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The CB1 Antagonist Rimonabant Decreases Insulin Hypersecretion in Rat Pancreatic Islets

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Abstract

Type 2 diabetes and obesity are characterized by elevated nocturnal circulating free fatty acids, elevated basal insulin secretion, and blunted glucose-stimulated insulin secretion (GSIS). The CB1 receptor antagonist, Rimonabant, has been shown to improve glucose tolerance and insulin sensitivity *in vivo* but its direct effect on islets has been unclear. Islets from lean littermates and obese Zucker (ZF) and Zucker Diabetic Fatty (ZDF) rats were incubated for 24 h *in vitro* and exposed to 11 mmol/l glucose and 0.3 mmol/l palmitate (GL) with or without Rimonabant. Insulin secretion was determined at basal (3 mmol/l) or stimulatory (15 mmol/l) glucose concentrations. As expected, basal secretion was significantly elevated in islets from obese or GL-treated lean rats whereas the fold increase in GSIS was diminished. Rimonabant decreased basal hypersecretion in islets from obese rats and GL-treated lean rats without decreasing the fold increase in GSIS. However, it decreased GSIS in islets from lean rats without affecting basal secretion. These findings indicate that Rimonabant has direct effects on islets to reduce insulin secretion when secretion is elevated above normal levels by diet or in obesity. In contrast, it appears to decrease stimulated secretion in islets from lean animals but not in obese or GL-exposed islets.

INTRODUCTION

The endocannabinoid system is a recently characterized endogenous signaling system that plays an important role in the integrated regulation of energy balance, feeding behavior, hepatic lipogenesis, and glucose homeostasis (1–5). The endocannabinoid system is overactive in human obesity (6–9) and in animal models of genetic and diet-induced obesity (10,11). Activation of the cannabinoid receptor CB1 by the endogenous cannabinoid receptor ligands anandamide (*N*-arachidonyl ethanolamine) and 2-AG (2-arachidonyl glycerol), both centrally and peripherally, favors metabolic processes that lead to weight gain, lipogenesis, insulin resistance, dyslipidemia, and impaired glucose homeostasis (12–16). Thus, the endocannabinoid system may be a novel mechanistic pathway for modulating cardiometabolic processes, energy intake and expenditure, and lipid and glucose metabolism.

Type 2 diabetes and obesity are characterized by elevated nocturnal circulating free fatty acids, elevated basal insulin secretion, and blunted glucose-stimulated insulin secretion (GSIS) (17). Exposure of rodent islets or β -cells to prolonged elevation of free fatty acids and high glucose recapitulates the increased basal and blunted GSIS characteristic of obesity (18). The CB1 receptor antagonists have been shown to improve glucose tolerance and

insulin sensitivity *in vivo* in both animal models (19,20) and humans (21,22), by regulating energy balance and metabolism through peripheral targets, such as adipose tissue (23). It has been proposed that the drug's effectiveness is due, at least in part, to the upregulated endocannabinoid system in obesity and type 2 diabetes (5,6).

It is still unknown whether the improvement in insulin resistance is also due to an effect of CB1 receptor antagonists on islet physiology. Cannabinoid CB1 and CB2 receptors have been identified in isolated mouse, rat, and human pancreatic islets, with CB1 receptors mainly expressed in non- β -cells, and CB2 receptors expressed in both β - and non- β -cells (24–27). It has also been shown, in a paper by Bermudez-Silva *et al.*, that a selective cannabinoid CB1 receptor agonist, arachidonyl-22-chloroethylamide caused glucose intolerance after a glucose load in male Wistar rats (28), suggesting that the CB1 receptor also plays a role in the regulation of insulin secretion. In contrast to the paper by Juan-Pico *et al.* (24), Nakata and Yada have recently reported mRNA for the CB1 receptor, but not the CB2 receptor, expressed in mouse pancreatic islets, and a further immunohistochemical study found the CB1 receptor expressed in β -cells (29). The basis for these discrepancies is not known; however, due to interactions among the different cell types of the islet through hormones and other secreted factors, it is possible that insulin secretion could be modified either directly via the β -cell or indirectly by acting on one of the other islet cell types (30). There is general agreement that endocannabinoids influence insulin secretion (5). The critical issue is how CB1 receptor antagonism influences insulin secretion by the islet in response to obesity and fuel excess.

To determine whether the CB1 receptor antagonist Rimonabant affected basal or stimulated insulin secretion, we studied isolated islets from lean siblings and obese Zucker (ZF) and Zucker Diabetic Fatty (ZDF) rats that were incubated for 24 h *in vitro* and exposed to 11 mmol/l glucose plus 0.3 mmol/l palmitate (GL) with or without Rimonabant. Insulin secretion was determined during incubation at basal or stimulatory glucose. As expected, basal secretion was significantly elevated in islets from obese or GL-treated lean rats whereas the fold increase in GSIS was diminished.

METHODS AND PROCEDURES

Animals

Islets were isolated from 7- to 11-week-old male ZF and Zucker diabetic rats and their lean siblings. The abbreviations used for lean siblings of the obese (153–353 g) and obese diabetic (178–396 g) are ZL and ZL-D, respectively. The abbreviations used for the Zucker obese (312–415 g) and Zucker Diabetic Fatty (260–340 g) rats are ZF and ZDF, respectively. The animals were housed in the Laboratory Animal Science Center at Boston University Medical Center. The experimental protocol was approved by the “Institutional Animal Care and Use Committee” at Boston University Medical Center. The animals were fed normal rat chow and water *ad libitum* until time of sacrifice.

Materials

The islet isolating buffer consisted of Hank's balanced salt solution (GIBCO, Billings, MT) containing 20 mmol/l HEPES (GIBCO) and 0.1% bovine serum albumin (fatty acid free; Serologicals, Pensacola, FL) at pH 7.4. Collagenase, type 4, was purchased from Worthington Biochemical (Lakewood, NJ). The islet cell culture media was RPMI 1640 (GIBCO) containing glucose (11 mmol/l), penicillin–streptomycin (5,000 U penicillin/ml, 5 mg streptomycin/ml; GIBCO). The islet secretion buffer consisted of Krebs buffer containing: 119 mmol/l NaCl, 20 mmol/l HEPES, 4.6 mmol/l KCl, 1 mmol/l MgSO₄, 0.15

mmol/l Na_2HPO_4 , 0.4 mmol/l KH_2PO_4 , 5 mmol/l NaHCO_3 , 2 mmol/l Ca^{2+} , and 0.05% bovine serum albumin.

Islet isolation

Pancreatic islets were isolated as previously described (31), hand-picked, and cultured overnight in islet cell culture media containing 11 mmol/l glucose and 10% fetal bovine serum (HyClone, Logan, UT).

Islet incubations

Following overnight culture, islets were again hand-picked and cultured for 24 h in islet cell culture media containing 11 mmol/l glucose, 1% fetal bovine serum and the following conditions: control (media only), Rimonabant (media + 1 $\mu\text{mol/l}$ Rimonabant), GL (media + 0.3 mmol/l palmitate), GL+Rimonabant (media + 1 $\mu\text{mol/l}$ Rimonabant + 0.3 mmol/l palmitate). Palmitate was bound to bovine serum albumin in a ratio of 1:3. Twenty-four-hour incubations were chosen because that time is sufficient to cause both recovery from the stress of collagenase isolation of islets, GL-induced basal hypersecretion, and GL-induced impairment of GSIS. The Rimonabant concentration of 1 $\mu\text{mol/l}$ was chosen to give significant inhibition of insulin secretion. This relatively high concentration, relative to its K_D for CB1 receptors (10 nmol/l), was probably due to poor penetrance of the drug into the -cell rich islet interior.

Insulin secretion assay

After the 24-h incubation, islets were hand-picked into micro-centrifuge tubes, washed, and incubated in islet secretion buffer under the following conditions: basal (3 mmol/l glucose), stimulated (15 mmol/l glucose), or stimulated + Rimonabant (15 mmol/l glucose + 1 $\mu\text{mol/l}$ Rimonabant) for 30 min at 37 °C. Insulin secretion was determined by enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH).

Statistics

Values are reported as mean $\pm$ s.e.m. of three independent experiments. Comparisons were made with Statview version 5.0.1 (SAS Institute, Cary, NC). Statistical significance was found using a two-way ANOVA. Fisher's PLSD post hoc test was employed for ANOVA results found to be significant ($P < 0.05$).

RESULTS

Comparison of islets from ZF and ZDF rats and their lean siblings

Islets from ZF and ZDF rats and their respective controls were isolated and incubated overnight then treated (or not) for 24 h. As expected, islets from ZF and ZDF rats exhibited basal insulin hypersecretion and a blunted 4.2- or 4.9-fold increase in GSIS compared to an 8.7- or 9.3-fold increase in lean ZL or ZL-D rat islets (Table 1) consistent with previous publications (32,33). Secretion from islets derived from lean littermates of ZF and diabetic fatty rats (ZL and ZL-D) were very similar with the exception that exposure to elevated glucose and lipid (GL) had no significant effect in the ZD-L (Table 1). Likewise, secretion from islets derived from ZF and ZDF rats were also quite similar with a tendency toward more secretion and slightly less effect of GL and Rimonabant on ZDF islets (Table 1). Therefore, in the following sections, data from the two lean and two obese models were pooled.

Effect of 24-h exposure to GL on insulin secretion

Islets from lean rats exhibited increased basal insulin secretion in response to GL treatment (Figure 1a). Unexpectedly, in hypersecreting obese rat islets, GL treatment lowered basal insulin hypersecretion (Figure 1b). GSIS was significantly decreased in GL-treated islets from lean rats (Figure 2a) and paradoxically increased in islets from the obese models (Figure 2b).

Effect of 24-h exposure to Rimonabant on lean and obese rat islet insulin secretion

In untreated islets from lean rats, Rimonabant did not affect basal insulin secretion (Figure 1a) but inhibited GSIS (Figure 2a). In obese rat islets, Rimonabant decreased basal insulin hypersecretion (Figure 1b) but did not affect the already blunted fold increase in GSIS (Figure 2b).

Effect of 24-h exposure to Rimonabant on GL-treated islets

In GL-treated lean rat islets, in contrast to the controls, Rimonabant significantly decreased basal insulin hypersecretion (Figure 1a) and increased the fold GSIS (Figure 2a). In GL-treated obese rat islets, Rimonabant had no further effect on the already lowered basal insulin hypersecretion; however, Rimonabant did slightly diminish the fold GSIS (Figure 2b).

Effect of acute Rimonabant treatment on insulin secretion

The preceding insulin secretion data with Rimonabant were obtained in the absence of Rimonabant during the 30-min collection period. Exposure to Rimonabant during the 30-min collection period lowered GSIS in untreated lean and obese rat islets, but had no further effect on lowering GSIS when Rimonabant had already been present for 24 h (data not shown).

Discussion

Insulin resistance is a prominent feature of obesity, metabolic syndrome, and type 2 diabetes. In human studies, the CB1 receptor antagonist Rimonabant decreases insulin levels without impairing glucose homeostasis indicating an improvement in insulin sensitivity (34–36). We found that Rimonabant directly influenced islet insulin secretion. The drug decreased basal insulin hypersecretion due to obesity or high glucose plus lipid treatment without further impairing GSIS. These findings indicate that Rimonabant has direct effects on islets to influence insulin secretion when secretion is elevated above normal levels as it is in obesity and insulin resistance. This unique feature is expected to be beneficial in states of hypersecretion. Our findings are also consistent with animal observations *in vivo* (14) and could, at least partially, explain the ability of the drug to lower insulin levels while improving glycemic status. Thus an important and novel benefit of Rimonabant is to improve glycemic control by decreasing basal hyperinsulinemia without impairing responsiveness to stimulatory glucose.

Hyperinsulinemia causes and maintains insulin resistance just as insulin resistance causes and maintains insulin hypersecretion. Because of this interdependency, it is impossible in most cases to determine which came first. There are no compelling data proving that either hyperinsulinemia or insulin resistance is the primary event: both usually coexist. Type 2 diabetes (T2D) occurs when the β -cell fails to compensate for insulin resistance. It is therefore unlikely that stimulation of the β -cell, the current therapeutic approach to early T2D, can provide long-term benefit, as it adds stress to an already hyperactive β -cell; conversely, decreasing insulin secretion at an early stage may be of value. Sustained basal elevation in insulin secretion develops through a mechanism that differs from the well-

established consensus pathway for fuel-stimulated secretion and this mechanism has been inadequately investigated, unlike the well-established molecular events accompanying insulin resistance. It appears likely that Rimonabant impacts on the yet unstudied pathway involved in basal insulin hypersecretion.

The ability of Rimonabant to decrease basal insulin hypersecretion occurs in response to both chronic and acute drug addition. The response is also sustained after drug removal following 24-h exposure and the subsequent further addition of Rimonabant acutely does not further decrease hypersecretion. This suggests that a maximum response is achieved rapidly, is sustained at least for an hour after drug removal, and not altered by additional drug exposure. The temporal details of the Rimonabant signaling cascade after binding with the CB1 receptor are not known. In fact, it has not been proven that the observed effects of Rimonabant are due to interaction with the CB1 receptor, although other targets are not known.

In contrast, Rimonabant appears to impair stimulated secretion in islets from lean animals but not obese or GL-exposed. However, the impairment in isolated islets is not reflected in either human or animal studies and would likely be prevented by counter-regulatory hormones *in vivo*.

Unexpectedly, the effect of GL on ZF and ZDF rats is to lower basal hypersecretion while actually improving GSIS, similar to that of Rimonabant. The mechanism for this contrary response in the obese models is not known. Possibilities include induction of lipid metabolizing enzymes, an increased ability to metabolize fat vs. carbohydrates, or a requirement for high fat to generate signals in the obese models compared to lean models. It should also be noted that in a high fat feeding study in Zucker fatty rats, insulin levels were lower *in vivo* in the fat fed than in the carbohydrate fed animals (37), indicating that this unusual feature characterizes the Zucker models both *in vivo* and *in vitro*.

In summary, Rimonabant lowered basal insulin secretion *in vitro* without impairing GSIS in basal hypersecreting rat models through a direct islet effect. This unique mechanism is predicted to improve insulin sensitivity and diminish stress on overactive β -cells in both obesity and T2D.

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DISCLOSURE

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Figure 1. Basal insulin secretion (3 mmol/l glucose) from islets isolated from obese Zucker rats and their lean siblings. **(a)** Islets from lean rats; **(b)** islets from obese rats. Secretion was measured for 30 min in 3–4 groups of six islets for each of three separate experiments after 24-h incubation at 11 mmol/l glucose in the presence or absence of 1 μmol/l Rimonabant (Rim) and/or 0.3 mmol/l palmitate (GL). Data are expressed as means ± s.e.m. of three separate experiments. Significant differences, determined using two-way ANOVA, from the bar labeled “None” is indicated with an asterisk.

Figure 2.

Ratio of insulin secretion from islets incubated at 15 mmol/l: 3 mmol/l glucose. **(a)** Islets from lean rats; **(b)** islets from obese rats. Secretion was measured for 30 min in 3–4 groups of six islets as described in Figure 1. Significant differences, determined using two-way ANOVA, from the bar labeled “None” is indicated with an asterisk, from the bar labeled GL is indicated with double asterisks.

Table 1

Insulin secretion from islets isolated from Zucker rats and their lean siblings

Treatment	Glucose (mmol/l)	Insulin secretion (ng/ μ l/6 islets)			
		ZL	ZL-D	ZF	ZDF
None	3	1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	7.7 $\pm$ 0.9	7.1 $\pm$ 0.1
	15	9.6 $\pm$ 0.7	10.1 $\pm$ 1.5	32.1 $\pm$ 2.9	34.6 $\pm$ 5.9
Rim	3	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1	3.5 $\pm$ 0.6	5.2 $\pm$ 0.1
	15	5.9 $\pm$ 0.6	5.9 $\pm$ 1.4	14.8 $\pm$ 1.4	22.1 $\pm$ 4.0
GL	3	2.3 $\pm$ 0.1	1.1 $\pm$ 0.1	3.2 $\pm$ 0.1	4.7 $\pm$ 0.1
	15	7.3 $\pm$ 0.4	7.7 $\pm$ 1.2	22.5 $\pm$ 5.5	24.5 $\pm$ 6.1
GL+Rim	3	1.4 $\pm$ 0.2	1.0 $\pm$ 0.1	3.8 $\pm$ 0.6	4.6 $\pm$ 0.5
	15	8.0 $\pm$ 0.9	8.2 $\pm$ 1.4	19.4 $\pm$ 4.8	20.9 $\pm$ 5.1

Islets were obtained from ZL, ZL-D, ZF, ZDF, Zucker diabetic fatty rats; ZF, Zucker fatty rats; ZL, lean siblings of Zucker fatty rats; ZL-D, lean siblings of Zucker diabetic fatty rats; Rim, 1 μ mol/l Rimonabant; GL, 11 mmol/l glucose plus 0.3 mmol/l palmitate.