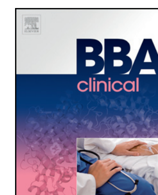
BBA Clinical 5 (2016) 143–150

Contents lists available at ScienceDirect

BBA Clinical

journal homepage: www.elsevier.com/locate/bbaclin



Prolonged activation of human islet cannabinoid receptors in vitro induces adaptation but not dysfunction

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ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 26 March 2016
Accepted 29 March 2016
Available online 30 March 2016

Keywords:
Human islets
Endocannabinoid system
Gene expression
Insulin
Glucagon
Apoptosis

ABSTRACT

Background: Although in vivo studies have implicated endocannabinoids in metabolic dysfunction, little is known about direct, chronic activation of the endocannabinoid system (ECS) in human islets. Therefore, this study investigated the effects of prolonged exposure to cannabinoid agonists on human islet gene expression and function.
Methods: Human islets were maintained for 2 and 5 days in the absence or presence of CB1r (ACEA) or CB2r (JWH015) agonists. Gene expression was quantified by RT-PCR, hormone levels by radioimmunoassay and apoptosis by caspase activities.
Results: Human islets express an ECS, with mRNAs encoding the biosynthetic and degrading enzymes NAPE-PLD, FAAH and MAGL being considerably more abundant than DAGLα, an enzyme involved in 2-AG synthesis, or CB1 and CB2 receptor mRNAs. Prolonged activation of CB1r and CB2r altered expression of mRNAs encoding ECS components, but did not have major effects on islet hormone secretion. JWH015 enhanced insulin and glucagon content at 2 days, but had no effect after 5 days. Treatment with ACEA or JWH015 for up to 5 days did not have marked effects on islet viability, as assessed by morphology and caspase activities.
Conclusions: Maintenance of human islets for up to 5 days in the presence of CB1 and CB2 receptor agonists causes modifications in ECS element gene expression, but does not have any major impact on islet function or viability.
General Significance: These data suggest that the metabolic dysfunction associated with over-activation of the ECS in obesity and diabetes in humans is unlikely to be secondary to impaired islet function.
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1. Introduction

Islets of Langerhans have a limited capacity to adapt to different environmental challenges such as hyperglycaemia, hyperlipidaemia, pregnancy and obesity. When this narrow threshold is insufficient to compensate for alterations in metabolic state, inadequate β-cell turnover and islet dysfunction may lead to the development of type 2 diabetes. In this context, accumulating evidence suggests that the endocannabinoid system (ECS) may be involved in the dysregulation of fuel homeostasis through the hypersecretion of the endocannabinoids anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) from expanding adipocytes in obesity [1–8]. Sustained elevations in endocannabinoid levels in the circulation that occur following ECS over-activation in obese and diabetic patients are reported to have detrimental effects in peripheral organs, leading to impaired insulin sensitivity, glucose intolerance and dyslipidaemia [3,4,7,9,10]. In addition, central CB1 receptor activation by endocannabinoids has orexigenic effects [11–14], and chronic exposure to cannabinoids is reported to lead to changes in the expression of lipogenic genes and lipid content in hepatocytes [4,15,16], and to the induction of apoptosis in sebocytes [5].

Rodent and human islets express a local ECS consisting of the enzymes required for the synthesis (NAPE-PLD and DAGL) and degradation (FAAH and MAGL) of AEA and 2-AG, and the endocannabinoids can activate the CB1 and CB2 cannabinoid receptors that are also expressed by islet cells [10,17–35]. There is some controversy over whether acute activation of CB1 and CB2 receptors is coupled to stimulation [17,19,23–27,29–31,33,34], or inhibition [10,18,22,28,32] of insulin secretion from rodent islets. However, our earlier studies in isolated human islets indicate that CB1 and CB2 receptor agonists can stimulate insulin secretion [17,30], suggesting that cannabinoids may have beneficial effects in human β-cells. Most studies investigating the effects of

Abbreviations: ACEA, *N*-(2-Chloroethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eiscosatetraenamide; AEA, anandamide; 2-AG, 2-arachidonoyl glycerol; CB1r, cannabinoid receptor type 1; CB2r, cannabinoid receptor type 2; DAGL, diacylglycerol lipase; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; JWH015, (2-methyl-1propyl-1*H*-indol-3-yl)-1-napthalenylmethanone; MAGL, monoacylglycerol lipase; NAPE-PLD, N-acyl-phosphatidyl ethanolamide-hydrolysing phospholipase D; PPG, preproglucagon; PPI, preproinsulin.

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http://dx.doi.org/10.1016/j.bbacli.2016.03.009
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cannabinoid agonists and antagonists on islet function in vitro have focused on acute signalling [10,18,19,22,23,25–34], but 2-AG and AEA levels are chronically elevated in the pancreas under conditions of diet-induced obesity [6,25], and these endocannabinoids are also increased in human islets in response to high glucose concentrations [3, 10]. Excess endocannabinoid production by the local islet ECS in obesity and diabetes could therefore have a chronic impact on islet function [35].

We have previously evaluated the long term effects of mouse islet CB1r or CB2r over-activation on ECS gene expression, on insulin and glucagon secretion and on islet viability, and found that there were no major functional effects [24]. However, similar studies on the effects of chronic exposure of human islets to cannabinoids have not been carried out although cannabinoids have been implicated in mouse β-cell death [22]. Therefore, the aim of the current study was to identify whether isolated human islets maintained chronically with cannabinoid agonists show modifications in expression of genes coding for elements of the ECS, and/or alterations in islet secretory function and viability.

2. Materials and methods

2.1. Materials

DMEM (5.5 mM glucose), penicillin/streptomycin, L-glutamine, collagenase (Type XI), and PCR primers for human CB1r, CB2r, MAGL, NAPE-PLD, FAAH, DAGL and 18s rRNA were purchased from Sigma Aldrich (Dorset, UK). Primers for human preproinsulin and preproglucagon, RNeasy mini kits, and QuantiFast SYBR Green PCR Kit, were obtained from Qiagen (West Sussex, UK). Real-time PCR master mix and plates were purchased from Roche Diagnostics Ltd. (West Sussex, UK). *N*-(2-chloroethyl)-5*Z*,8*Z*,11*Z*,14*Z*-eiscosatetraenamide (ACEA) and (2-methyl-1propyl-1*H*-indol-3-yl)-1-napthalenylmethanone (JWH015) were from Tocris Biosciences (Bristol, UK). Fetal bovine serum and reverse transcription reagents were purchased from Invitrogen (Paisley, UK), and Caspase-3/7-Glo apoptosis kits were obtained from Promega UK (Southampton, UK).

2.2. Human islet exposure to cannabinoids

Human islets were isolated and purified from non-diabetic, heart-beating donors (with ethical permission) by the King's College Islet Transplantation Unit, as previously reported [36]. Human islets from eight separate donors with the following characteristics were used in this study: 5 male, 3 female; age 45 ± 4.2; BMI 27 ± 1.0. Hand-picked, size-matched islets were maintained in culture with DMEM containing 5.5 mM glucose, 10% FBS, 2 mM L-glutamine, and 100 units/ml penicillin/100 μg/ml streptomycin, for 2 or 5 days in the presence or absence of either 100 nM of the CB1r-selective agonist ACEA, or 100 nM of the CB2r-selective agonist JWH015. Control groups of islets were incubated in the presence of 0.001% DMSO, the vehicle used for preparation of ACEA and JWH015. Culture media replacement and microscopic observation of the islets was performed daily.

2.3. Polymerase chain reaction (PCR)

Total RNA was isolated using RNeasy mini kits from groups of 150–200 human islets previously treated for 2 or 5 days in the absence or presence of ACEA or JWH015 except for quantification of mRNAs encoding ECS elements, where four groups of 150–200 human islets were incubated overnight, without treatment, immediately after provision by the King's College Islet Transplantation Unit. RNA samples were adjusted to obtain 20–50 ng/μl of cDNA by reverse transcription reactions, as previously reported [24]. Real-time SYBR Green PCR amplifications were performed with a LightCycler 480 96 well-plate system, using the primers and conditions described in Table 1. Relative expression of mRNAs was determined after normalisation against 18s rRNA as an internal reference gene, and calculated by the $2^{-\Delta\Delta}$Ct method [37].

Table 1
Primer sequences and annealing temperatures used in real-time PCR.

Gene name for *Homo sapiens*	Primer sequences	Annealing temp.
Cannabinoid receptor 1 (CB1r)	F 5′-CACCTTCCGCACCATC ACCAC-3′ R 5′-GTCTCCCGCAGTCA TCTTCTCTTG-3′	60 °C
Cannabinoid receptor 2 (CB2r)	F 5′-CATGGAGGAATGCT GGGTGAC-3′ R 5′-GAGGAAGGCGATGA ACAGGAG-3′	62 °C
Diacylglycerol lipase alpha (DAGLα)	F 5′-AGAATGTCACCCTC GGAATGG-3′ R 5′-GTGGCTCTCAGCTT GACAAAGG-3′	62 °C
Monoacylglycerol lipase (MAGL)	F 5′-CAAGGCCCTCATCTTT GTGT-3′ R 5′-ACGTGGAAGTCAGA CACTAC-3′	60 °C
Fatty acid amide hydrolase (FAAH)	F 5′-CCCAGATGGAACAT TACAGG-3′ R 5′-CAGGATGACTGGTT TTCAGG-3′	60 °C
N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD)	F 5′-CACGGTAATGGTGG AAATGG-3′ R 5′-GTCCAGATGGTCAT AGTGGTTG-3′	62 °C
18s rRNA	F 5′-GGGAGCCTGAGAAA CGG-3′ R 5′-GGGTCGGGAGTGGG TAATTT-3′	60 °C
Preproinsulin	Qiagen Quantitect primer	60 °C
Preproglucagon	Qiagen Quantitect primer	60 °C

2.4. Insulin and glucagon secretion

Human islets that had been maintained in culture for 2 or 5 days in the absence or presence of 100 nM ACEA or 100 nM JWH015 were washed then pre-incubated for one hour at 37 °C in 400 μl of physiological buffer [38] containing 2 mM glucose. For measurements of insulin and glucagon secretion groups of 5 and 10 islets respectively were transferred to Eppendorf tubes and incubated for one hour at 37 °C in 500 μl of buffer supplemented with 16 mM glucose or 10 mM arginine. Insulin and glucagon secreted from the islets in these static incubation experiments was quantified by radioimmunoassay [17,39].

2.5. Insulin and glucagon content

Acidified ethanol (100 μl) was added to four groups of 10 human islets that had been exposed for 2 or 5 days to DMEM (5.5 mM glucose) alone or with DMEM supplemented with 100 nM of either ACEA or JWH015. The islet samples were sonicated and stored at −20 °C until radioimmunoassays were performed to measure insulin and glucagon contents.

2.6. Caspase-3/7 activities

The extent of apoptosis of human islets previously maintained for 2 or 5 days in the absence or presence of cannabinoid agonists was determined by detection of caspase-3/7 activities with a luminometer following cleavage of a proluminescent substrate (Z-DEVD-aminoluciferin), as previously reported [40]. Apoptosis was induced in these experiments by exposing five islets from each group to a cytokine cocktail (0.5 U/μl IL-1β, 5 U/μl TNFα, and 5 U/μl IFNγ) for the final 20 h of incubation.

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