Islet Cannabinoid Receptors

Cellular Distribution and Biological Function

Luis E. Flores, PhD, María E. Alzugaray, PhD,* Marisa A. Cubilla, PhD,† María A. Raschia, MS,* Héctor H. Del Zotto, MD, PhD,* Carolina L. Román, MS,* Ángela M. Suburo, MD, PhD,† and Juan J. Gagliardino, MD, PhD**

Objectives: This study aimed to determine the cellular distribution of islet cannabinoid receptors (CBs) and their involvement in the development of metabolic and hormonal changes in rats fed a fructose-rich diet (F).

Methods: In normal rat islets, we determined CBs (immunofluorescence and retrotranscription–polymerase chain reaction) and glucose-stimulated insulin secretion (GSIS) of isolated islets incubated with the CB1 antagonist rimonabant (R) and/or different CBs agonists. In 3-week F-fed rats, we determined the in vivo effect of R on serum glucose, triglyceride, and insulin levels; homeostasis model assessment for insulin resistance, GSIS, and CBs and insulin receptor substrate gene expression levels (real-time polymerase chain reaction).

Results: Cannabinoid receptors appeared exclusively in islet α cells. Whereas different CB agonists enhanced GSIS in normal rat islets, R did not affect it. F rats had higher serum triglyceride and insulin levels and homeostasis model assessment for insulin resistance than control rats; these alterations were prevented by R coadministration. Although R did not correct the increased GSIS observed in F islets, it modulated CBs and insulin receptor substrate gene expression.

Conclusions: Islet CBs would exert an important modulatory role in metabolic homeostasis. Administration of R and F affected islet CB expression and prevented the development of F-induced metabolic impairment. Selective islet CB1 blockers could be useful to prevent/treat the alterations induced by the intake of unbalanced/unhealthy diets.

Key Words: cannabinoid receptors, pancreatic islets, metabolic syndrome, β-cell function, dyslipidemia, metabolic homeostasis

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The endocannabinoid system comprises 2 cannabinoid receptor (CB) subtypes, CB1 and CB2, with specific endogenous ligands (anandamide [AEA] and 2-arachidonoylglycerol) as well as specific enzymes for ligand biosynthesis and degradation, that is, sn-1-selective diacylglycerol lipase-α, monoacylglycerol lipase, *N*-arachidonoyl phosphatidyl ethanolamine phospholipase D, and fatty acid amide hydrolase.[1]

It has been claimed that these receptors actively participate in the control of energy homeostasis,[2,3] an assumption supported by the transient reduction of food intake induced by the administration of a CB1 blocker, rimonabant (R), to either food-deprived lean or ad libitum-fed obese animals.[3–5] Further, published evidence strongly suggests that the endocannabinoid system exerts a tonic modulation on appetite and other metabolic functions.[3–5] In this sense, other reported data support this concept, namely, (*a*) the persistent weight loss induced by chronic CB1 blockade is independent from food intake inhibition[6,7] and (*b*) CB1-deficient mice have significantly less fat mass than wild-type mice and are not susceptible to develop obesity when fed with a high-fat diet.[8] Altogether, these findings suggest that the endocannabinoid system contributes positively to obesity development, probably via the up-regulation of liver receptors, as shown in mice with dietary-induced obesity.[9]

Obesity is accompanied by a decrease in insulin sensitivity and a compensatory increase in β-cell function/mass, with the consequent increase in serum insulin levels.[10] At early stages, a modest increase in serum glucose levels within reference range can also be observed[11]; at later stages and in people at risk, this situation leads to the development of overt type 2 diabetes (T2DM).[12] It is clear that obesity represents an overload of the β-cell function and contributes to its failure in subjects with genetic predisposition.[13] On the other hand, it is accepted that CB1 and CB2 are present in pancreatic islets, despite some controversies about their cellular distribution and their effect on insulin and glucagon secretion.[14–25] Conversely, the potential role of such receptors in the cross-talk between obesity (adipose tissue) and β-cell function in normal and pathological conditions is not completely clear as yet.

Administration of high sucrose or fructose (F) diets to normal rats induces early hormonal and metabolic changes that resemble the human prediabetes, diabetes, and metabolic syndrome profiles, that is, high serum insulin and triglyceride levels, insulin resistance (IR), impaired glucose tolerance, increased abdominal adipose tissue with impaired adipokine release, and decreased β-cell mass ascribed to an increased apoptosis rate.[26–30] Consequently, F might be a suitable model to answer the previously mentioned question.

In an attempt to obtain new evidence of the possible role of the cannabinoid system in the regulation of the β-cell secretory function and metabolic homeostasis, we studied the effect of F and R coadministration to normal rats for 3 weeks. Our findings demonstrate that (*a*) R administration prevents the development of multiple F-induced hormonal and metabolic disturbances, including hyperinsulinemia, and (*b*) in isolated pancreatic islets, the characterized functional CBs would play an active role in the mechanism by which R exerts such effect.

From the *CENEXA - Centro de Endocrinología Experimental y Aplicada (UNLP-CONICET LA PLATA, Centro Colaborador OPS/OMS para Diabetes), Facultad de Ciencias Médicas, UNLP, La Plata; and †Facultad de Ciencias Biomédicas, Universidad Austral, Pilar, Buenos Aires, Argentina.
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Reprints: Juan J. Gagliardino, MD, PhD, CENEXA - Centro de Endocrinología Experimental y Aplicada (UNLP-CONICET LA PLATA, Centro Colaborador OPS/OMS para Diabetes), Facultad de Ciencias Médicas, UNLP, 60 y 120, 1900 La Plata, Argentina (e-mail: cenexa@speedy.com.ar).
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LEF and MEA contributed equally to the development of this work.
LEF and JJG conceived and designed the study, performed the statistical analysis, and drafted the manuscript; LEF, MEA, MAR, and CLR carried out the experiments and metabolic determinations; HDZ, MAC, and AMS performed the histochemical and immunofluorescent studies. All authors read and approved the final manuscript.
LEF, AMS, HDZ, and JJG are members of the research career of CONICET. MAC is a fellow from CONICET and CLR is a fellow from the National University of La Plata. MAR and MEA were fellows from CONICET.
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MATERIALS AND METHODS

Chemicals and Drugs

Collagenase was obtained from Serva Feinbiochemica (Heidelberg, Germany); bovine serum albumin (BSA) fraction V, AEA, arachidonyl-2-chloroethylamide (ACEA), JWH-015, and other reagents were from Sigma Chemical Co (St Louis, Mo); R was from Sanofi-Aventis and F was obtained from Corn Products International Inc (Westchester, Ill).

Experimental Animals

Normal adult male Wistar rats (180–200 g bw) were kept in a temperature-controlled environment (23°C) on a fixed 12-hour light/dark cycle and fed ad libitum for 1 week (stabilization period) with a standard commercial diet (rat chow, Ganave; Argentina). This study protocol complies with the NIH guide for the care and use of laboratory animals (National Research Council, 1985).

CB1 and CB2 Immunohistochemical Detection in Normal Rat Pancreases

Normal rats were anesthetized and perfused with 4% paraformaldehyde in phosphate buffer. Pancreases were carefully dissected and cryopreserved in graded sucrose solutions and a sucrose-OCT compound mixture. Tissue blocks were frozen in N_2-cooled acetone and sectioned at 7 μm.

Cryosections were incubated with dilutions of our own guinea pig insulin antibody, rabbit anti-glucagon (kindly provided by Novo Nordisk, Copenhagen, Denmark), and/or anti-somatostatin antibody (a gift from Dr S. Efendic, Department of Endocrinology, Karolinska Institute, Stockholm, Sweden). CB1 was detected with 2 specific antibodies, OPA1-15297 (1:500; Affinity BioReagents, Golden, Co) when immunofluorescence was performed in triple combination with either insulin and glucagon or insulin and somatostatin, and p-CB1 (Ser 316): sc-17555 (1:50; Santa Cruz Biotechnology, Inc) in combination with insulin. Two different CB2 antibodies were used to detect CB2: PA1-746A CB2 (1:1000; Affinity BioReagents) in combination with either glucagon or somatostatin, and CB2 (M-15): sc-10076 (1:50; Santa Cruz Biotechnology, Inc) in combination with insulin.[23] The reaction was completed by incubation with anti-rabbit, -mouse, or -guinea pig antibodies conjugated with fluorescein isothiocyanate, lissamine rhodamine or Cy5 (Jackson Immunoresearch Laboratories, West Grove, Pa).

Confocal images were obtained with a Laser Scanning System Radiance 2000 (BioRad, Hemel Hempstead, UK) and a Confocal LEICA TCS SP5 AOBS microscope from the National University of La Plata Microscopy Platform. The 488 line of an argon laser, the 543 line of a helium-neon laser, and the 633-nm line from a diode laser were sequentially used. Cytoplasmic colocalization was evaluated in 1-μm-thick optical sections. Optic projections and merged images were produced with Confocal Assistant Software (BioRad). In all cases, images were obtained from 3 pancreases and at least 3 sections from 3 different levels of the block. Control samples omitting incubation with primary antibodies were run in parallel for every immunostaining procedure tested.

Glucose-Stimulated Insulin Secretion, Islet Insulin, and DNA Content

Pancreases from normal animals were removed to isolate islets by collagenase digestion.[31] Groups of 5 islets with similar shape and size were incubated for 60 minutes at 37°C in 0.6 mL Krebs-Ringer bicarbonate buffer, pH 7.4, previously gassed with a mixture of CO_2/O_2 (5%/95%), containing 1.5% (wt/vol) BSA and 3.3 or 16.7 mM glucose for glucose-stimulated insulin secretion (GSIS) determination. Under these conditions, we tested separately the effect of the endocannabinoid AEA (Sigma-Aldrich, 1–200 μM), of 2 different specific CB agonists: the specific CB1 agonist ACEA (0.1–20 μM, Sigma-Aldrich) and the specific CB2 ligand JWH-015 (CB2 agonist, Sigma-Aldrich, 0.1–20 μM), and R (CB1 antagonist, Sanofi-Aventis, France, 0.01–1 μM). We also studied the effect of the combination of R and AEA or ACEA. Because all these compounds were dissolved in DMSO, its concentration was kept identical in each condition/tube tested (final DMSO concentration 1:1000). At the end of the incubation period, aliquots from the medium were taken and kept frozen for insulin assay,[32] whereas the islets were suspended in water and immediately sonicated to extract and measure their insulin content by radioimmunoassay (RIA).[32] The amount of DNA per islet was also measured to express GSIS as a function of its content.

Total RNA Isolation, Retrotranscription, and Polymerase Chain Reaction

After killing, the brain, spleen, and pancreas from normal animals were removed. Total RNA was obtained from isolated rat islets, brain, and spleen using TRIzol Reagent (Gibco-BRL, Rockville, Md) following the manufacturer's instructions. The integrity of the isolated RNA was checked by 1% agarose-formaldehyde gel electrophoresis. Possible contamination with protein or phenol was controlled by measuring the 260/280-nm absorbance ratio, whereas DNA contamination was avoided using 1 U/μL DNAase I (Gibco-BRL).

Retrotranscription (RT) reaction was performed using 200 U/μL Super Script III reverse transcription (Gibco-BRL), oligo dT (Invitrogen), and 1-μg total RNA as template. Negative controls were performed by omission of SuperScript III in the reaction mix.

For polymerase chain reaction (PCR), specific primers based on the rat CB1 and CB2 complementary DNA (cDNA) sequences were used: sequence 5′ to 3′, forward primer (A) and reverse primer (B): CB1 (NM_012784.3): A, ttccgtaccatcaccacaga; B, agtgcaggatgacacacagc; product size, 397 bp. CB2 (AF218846.1): A, atctttgcctgcaacttcgt; B, acatgtggtgtgctttcca; product size, 404 bp. β-Actin primers were used as a positive control of the PCR (V01217: A, cgtaaagacctctatgccaa; B, agccatgccaaatgtctcat, product size, 473 bp).

Polymerase chain reactions were run using 1/40th of the cDNA or negative control as template, 1 μmol/L forward and reverse primers, 40 mU/μL Platinum Taq DNA polymerase (Invitrogen), 3 mM $MgCl_2$ and 0.2 mM dNTPs. The cycling profile was as follows: a first step of 2 minutes at 94°C followed by 35 cycles of 30 seconds at 94°C, 1 minute at 60°C, and 1.5 minutes at 72°C with a final step of 10 minutes at 72°C. Polymerase chain reaction products were separated by electrophoresis on a 2% (wt/vol) agarose gel and stained with ethidium bromide.

In Vivo Studies

Normal rats were randomly divided into 3 groups (n = 12 each group) and received the following diets during 21 days: a powdered standard commercial diet and tap water ad libitum (control, C); the same diet as C plus 10% (wt/vol) fructose in the drinking water (F); the same diet as F plus 105 mg R per kg of food powder (R-F; under our conditions, this concentration assures a mean R intake of 2 mg/rat per day). Body weight together with food and water intake were checked every second day.

Blood Measurements and GSIS

Blood samples were drawn from the retroorbital plexus of nonfasted animals at the time of killing (around 9:00 A.M.) under

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light isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether; Abbott, Ill) anesthesia to measure serum glucose, insulin, and triglyceride levels. Thereafter, animals were killed by cervical dislocation.

Glucose levels were measured in each animal using the Accu-Check Performa glucometer (Roche Diagnostics, Germany). Triglyceride levels were assessed with enzymatic commercial kits (Bayer Diagnostics, Argentina) implemented in an automated clinical analyzer. Insulin levels were determined by RIA using rat insulin standard.[32] Insulin resistance and the β-cell reserve were assessed with the homeostasis model assessment for IR (HOMA-IR) and the HOMA for β-cell function (HOMA-β) scores, respectively, calculated with the formula described by Matthews and coworkers.[33]

Glucose-stimulated insulin secretion was performed in islets isolated from all experimental groups[31] and incubated for 60 minutes in the presence of different glucose concentrations (3.3, 8.0, and 16.7 mM). Insulin released to the incubation media was determined by RIA[32] as described before. Insulin release was thereafter expressed as ng per μg of DNA in 1 hour (ng/μg DNA per hour).

Analysis of Islet Gene Expression by Real-Time PCR

cDNA of islets isolated from each study group was obtained as described before. Real-time PCR (qPCR) was performed with a Mini Opticon Real-Time PCR Detector Separate MJR (BioRad), using SYBR Green I as a fluorescent dye and 1/40th of the islet cDNA as template. Reactions were performed in a 25-μL qPCR reaction mixture containing 0.36 μM of each primer, 3 mM $MgCl_2$, 0.2 mM dNTPs, and 40 mU/μL Platinum Taq DNA polymerase (Invitrogen). Polymerase chain reaction profile was as follows: a first step of 3 minutes at 95°C followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 65°C, and 45 seconds at 72°C, with a final extension step of 10 minutes at 72°C followed by a melting curve from 55°C to 90°C. The optimal parameters for the PCR reactions were empirically defined. Each PCR amplification was performed in triplicate. The oligonucleotide primers (Invitrogen) used were as follows: sequence 5′ to 3′, forward primer (A) and reverse primer (B): CB1 (NM_012784.3): A, cgtaaagacagccccaatgt and B, tacctgtcgatggctgtgag; CB2 (AF218846.1): A, cctgttgaagatcggcagcg and B, ggtaggagatcaacgccgag; insulin receptor substrate 1 (IRS1, NM_012969.1), A, tgtgccaagcaacaagaaag and B, acggtttcaga gcagaggaa; IRS2 (NM_001168633.1): A, ctacccactgagcccaagag and B, ccagggatgaagcaggacta. All amplicons were in a size range of 90 to 250 bp. β-Actin (V01217) was used as housekeeping gene (primer sequences: A, agagggaaatcgtgcgtgac and B, cgatagtgatgacctgaccgt). The purity and specificity of the amplified PCR products were verified by performing melting curves and were further checked by 2% (wt/vol) agarose gel

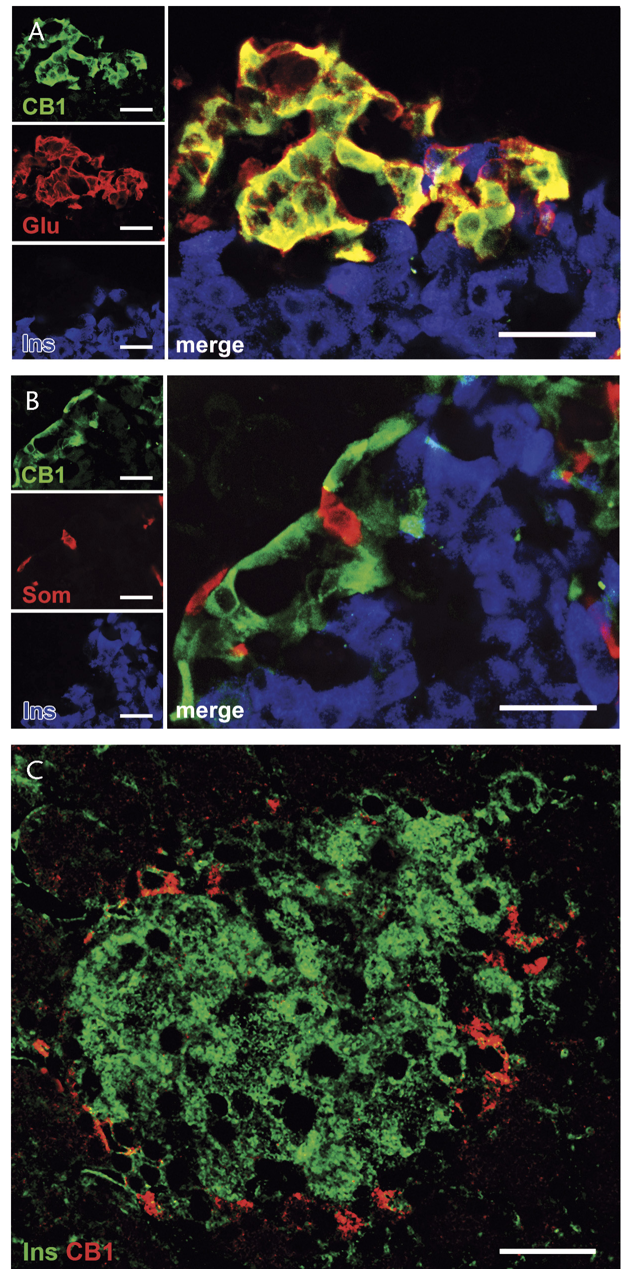

FIGURE 2. CB1 identification in islet cells. Confocal optical sections (1 μm) showing cellular CB1 localization in rat islets. A, Left panels show the same section immunostained for CB1 (green), glucagon (Glu, red) and insulin (Ins, blue). In the right panel, the 3 images were merged, demonstrating the presence of CB1 in α cells. B, Left panels show the same section immunostained for CB1 (green), somatostatin (Som, red), and insulin (Ins, blue). The merged image on the right shows the lack of CB1 colocalization with somatostatin or insulin. C, Double immunofluorescent labeling of CB1 (red) and insulin (Ins, green), demonstrating the peripheral distribution of CB1 immunoreactive-positive cells. In all cases, images are representative of 3 pancreases and at least 3 sections from 3 different levels of the block, calibration bar equals 20 μm.

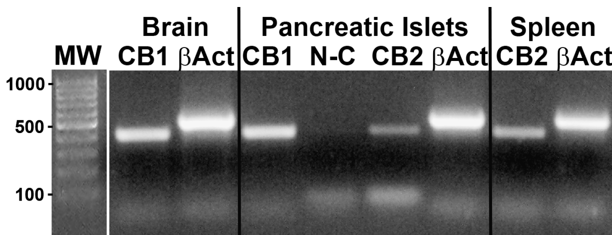

FIGURE 1. Cannabinoid receptor expression in normal rat tissues (RT-PCR). Lanes 1 and 2, normal rat brain; lane 1, CB1; lane 2, β-actin. Lanes 3–6, normal rat islets; lane 3, CB1; lane 4, RT-negative control; lane 5, CB2; lane 6, β-actin. Lanes 7 and 8, normal rat spleen; lane 7, CB2; lane 8, β-actin. MW indicates molecular weight marker. Polymerase chain reaction product sizes: CB1, 397 bp; CB2, 404 bp; β-actin, 473 bp.

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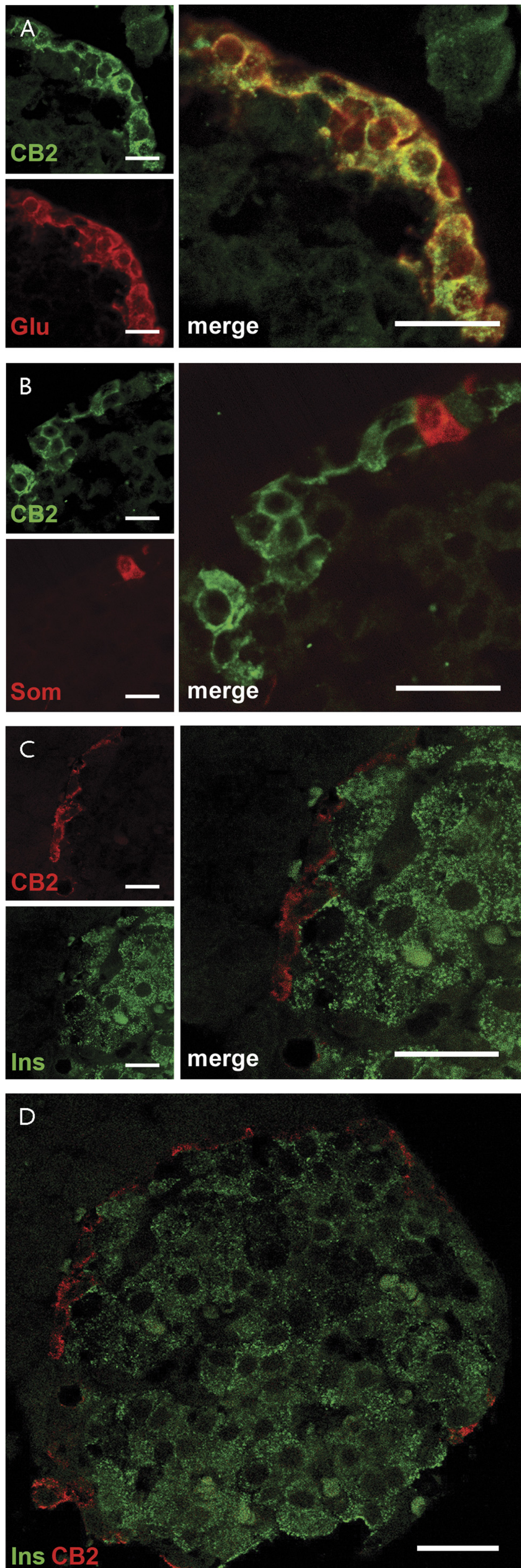

electrophoresis and ethidium bromide staining. Data are expressed as relative gene expression respect to control group after normalization to the β-actin housekeeping gene, by using the δ-δ Ct method.

Statistical Analysis

The statistical analysis was performed using 1-way analysis of variance followed by Bonferroni *t* test considering differences as significant when *P* was less than 0.05.

RESULTS

Islet Expression and Cellular Distribution of CB1 and CB2 in Normal Rats

Retrotranscription–polymerase chain reaction (RT-PCR) demonstrated the expression of CB1 and CB2 genes in islets isolated from normal rat pancreases (Fig. 1). The brain and spleen cDNA was used as positive control. The immunohistochemical identification showed that CBs were always present in the periphery of the islets (Figs. 2 and 3). Whereas double and triple immunofluorescent labeling revealed CB1 (Fig. 2) and CB2 (Fig. 3) colocalization in glucagon-producing cells, they were not detected in somatostatin- or insulin-producing cells.

Direct Effect of AEA, ACEA, JWH-015, and R on GSIS In Vitro

In all cases, islets isolated from normal rats were challenged with low (3.3 mM) and high (16.7 mM) glucose and insulin was expressed as the increase of the latter above low glucose (Fig. 4). Addition of AEA or either the specific CB1 (ACEA) or CB2 (JWH-015) agonists to the incubation media did not affect insulin release in response to a low glucose concentration (data not shown). Conversely, all these agents significantly affected insulin secretion unevenly in response to 16.7 mM glucose (Fig. 4A), whereas AEA enhanced it in a dose-response fashion ($P < 0.05$ at 100 and 200 μM concentration), both CB agonists tested showed a significant enhancement at 1 μM ($P < 0.05$), followed by a decrease at higher concentrations ($P < 0.05$ at 20 μM).

No significant changes in insulin secretion were recorded when the islets were incubated with different R concentrations; however, the simultaneous addition of R and stimulatory concentrations of either AEA or ACEA to the incubation media significantly decreased the amount of insulin released in response to 16.7 mM glucose ($P < 0.05$, Fig. 4B).

In all the experimental groups, insulin content in the islets after GSIS was significantly higher in islets incubated with 3.3 mM glucose as compared to those challenged with 16.7 mM glucose ($P < 0.05$, not shown). On the other hand, no differences were recorded in insulin content when comparing groups incubated with the same glucose concentration.

FIGURE 3. CB2 identification in islet cells. Confocal optical sections (1 μm) showing CB2 localization in rat islets. A, Left panels show the same section immunostained for CB2 (green) and glucagon (Glu, red). The merged image on the right shows complete colocalization of CB2 and glucagon signals, indicating the presence of this receptor in α-cells. B, Left panels show the same section immunostained for CB2 (green) and somatostatin (Som, red). The merged image on the right demonstrates the lack of CB2 colocalization with somatostatin. C, Left panels show the same section immunostained for CB2 (red) and insulin (Ins, green). On the right panel, merged images show the lack of CB2 colocalization with insulin. D, The same islet as in panel C labeled with CB2 (red) and insulin (green) shows all CB2 positive cells located in the periphery of the islets. In all cases, images are representative of 3 pancreases and at least 3 sections from 3 different levels of the block, calibration bar equals 20 μm.

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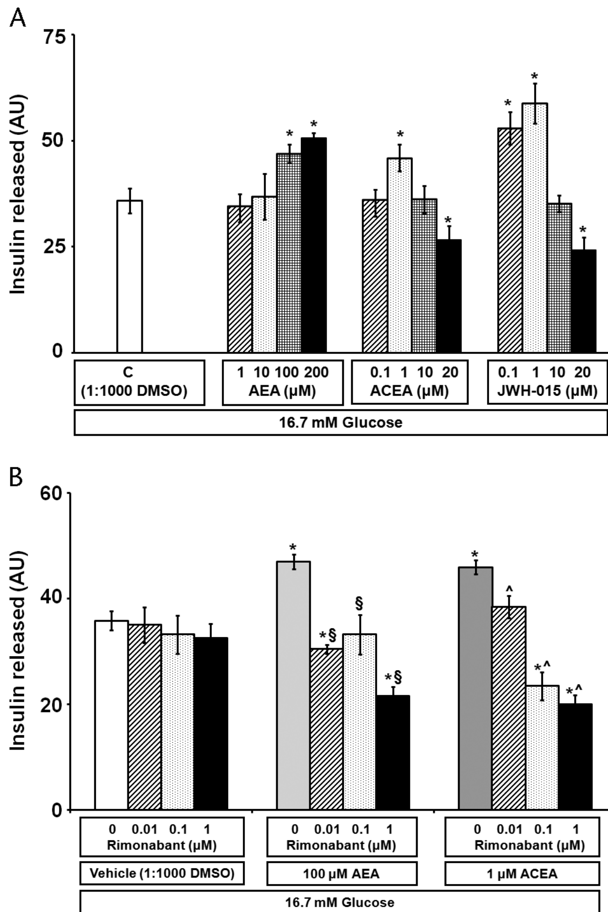

FIGURE 4. Insulin released by islets isolated from normal rats challenged with 16.7 mM glucose. A, Islets incubated in the presence of vehicle (1:1000 DMSO, Control [C] white column), 1–200 μM AEA, 0.1–20 μM ACEA, and 0.1–20 μM JWH-015. *$P < 0.05$ compared to C. B Islets incubated in the presence of vehicle (1:1000 DMSO), 100 μM AEA, or 1 μM ACEA combined with 0–1 μM R. *, §, and ^ $P < 0.05$ compared to C (white column), 100 μM AEA without R (light gray bar) and 1 μM ACEA without R (gray column), respectively. In all cases, values were calculated as ng of insulin released per μg of islet DNA in 1 hour (ng/μg DNA per hour). Values are expressed in arbitrary units (AU) with respect to the amount of insulin released by the islets exposed to 3.3 mM glucose (n = 15 in all cases).

Effect of R and F Coadministration on Body Weight and Metabolic Parameters

F-fed rats significantly increased energy and water intake, body weight as well as serum insulin and triglyceride levels as compared to C ($P < 0.05$; Tables 1 and 2). These changes were accompanied by a significant increase in the HOMA-IR index ($P < 0.05$), thus demonstrating the presence of an IR state in these rats. No significant changes in the HOMA-β index were detected among the experimental groups.

Coadministration of F and R significantly decreased the daily food intake ($P < 0.05$); this effect was accompanied by the disappearance of F-induced changes in serum triglyceride and insulin levels, as well as by a significant decrease in the HOMA-IR index ($P < 0.05$).

Effect of R on CB1 and CB2 Gene Expression

Islets from F rats showed a higher CB1 mRNA level ($P < 0.05$) than those from C, whereas CB2 mRNA level did not change after F administration (Fig. 5). CB1 blockage by R induced a significant CB2 increase in F rats ($P < 0.05$) and did not modify the F-induced changes recorded in CB1 gene expression (the increment found in R-F with respect to F islets was not significant).

Effect of R on GSIS and IRS Gene Expression

Islets isolated from F rats released significantly more insulin than those from C ones in response to 8.0 and 16.7 mM glucose ($P < 0.05$; Fig. 6), whereas administration of R to F rats did not affect the changes observed in GSIS (Fig. 6).

F unevenly modified the expression levels of IRS genes (Fig. 7), decreasing significantly IRS1 ($P < 0.05$) and increasing IRS2 ($P < 0.05$) mRNA levels with respect to C. As shown in Figure 7, coadministration of R and F recovered IRS1 gene expression level (F vs R-F, $P < 0.05$), but failed to fully correct the changes observed in IRS2 mRNA level: it decreased with reference to F (NS), but was still higher than in C islets ($P < 0.05$).

DISCUSSION

It is well known that several components of the endocannabinoid system are present in islet cells. In fact, Li et al[34] have recently shown that endocannabinoid 2-arachidonoylglycerol and monoacylglycerol lipase, the enzyme that catalyzes its degradation, are involved in the regulation of islet function.[34] Great efforts have been made in the last 10 years to understand the presence, cell distribution, and role of islet CBs in the control of β-cell function and glucose homeostasis. Different CB1 and CB2 localizations in islet cells have been reported, depending on the species or cell line used. In rat and mouse islets, CB1 has been identified in non-β cells,[14,15] in either α and δ cells,[16–18] or specifically in glucagon producing α cells,[19] whereas its presence in δ[18] and β cells[20,21] has been reported in human islets. Our findings confirm the presence of CB1 in α cells but not in other rat islet cells. Because previous confocal colocalization studies did not report the use of single (1 μm) optical sections, the presence of a minor proportion of β cells displaying CB1 might be rather artifactual.

TABLE 1. Body Weight and Food Consumption

	Body Weight Increment, g/rat per day	Food Consumption, g/rat per day	Water Intake, mL/rat per day	Energy Intake, cal/rat per day
C	2.27 (0.57)	20.4 (0.5)	24.6 (0.8)	59.1 (1.5)
F	3.43 (0.45)*	20.9 (0.7)	64.6 (9.1)*	86.2 (1.8)*
R-F	2.30 (0.52)†	15.8 (1.1)†	36.3 (5.1)†	60.1 (3.5)†

Values represent means (SEM) of the parameters recorded in 12 animals from each experimental group. $P < 0.05$ *compared to C, †compared to F.

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TABLE 2. Serum Metabolites, Insulin Concentration, and HOMA Scores

	Glucose, mmol/L	Insulin, μU/mL	Triglyceride, mg/dL	HOMA-IR	HOMA-β
C	5.04 (0.13)	10.54 (0.81)	56.0 (5.3)	2.34 (0.15)	159.6 (28.7)
F	5.53 (0.15)	15.88 (0.91)*	73.7 (3.5)*	3.90 (0.24)*	165.0 (16.5)
R-F	5.21 (0.34)	11.12 (0.95)†	63.7 (1.6)†	2.63 (0.28)†	174.5 (49.5)

Values represent means (SEM) of the parameters recorded in 12 animals from each experimental group. $P < 0.05$ *compared to C, †compared to F.

CB2 localization was more disputable: in islet cell suspensions, they appeared in β and non-β cells,[14,15] whereas in intact islets a small CB2 immunoreactivity in somatostatin (δ)-[17], glucagon (α)- and insulin-producing cells[19] has been reported. Conversely, other authors failed to demonstrate their presence using either immunohistochemistry or RT-PCR[16] techniques. Our results demonstrate the presence of CB2 mRNA and its immunocytochemical localization in non-β cells, as reported by Starowicz et al.[19] Although at present we cannot exclude the presence of CB2 in δ cells, our findings suggest that if that were the case, they would only represent a minor subpopulation.

Our data show that the endogenous cannabinoid AEA enhances GSIS in vitro in normal rat islets, and that different CB1 and CB2 agonists (at appropriate concentrations) also exert such effect. These results lend support to similar results previously reported in a rat insulinoma cell line,[20] in fasting rats,[23] and in either perifused mouse islets[24] or Min6 cells.[25] Further, the inhibition of insulin secretion currently recorded at high concentrations of CBs agonists could explain earlier reports[14–19] showing that cannabinoids inhibit the hormone's secretion.

A recent publication shows that islets isolated from normal rats and incubated with R did not change their response to low glucose, but significantly decreased the one elicited by high glucose.[35] Under our experimental conditions, addition of R to the incubation media did not cause any significant effect on insulin secretion, thus suggesting the lack of a direct effect on this process. However, when R was simultaneously added with stimulatory concentrations of CB1 ligands (either AEA or ACEA) to the incubation media, not only did they abolish their GSIS enhancing effect, but it also decreased insulin secretion, thus suggesting that the effect could be mediated by islet CB1. Taken together, our results suggest that even when CB1 are present and can modulate GSIS, they do not exert a tonic effect on such process, at least under the experimental condition tested.

On the basis of the presence and cellular distribution of CB1 and CB2 in non-β cells, we may assume that the effect of these receptor agonists on insulin release depends on some type of paracrine β- and α-cell interaction. This assumption is supported by the early report of Pipeleers et al[36] showing that the potent insulin-releasing action of glucose in intact islets not only depends on its fuel capacity but also on the concurrent cAMP levels in β cells that were probably modulated by endogenously released glucagon. The existence of a potential tonic effect of the cannabinoid system on glucagon secretion remains to be demonstrated.

There is clear evidence for an overactivity of the endocannabinoid system during conditions of disrupted energy homeostasis (eg, obesity and hyperglycemia) and for its active role in the pathogenesis of the metabolic disorders present under those conditions.[2,3] In animals fed a high-fat diet, this overactivity occurs simultaneously at central (hypothalamus) and peripheral (liver, pancreas, and adipose tissue) level.[19] In this regard, it has been previously shown that F administration to normal rats induces a state characterized by high serum triglyceride,[37] insulin and leptin[30] levels, high HOMA-IR index,[26–30,37] impaired glucose tolerance, abnormal fat[29] and liver[27] metabolism, and a significant increase of glucoxidative markers,[28] characteristics that resemble those present in human diabetes and metabolic syndrome.[38] Administration of the CB1 blocker R to these animals prevented the development of most of these metabolic abnormalities and would, consequently, protect sensitive tissues, such as pancreatic β cells,[39] against the deleterious

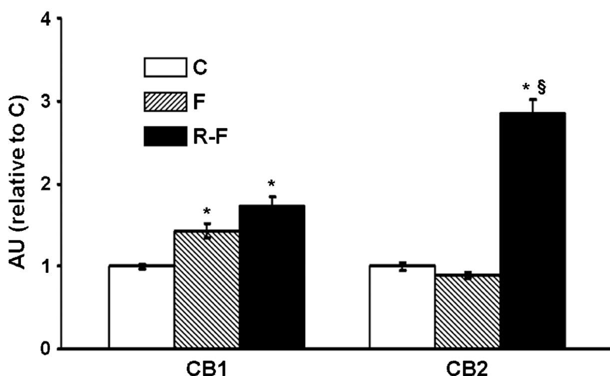

FIGURE 5. mRNA levels of CB1 and CB2 determined by qPCR in islets isolated from C, F, and R-F rats. Values are expressed in AU relative to C and represent the mean of 3 independent experiments. *Compared to C and §compared to F; in all cases, $P < 0.05$.

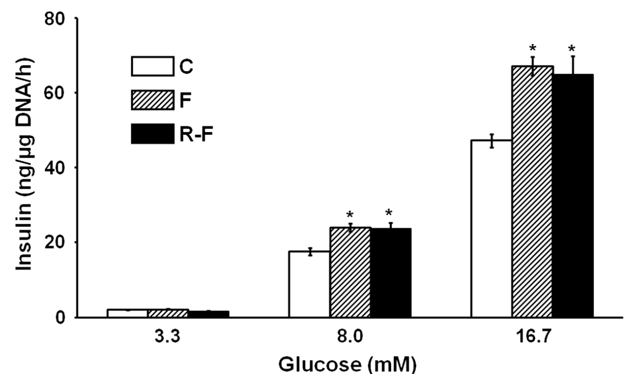

FIGURE 6. GSIS. Insulin released by islets isolated from C, F, and R-F rats in response to different glucose concentrations (3.3, 8.0, and 16.7 mM). Values are expressed as ng of insulin released per μg of DNA in 1 hour (ng/μg DNA per hour). In all cases, $P < 0.05$ for 3.3 mM compared to 8.0 mM and 16.7 mM glucose and *compared to C (n = 15 in all cases).

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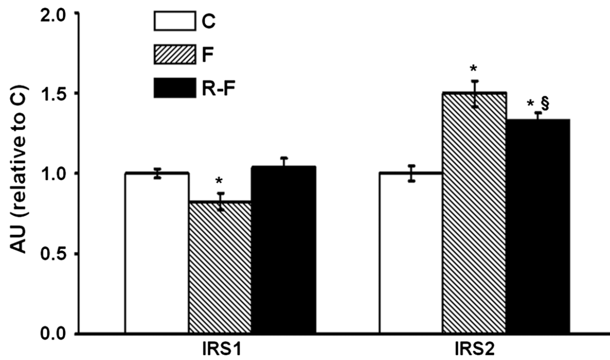

FIGURE 7. mRNA levels of IRS1 and IRS2 determined by qPCR in islets isolated from C, F and R-F rats. Values are expressed in AU relative to C and represent the mean of 3 independent experiments. *Compared to C and §compared to F; in all cases, $P < 0.05$.

effect of lipotoxicity and IR,[40] further preventing the increased triglyceride accumulation in liver.[41] R coadministration to F rats also prevented the significant decrease recorded in islet IRS1 expression, whose active positive role in the control of β-cell function[42–44] would help to understand how islet CBs modulate such function and participate in overall metabolic homeostasis.

Although the beneficial effects of R on IR,[45] lipid metabolism[46] and metabolic lipid abnormalities present in people with obesity[47] have been previously reported, our results would be the first to demonstrate a preventive effect of this compound. The participation of islet CBs in the preventive effect of R is also supported by the changes recorded in their expression at islet level. In fact, as seen in obesity[48] and diabetes,[49] we observed a significant increase in CB1 expression in F rat islets, and the apparent discrepancy represented by the further increase after R coadministration might only represent an attempt to overcome CB1 blockade. The simultaneous change recorded in CB2 expression would just reinforce the possible overall involvement of the islet cannabinoid system in the pathogenesis of F-induced metabolic dysfunction and its important role on islet gene expression.[23]

In a recent publication, Vilches-Flores et al[50] did not find substantial changes in islet function when normal mouse islets were treated in vitro with CBs agonists for up to 7 days; accordingly, they concluded that the effect of an overactivation of the endocannabinoid system should act on IR rather than directly on β-cell function.[50] Supporting this assumption, our in vivo study demonstrated that R coadministration with F prevented the diet-induced IR but did not improve GSIS.

Despite we have ascribed the preventive effect of R on F-induced abnormalities to its blocking activity on CB1, we cannot discard that some of these abnormalities might also depend on its reported interaction with orphan G-coupled receptor (GPR55).[51,52]

In brief, our results show that (*a*) in normal rats, CB1 and CB2 are present in the islets with a clear cell distribution, and in intact animals, they would exert an important modulatory role in metabolic homeostasis, (*b*) such role can be seriously affected by administration of unbalanced diets (F in our case), and CBs would thus participate in the pathogenesis of impaired metabolic homeostasis, and (*c*) in that situation, administration of a CB1 blocker was an effective preventive alternative. Because R is not used any longer in clinics due to its off target effects, the development of selective blockers directed to islet CB1 could be a useful tool to prevent/treat the hormonal and metabolic alterations induced by the intake of unbalanced/unhealthy diets.

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