects of RX871024 on responses of the α- and δ-cell may constitute two additional targets for the antidiabetogenic action of the compound. The suppression of glucagon release results in decreased hepatic glucose production (22, 23), while the enhanced somatostatin release prolongs the rate of absorption of nutrients and attenuates hyperglycemia (24).

In conclusion, the imidazoline compound RX871024 exerts a complex effect on the endocrine pancreas by stimulating insulin and somatostatin release and inhibiting glucagon release. These effects are likely to explain the antidiabetogenic action of this class of compounds.

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