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European Journal of Pharmacology 294 (1995) 117–123

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Effects of imidazoline antagonists of α_2-adrenoceptors on endogenous adrenaline-induced inhibition of insulin release

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Received 25 April 1995; revised 10 August 1995; accepted 15 August 1995

Abstract

We studied the effects of adrenoceptor antagonists and imidazoline derivatives on endogenous adrenaline-induced inhibition of insulin release in anesthetized rats. The intracerebroventricular injection of neostigmine increased plasma levels of catecholamines and glucose but not insulin. Pretreatment with an i.p. injection with phentolamine caused a dose-dependent increase in insulin secretion. When atropine was coadministered with phentolamine, the phentolamine-induced increase in insulin secretion was inhibited. Neither phentolamine nor atropine affected plasma levels of catecholamine. Yohimbine and idazoxan, which are α_2-adrenoceptor antagonists, and tolazoline, a non-selective α-adrenoceptor antagonist, also reversed adrenaline-induced inhibition of insulin secretion. Phenoxybenzamine, prazosin, propranolol, and antazoline, an imidazoline without α_2-adrenoceptor activity, did not affect insulin levels. When agents were preinjected i.p. in rats that were given saline into the third cerebral ventricle, phentolamine and antazoline, but not yohimbine and idazoxan, increased plasma levels of insulin. The results suggest that the inhibition of insulin release induced by adrenaline was reversed by antagonism of α_2-adrenoceptors. Phentolamine and antazoline, both of which are imidazoline derivatives, induced insulin secretion independently of the adrenoceptors only under the resting conditions.

Keywords: Imidazoline; α_2-Adrenoceptor; Adrenaline; Insulin release; (In vivo); (Rat)

1. Introduction

Insulin secretion is suppressed under various conditions that stimulate sympathoadrenal activity such as hypoxia (Baum and Porte, 1972), hypothermia (Baum et al., 1968; Baum and Porte, 1971), exercise (Cochran et al., 1966; Schalch, 1966; Nikkilä et al., 1968), and psychological stress (Mason et al., 1968). Phentolamine, a non-selective α-adrenoceptor antagonist, (Baum and Porte, 1971, 1972; Robertson and Porte, 1973; Wollheim et al., 1977), and selective α_2-adrenoceptor antagonists (Nakaki et al, 1981; Yamazaki et al., 1982; Tamagawa and Henquin, 1983) have been shown to reverse the catecholamine-induced inhibition of insulin secretion, suggesting that the inhibitory effect of the sympathoadrenal system depends on catecholamine-induced activation of α_2-adrenoceptors.

Phentolamine enhances insulin release even in the absence of elevated catecholamine levels, suggesting that pancreatic β-cell function is influenced by the basal adrenergic inhibitory tone (Robertson and Porte, 1973; Robertson et al., 1976; Broadstone et al., 1987). However, Henquin et al. (1982) and Schulz and Hasselblatt (1989) have suggested that phentolamine and other imidazoline receptor antagonists of α_2-adrenoceptors possess an insulin-releasing property unrelated to the blockade of adrenoceptors. Subsequent studies have shown that several imidazoline antagonists of α_2-adrenoceptors increase insulin release by inhibiting ATP-sensitive K^+ channels in β-cells (Chan and Mor-

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SSDI 0014-2999(95)00519-6

gan, 1990, 1991; Plant and Henquin, 1990; Jonas et al., 1992). However, these channels are not activated by adrenaline (Hsu et al., 1993; Plant et al., 1991). Thus, the pharmacological effects of agents previously assumed to be specific antagonists of α-adrenoceptors on insulin secretion require reinterpretation.

We previously reported that the insulin secretion induced by stimulation of central cholinergic neurons with neostigmine, an acetylcholinesterase inhibitor, in adrenalectomized rats was mediated by the parasympathetic pathway in the pancreas (Gotoh et al., 1989). Stimulation of the central nervous system of intact rats increased plasma levels of catecholamines and caused marked hyperglycemia without causing an increase in the plasma levels of insulin (Iguchi et al., 1989), indicating that the increased circulating level of adrenaline suppressed insulin secretion from the pancreas despite the presence of hyperglycemia. In the present study, we investigated the effects of α-adrenoceptor antagonists and imidazoline derivatives on the endogenous catecholamine-induced inhibition of insulin secretion in vivo.

2. Materials and methods

2.1. Animal preparation

Male albino Wistar rats (Keary, Nagoya, Japan) weighing 250–300 g were kept in individual cages in a temperature-controlled room (24 ± 1°C) with a 12-h light cycle. The rats had free access to laboratory chow and water until the start of the experiment. Experiments were performed between 09.00 and 12.00 h. The rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.). A Silastic catheter was inserted into a hepatic vein, using the transjugular hepatic vein cannulation technique (Iguchi et al., 1979) for collecting of blood samples.

2.2. Experimental protocol

The rats were placed in a stereotaxic apparatus 45 min after the cannulation procedure. Saline (1 μl) with or without 5×10^{-8} mol neostigmine was injected into the third cerebral ventricle, according to Pellegrino's atlas (Pellegrino and Cushman, 1967). Blood samples (0.5 ml) were obtained from the hepatic venous cannula 15 min before and 0, 10, 30, 60 and 120 min after the intracerebroventricular (i.c.v.) injection. To minimize the potential influence of blood withdrawal, an equal amount of saline was administered via the hepatic venous cannula each time blood was withdrawn.

The rats were laparotomized after each experiment and the location of the tip of the hepatic venous cannula was verified by inspection and palpation. They were then killed by decapitation, and their brains were fixed in 10% neutral formaldehyde solution, embedded in paraffin, and stained. The site of the injection was then examined by microscopy of histological sections.

The protocol was reviewed and approved by the Laboratory Animal Research Committee of Nagoya University School of Medicine.

2.2.1. Effects of phentolamine on endogenous adrenaline-induced inhibition of insulin release

The rats were pretreated with an i.p. injection of 0.2 ml saline with or without phentolamine in doses of 5×10^{-8}, 5×10^{-7}, and 1×10^{-6} mol 15 min before injection of neostigmine. The plasma levels of immunoreactive insulin and glucose were measured. In a separate group of rats, in order to determine whether the rise in insulin levels observed after phentolamine is partially due to direct parasympathetic neural drive to the pancreas, the rats received an i.p. injection of 1×10^{-8} or 1×10^{-7} mol of atropine together with 10^{-6} mol of phentolamine 15 min before the injection of neostigmine and plasma levels of insulin, glucose, adrenaline, and noradrenaline were determined. Blood samples for determinations of insulin and catecholamines were obtained from different groups of rats.

2.2.2. Effects of other adrenoceptor antagonists and imidazoline derivatives on endogenous adrenaline-induced inhibition of insulin release

The rats were pretreated with an i.p. injection of 0.2–0.4 ml of saline or distilled water mixed with one of the following agents 15 min before the injection of neostigmine: yohimbine in doses of 5×10^{-8}, 5×10^{-7}, and 1×10^{-6} mol; idazoxan and antazoline in doses of 1×10^{-6} and 1×10^{-5} mol; and tolazoline, phenoxybenzamine, prazosin, or propranolol in a dose of 1×10^{-6} mol. Plasma levels of insulin and glucose were determined.

2.2.3. Effects of adrenoceptor antagonists and imidazoline derivatives administered before i.c.v. injection of saline on insulin release

The rats received i.p. injections of phentolamine and yohimbine in a dose of 1×10^{-6} mol, and idazoxan or antazoline in a dose of 1×10^{-5} mol 15 min before i.c.v. injection of 1 μl of saline. Plasma levels of insulin and glucose were measured.

2.3. Assays

The plasma concentration of glucose was determined enzymatically using a YSI 23A glucose autoanalyzer (Yellow Springs Instrument Co., Yellow Springs, OH, USA). The plasma level of insulin was assayed by the double-antibody radioimmunoassay method with a

commercial insulin assay kit (SRL, Tokyo, Japan) using rat insulin as the standard. The sensitivity of the insulin assay was 100 pg/ml. The intra- and inter-assay coefficients of variation were 7.9 and 8.5%, respectively. The plasma levels of adrenaline and noradrenaline were determined by high-performance liquid chromatography using an electrochemical detector (Iguchi et al., 1986).

2.4. Drugs

Neostigmine, phenoxybenzamine hydrochloride, tolazoline hydrochloride, propranolol hydrochloride, prazosin hydrochloride, yohimbine hydrochloride, idazoxan hydrochloride, antazoline hydrochloride, and atropine sulfate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Phentolamine mesylate (Regitine) was obtained from Ciba-Geigy (Hyogo, Japan).

2.5. Data analysis

The data are the means ± S.E. and were analyzed by factorial analysis of variance or analysis of variance with repeated measures including a grouping factor (Drug) and a within factor (Time). Post-hoc, pairwise comparisons were performed using Fisher's protected least-significant difference method only when the Drug × Time interaction was significant. A difference of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of phentolamine on endogenous adrenaline-induced inhibition of insulin release

No increase in insulin secretion was observed despite the presence of marked hyperglycemia in control rats preinjected with saline (Fig. 1). Pretreatment with phentolamine increased insulin secretion in a dose-dependent manner. Insulin levels were significantly higher in rats pretreated with 5×10^{-7} and 1×10^{-6} mol of phentolamine than in saline-treated controls. Hyperglycemia was significantly suppressed in all phentolamine-treated groups.

When 1×10^{-7} mol of atropine was administered concomitantly with 1×10^{-6} mol of phentolamine, the phentolamine-induced increase in insulin secretion was significantly suppressed (Fig. 2). The plasma level of glucose was higher at 120 min in the atropine-treated groups than in the control group, but the difference was not significant.

Adrenaline secretion increased significantly in all three groups after injection of neostigmine (Table 1). Pretreatment with phentolamine or the combination of phentolamine and atropine had no effect on the neostigmine-induced increase in adrenaline. Noradrenaline also increased 4- to 5-fold above the baseline value after injection of neostigmine. Phentolamine or the combination of phentolamine and atropine did not influence this increase.

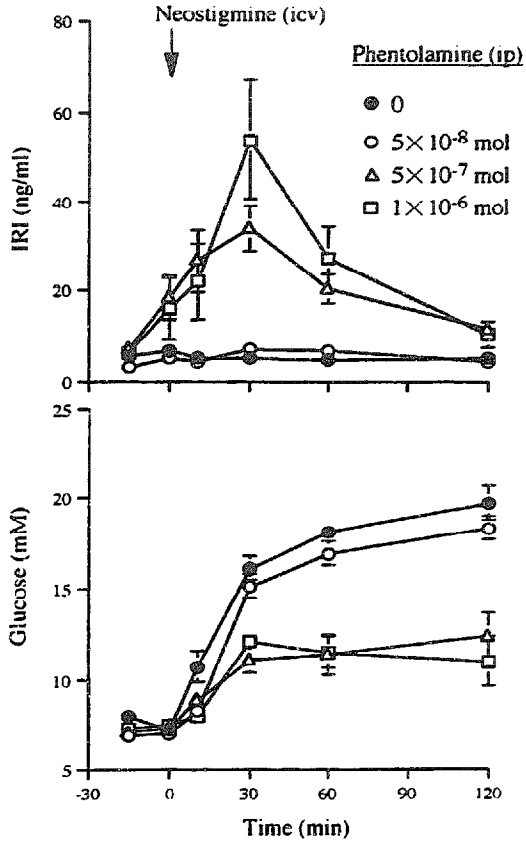

Fig. 1. Effects of phentolamine on the hepatic venous plasma levels of insulin and glucose after intracerebroventricular (i.c.v.) injection of neostigmine (5×10^{-8} mol/l μl saline). Saline (0.2 ml) was injected i.p. with or without phentolamine (5×10^{-8}, 5×10^{-7}, and 1×10^{-6} mol) at −15 min. Neostigmine was injected at 0 min. Values are expressed as the means ± S.E. for 5–9 rats. Statistically significant differences from the saline-treated control were observed in the 5×10^{-7} ($P < 0.0001$) and 1×10^{-6} mol ($P < 0.0001$) groups with regard to insulin and in the 5×10^{-8} ($P = 0.0353$), 5×10^{-7} ($P < 0.0001$) and 1×10^{-6} mol ($P < 0.0001$) groups for glucose (Fisher's protected least-significant difference (PLSD) method after analysis of variance (ANOVA) with repeated measures).

3.2. Effects of other adrenoceptor antagonists and imidazoline derivatives on endogenous adrenaline-induced inhibition of insulin release

Pretreatment with 1×10^{-6} mol of yohimbine, but not 5×10^{-8} or 1×10^{-7} mol, significantly increased insulin secretion (Fig. 3) and significantly reduced the hyperglycemia induced by neostigmine.

Tolazoline, a non-selective α-adrenoceptor antagonist, significantly increased the insulin levels (Table 2). Idazoxan, which is an imidazoline α_2-adrenoceptor an-

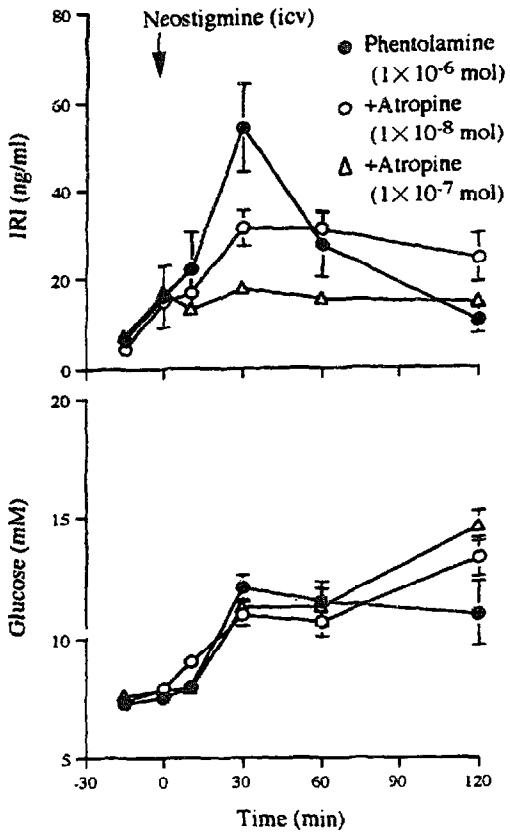

Fig. 2. Effects of coadministration of atropine with phentolamine on the hepatic venous plasma levels of insulin and glucose after i.c.v. injection of neostigmine (5×10^{-8} mol/1 μl saline). Phentolamine (1×10^{-6} mol/0.2 ml saline) with or without atropine (1×10^{-8} and 1×10^{-7} mol) was administered i.p. at −15 min and neostigmine was injected at 0 min. Values are expressed as the means ± S.E. for 6–8 rats. A statistically significant difference from the phentolamine alone group was observed in the 1×10^{-7} mol group ($P = 0.0500$) with regard to insulin (Fisher's PLSD method after ANOVA with repeated measures).

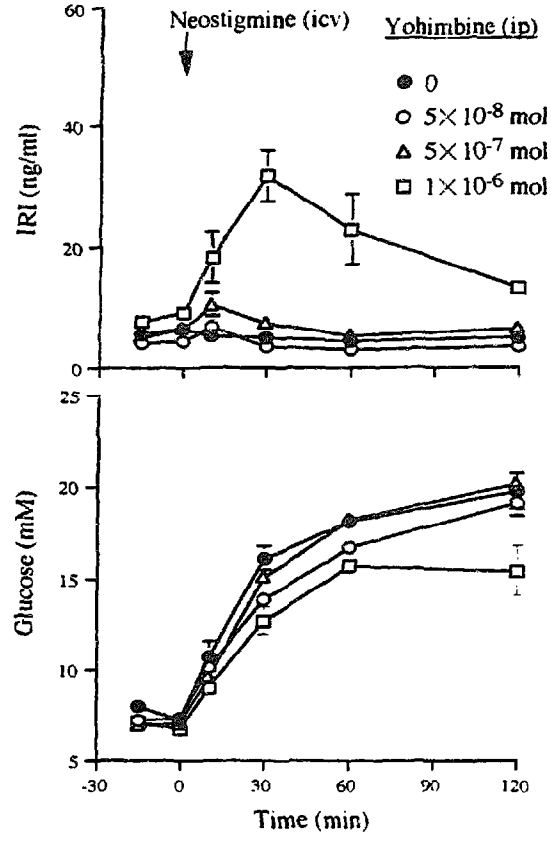

Fig. 3. Effects of yohimbine on the hepatic venous plasma levels of insulin and glucose after i.c.v. injection of neostigmine (5×10^{-8} mol/1 μl saline). Distilled water (0.2 ml) with yohimbine (5×10^{-8}, 5×10^{-7}, and 1×10^{-6} mol) was administered i.p. at −15 min and neostigmine was injected at 0 min. Values are expressed as the means ± S.E. for 5–7 rats. A statistically significant difference from the saline-treated control was observed in the 1×10^{-6} mol group with regard to both insulin ($P < 0.0001$) and glucose ($P = 0.0003$) (Fisher's PLSD method after ANOVA with repeated measures).

tagonist, significantly increased insulin secretion in a dose of 1×10^{-5} mol, which was 10 times greater than the doses of yohimbine required to increase insulin secretion. Antazoline, which possesses an imidazoline structure but has little potential to block α-adrenoceptors, had no significant effect on insulin secretion even in a dose of 1×10^{-5} mol. Phenoxybenzamine, propranolol, and prazosin lacked any significant effect on insulin secretion.

Only tolazoline significantly suppressed the neostigmine-induced hyperglycemia (Table 3).

3.3. Effects of adrenoceptor antagonists and imidazoline derivatives on insulin release after i.c.v. injection of saline

When adrenoceptor antagonists and imidazoline derivatives were injected i.p. 15 min before injection of 1 μl of saline into the third cerebral ventricle, phentolamine and antazoline caused a slight but significant

Table 1
Effects of phentolamine with or without atropine on neostigmine-induced adrenaline secretion

Substance	Dose (mol)	n	Adrenaline (ng/ml)					
			−15 min	0 min	10 min	30 min	60 min	120 min
Neo (i.c.v.)	5×10^{-8}	8	0.02 ± 0.00	0.10 ± 0.07	2.80 ± 1.33	4.40 ± 1.07	4.10 ± 1.36	1.69 ± 0.58
+ Phent (i.p.)	1×10^{-6}	6	0.12 ± 0.14	0.20 ± 0.18	2.40 ± 0.51	6.05 ± 1.31	4.03 ± 0.65	3.53 ± 1.81
+ Phent (i.p.) and Atro (i.p.)	1×10^{-6} 1×10^{-7}	6	0.10 ± 0.02	0.09 ± 0.02	1.37 ± 0.33	4.54 ± 0.74	4.22 ± 0.75	2.41 ± 0.91

Phentolamine (Phent) with or without atropine (Atro) was injected i.p. 15 min before intracerebroventricular (i.c.v.) injection of neostigmine (Neo). Values are expressed as the means ± S.E. There is no significant difference between the three groups (Fisher's protected least-significant difference (PLSD) method after analysis of variance (ANOVA) with repeated measures).

increase in the plasma level of insulin (Table 4). The areas under the insulin curves were 1777 ± 144 ng/ml/min for saline, 2652 ± 209 for phentolamine, 1529 ± 99 for yohimbine, 1641 ± 129 for idazoxan, and 2328 ± 220 ng/ml/min for antazoline. The values for phentolamine and antazoline were significantly higher than the value for saline (factorial analysis of variance with Fisher's protected least-significant difference method). Glucose levels were unchanged after the i.c.v. injection of saline, with or without pretreatment with the agents tested (Table 4).

4. Discussion

Central cholinergic stimulation activates the sympathoadrenal system as well as the parasympathetic neurons, which in turn stimulate the release of insulin in adrenalectomized rats (Gotoh et al., 1989). In intact rats, insulin release was completely suppressed by adrenaline secretion (Iguchi et al., 1988).

In the present study, the phentolamine-induced increase in insulin secretion following the injection of neostigmine was suppressed by i.p. injection of at-

Table 2
Effects of adrenoceptor antagonists and imidazoline derivatives on endogenous adrenaline-induced inhibition of insulin secretion caused by central nervous system stimulation

Substance	Dose (mol)	n	Insulin (ng/ml)					
			− 15 min	0 min	10 min	30 min	60 min	120 min
Saline		9	5.6 ± 0.5	6.5 ± 0.5	5.2 ± 0.5	5.1 ± 0.4	4.5 ± 0.3	4.9 ± 0.3
Tolazoline [a]	1×10^{-6}	6	3.2 ± 0.2	5.1 ± 0.7	9.7 ± 2.3	20.3 ± 8.0	19.4 ± 10.6	8.6 ± 2.8
Idazoxan	1×10^{-6}	8	5.7 ± 0.3	9.2 ± 0.9	8.2 ± 0.7	10.7 ± 1.3	5.2 ± 0.4	3.4 ± 0.4
Idazoxan [a]	1×10^{-5}	7	7.5 ± 0.4	8.7 ± 0.8	19.0 ± 2.5	45.3 ± 6.1	43.3 ± 8.3	17.0 ± 4.2
Antazoline	1×10^{-6}	6	7.6 ± 0.6	7.2 ± 0.5	7.5 ± 0.8	4.2 ± 0.5	3.2 ± 0.2	3.2 ± 0.3
Antazoline	1×10^{-5}	6	6.6 ± 1.7	9.5 ± 1.0	7.8 ± 1.3	5.9 ± 1.1	3.5 ± 0.7	3.7 ± 0.9

Adrenoceptor antagonists or imidazoline derivatives were injected i.p. 15 min before i.c.v. injection of neostigmine. Values are expressed as the means ± S.E. [a] $P < 0.05$ vs. saline-treated control (Fisher's PLSD method after ANOVA with repeated measures).

Table 3
Effects of adrenoceptor antagonists and imidazoline derivatives on central nervous system stimulation-mediated hyperglycemia

Substance	Dose (mol)	n	Glucose (mM)					
			− 15 min	0 min	10 min	30 min	60 min	120 min
Saline		9	8.3 ± 0.3	7.2 ± 0.4	10.7 ± 0.8	16.1 ± 0.6	18.1 ± 0.5	19.7 ± 0.9
Tolazoline [a]	1×10^{-6}	6	7.1 ± 0.2	6.9 ± 0.1	8.8 ± 0.3	14.2 ± 0.6	15.4 ± 0.6	15.9 ± 0.6
Idazoxan	1×10^{-6}	8	7.7 ± 0.2	8.1 ± 0.2	8.7 ± 0.2	13.0 ± 0.5	17.1 ± 0.7	20.6 ± 1.0
Idazoxan	1×10^{-5}	7	8.8 ± 0.3	8.8 ± 0.3	10.0 ± 0.3	14.1 ± 0.8	19.9 ± 0.8	23.3 ± 0.5
Antazoline	1×10^{-6}	6	7.9 ± 0.3	7.2 ± 0.4	10.7 ± 0.8	16.1 ± 0.6	18.1 ± 0.5	19.7 ± 0.9
Antazoline	1×10^{-5}	6	8.3 ± 0.3	7.2 ± 0.4	10.7 ± 0.8	16.1 ± 0.6	18.1 ± 0.5	19.7 ± 0.9

The experimental procedure is described in Table 2. Values are expressed as the means ± S.E. [a] $P < 0.05$ vs. saline-treated control (Fisher's PLSD method after ANOVA with repeated measures).

Table 4
Effects of adrenoceptor antagonists and imidazoline derivatives on insulin secretion and plasma glucose levels in i.c.v. saline-injected control rats

Substance	Dose (mol)	n	Insulin (ng/ml)					
			− 15 min	0 min	10 min	30 min	60 min	120 min
Saline		10	6.5 ± 0.4	4.7 ± 0.5	5.9 ± 0.7	6.6 ± 0.6	7.0 ± 0.9	7.5 ± 1.1
Phentolamine [a]	1×10^{-6}	12	7.9 ± 0.9	11.1 ± 1.5	17.9 ± 2.6	14.4 ± 2.1	22.7 + 3.1	13.2 ± 1.2
Yohimbine	1×10^{-6}	12	4.8 ± 0.2	6.8 ± 0.9	5.7 ± 0.5	4.8 ± 0.5	7.8 ± 1.2	8.0 ± 1.0
Idazoxan	1×10^{-5}	12	6.4 ± 0.5	7.1 ± 0.9	7.7 ± 0.6	7.7 ± 0.5	5.7 ± 1.0	5.3 ± 0.9
Antazoline [a]	1×10^{-6}	12	7.4 ± 0.9	10.1 ± 1.8	12.8 ± 1.6	10.4 ± 1.7	10.1 ± 2.0	10.2 ± 1.0
Substance	Dose (mol)	n	Glucose (mM)					
			− 15 min	0 min	10 min	30 min	60 min	120 min
Saline		10	8.0 ± 0.1	8.1 ± 0.1	8.5 ± 0.2	8.4 ± 0.2	8.1 ± 0.1	8.6 ± 0.2
Phentolamine	1×10^{-6}	12	8.2 ± 0.2	7.8 ± 0.1	8.5 ± 0.2	8.3 ± 0.1	8.7 ± 0.1	8.6 ± 0.2
Yohimbine	1×10^{-6}	12	8.2 ± 0.2	8.2 ± 0.1	8.1 ± 0.1	8.1 ± 0.2	8.3 ± 0.2	8.2 ± 0.2
Idazoxan	1×10^{-5}	12	8.0 ± 0.1	8.3 ± 0.1	8.4 ± 0.1	8.2 ± 0.2	8.5 ± 0.2	8.7 ± 0.3
Antazoline	1×10^{-6}	12	8.4 ± 0.3	7.9 ± 0.2	8.3 ± 0.1	8.0 ± 0.3	8.6 ± 0.2	8.8 ± 0.2

Adrenoceptor antagonists or imidazoline derivatives were injected i.p. 15 min before saline injection into the third cerebral ventricle. Values are the means ± S.E. [a] $P < 0.05$ vs. saline-treated control (Fisher's PLSD method after ANOVA with repeated measures).

ropine, which is consistent with our previous finding. Neither phentolamine nor the combination of phentolamine and atropine influenced catecholamine secretion, suggesting that both agents acted directly on the pancreas.

Yohimbine, a non-imidazoline selective α_2-adrenoceptor antagonist, also increased insulin secretion but to a lesser degree than phentolamine, suggesting that phentolamine's effect depended on its α_2-adrenoceptor blocking activity. Idazoxan, an imidazoline α_2-adrenoceptor antagonist, increased insulin secretion, but antazoline, which has an imidazoline structure but little α_2-adrenoceptor blocking activity, did not reverse the adrenaline-induced inhibition of insulin secretion, even in doses 10 times higher than the doses of phentolamine or yohimbine.

Phenoxybenzamine had no effect on insulin secretion although tolazoline, which is an imidazoline derivative, increased insulin secretion. Both agents are considered to be classic non-selective α-adrenoceptor antagonists and phenoxybenzamine has been found to block the noradrenaline-induced inhibition of insulin secretion (Roy et al., 1984). However, phenoxybenzamine is more selective for α_1-adrenoceptors (Doxey et al., 1977; Wood et al., 1979) and has been found to be less potent than phentolamine or yohimbine to reverse adrenaline-induced inhibition of insulin release in in vitro (Nakaki et al., 1981) and in vivo (Nakadate et al., 1980) studies. We hypothesize that phenoxybenzamine did not reverse the adrenaline-induced inhibition of insulin release, because its antagonistic effect on α_2-adrenoceptors of β-cells was not sufficient to cause reversal of the effect of endogenously secreted adrenaline and not because it lacked an imidazoline structure.

The relative potencies of the agents now examined to reverse the adrenaline-induced inhibition of insulin secretion were as follows: phentolamine > yohimbine > tolazoline > idazoxan. Antazoline, phenoxybenzamine, prazosin, and propranolol had no effect on adrenaline-induced inhibition of insulin release. Jonas et al. (1992) demonstrated that imidazolines antagonized the effects of clonidine on α_2-adrenoceptors in mouse islets in the following order: phentolamine > yohimbine > antazoline > tolazoline. Except for antazoline, our results are similar to theirs. Antazoline is generally classified as a histamine receptor antagonist and was previously found not to antagonize the effect of clonidine in mouse β-cells (Schulz and Hasselblatt, 1989).

The release of insulin from the pancreas is stimulated by the direct action of glucose on β-cells. However, our present and previous results indicate that neostigmine-induced insulin secretion is not secondary to an increased release of glucose. When neostigmine was injected into the third cerebral ventricle in fasting adrenalectomized rats, insulin release increased significantly in the absence of an increase in the plasma level of glucose (Gotoh et al., 1989). In the present study, i.p. injection of atropine had no significant effect on the plasma level of glucose but was associated with a decrease in insulin secretion.

Antazoline increased insulin levels slightly but significantly in rats treated with an i.c.v. injection of saline, because under these conditions the release of adrenaline was not stimulated. In rats treated with an i.c.v. injection of neostigmine, neostigmine-induced adrenaline release inhibited the effect of antazoline on insulin release and its insulin-releasing property was cancelled. The mechanism by which phentolamine and antazoline enhanced insulin secretion in rats treated with an i.c.v. injection of saline could not be established with our experimental protocol. However, both agents have an imidazoline structure which is probably involved in stimulation of insulin release. Idazoxan also has an imidazoline structure but failed to increase insulin levels. However, this finding is consistent with the results of previous studies, which showed that antazoline (Schulz and Hasselblatt, 1989; Berdeu et al., 1994) but not idazoxan (Berdeu et al., 1994) potentiated the release of insulin. The increase in insulin secretion induced by phentolamine and antazoline in rats treated with i.c.v. saline was less than that induced by the α_2-adrenoceptor antagonists, phentolamine and yohimbine, in neostigmine-treated rats.

In conclusion, imidazoline receptor antagonists of α_2-adrenoceptors reversed the endogenous adrenaline-induced inhibition of insulin release caused by i.c.v. injection of neostigmine by blocking α_2-adrenoceptors in anesthetized rats. These agents also increased insulin release independently of their adrenoceptor blocking activity in rats treated with an i.c.v. injection of saline, but their effect was less potent and was overcome by the inhibitory effect of adrenaline in the presence of neostigmine-induced stimulation of the sympathoadrenal system.

Acknowledgments

The authors thank Miss K. Mizuno for her technical and secretarial assistance.

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