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

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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.

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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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.

2.7. Statistical analysis

Data are expressed as means ± SEM of 3 or 4 individual experiments. One-way and two-way ANOVA with Bonferroni's test were used for analyses and differences between treatments were considered statistically significant at $p < 0.05$.

3. Results

3.1. Relative mRNA expression of the endocannabinoid signalling elements in human islets

Quantitative RT-PCR using human islet cDNAs indicated that the mRNA encoding FAAH, an enzyme responsible for degradation of the endocannabinoid AEA, was the most abundant component of the endocannabinoid system in human islets. It was expressed at levels similar to the mRNA coding for NAPE-PLD, which catalyses biosynthesis of AEA from the membrane phospholipid *N*-acylphosphatidylethanolamine, and both mRNAs were of similar abundance to preproglucagon mRNA (Fig. 1). The mRNAs encoding the enzymes that regulate the synthesis (DAGLα) and degradation (MAGL) of the other major endocannabinoid, 2-AG, were also detected in human islets but these, in particular DAGLα, showed lower levels of expression than NAPE-PLD and FAAH mRNAs (Fig. 1). Fig. 1 also indicates that CB1 and CB2 receptor mRNAs were detectable in human islets, but their expression levels were only approximately 0.1% of those of preproinsulin mRNA, which encodes the major islet hormone.

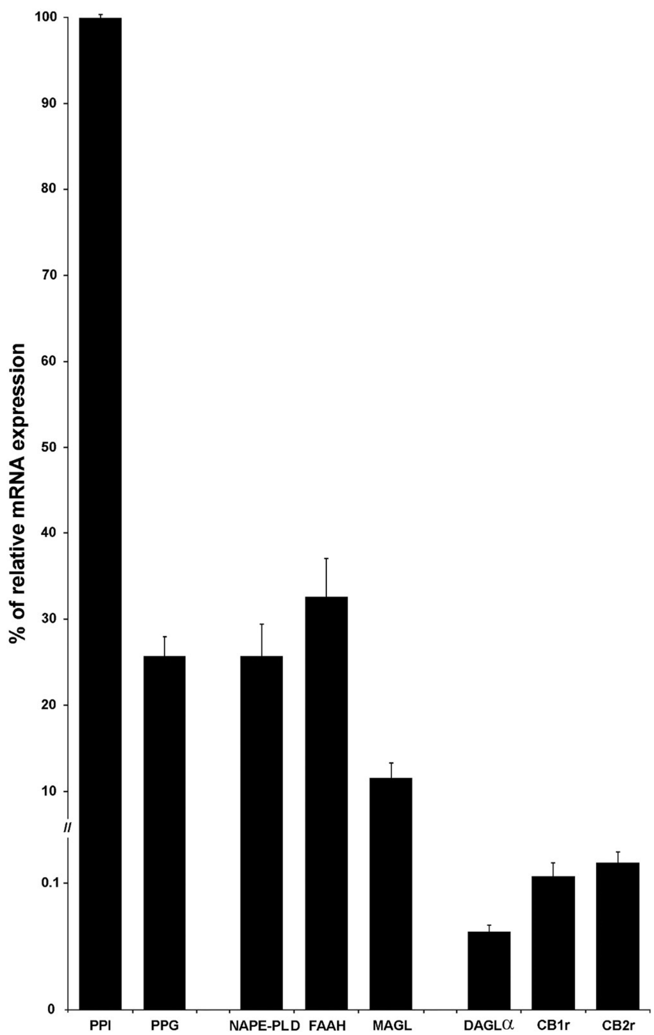


Fig. 1. Groups of 150–200 human islets were incubated overnight after isolation under standard culture conditions and qPCR was used to quantify relative expression of human islet mRNAs encoding preproinsulin (PPI), preproglucagon (PPG), and the enzymes and receptors of the endocannabinoid system. Data are expressed as relative percentage with respect to PPI mRNA levels, obtained from real-time PCR amplification values, normalised to 18s rRNA as an internal reference (means + SEM, n = 4).

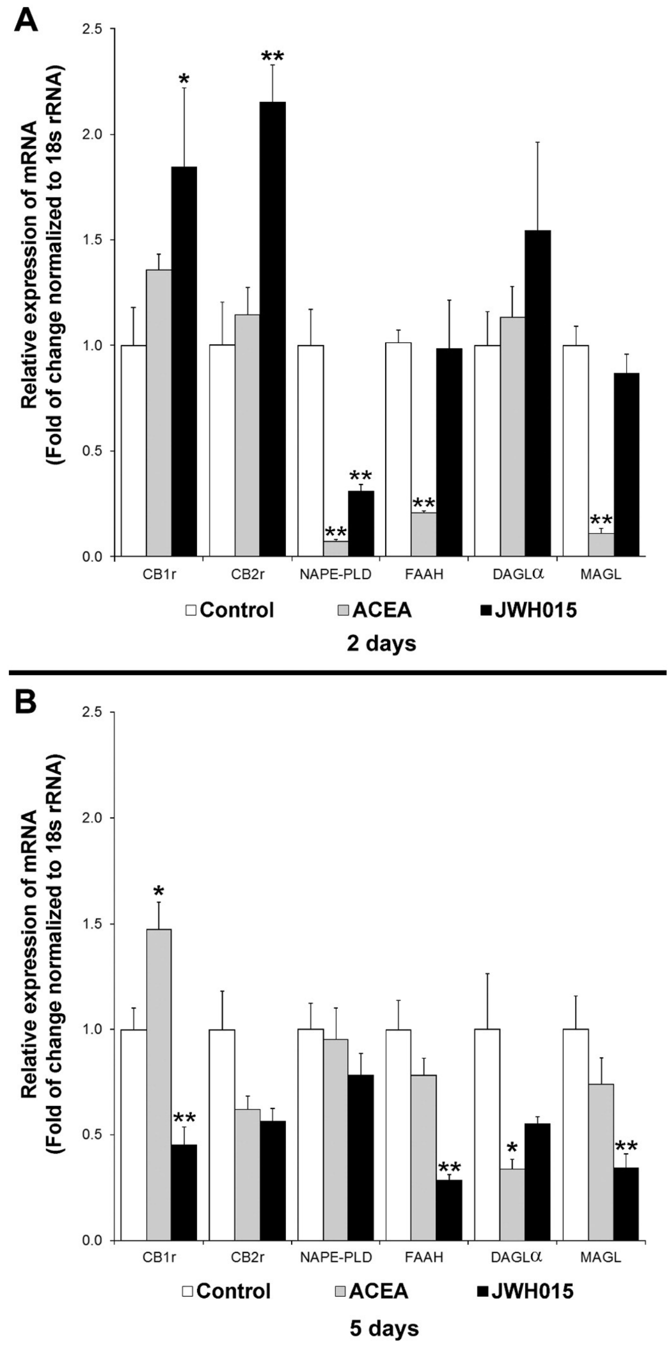


Fig. 2. Quantification of mRNAs encoding elements of the ECS in human islets after maintenance for 2 (A) and 5 days (B) in DMEM (5.5 mM glucose) alone (open bars) or supplemented with 100 nM ACEA (grey bars) or 100 nM JWH015 (black bars). Data are expressed relative to the levels present in control islets in the absence of cannabinoid receptor agonists and normalised to 18s rRNA levels in the same samples; means + SEM, n = 4 experiments. Data were analysed by one-way ANOVA; *$p < 0.05$, **$p < 0.01$ compared to the appropriate control.

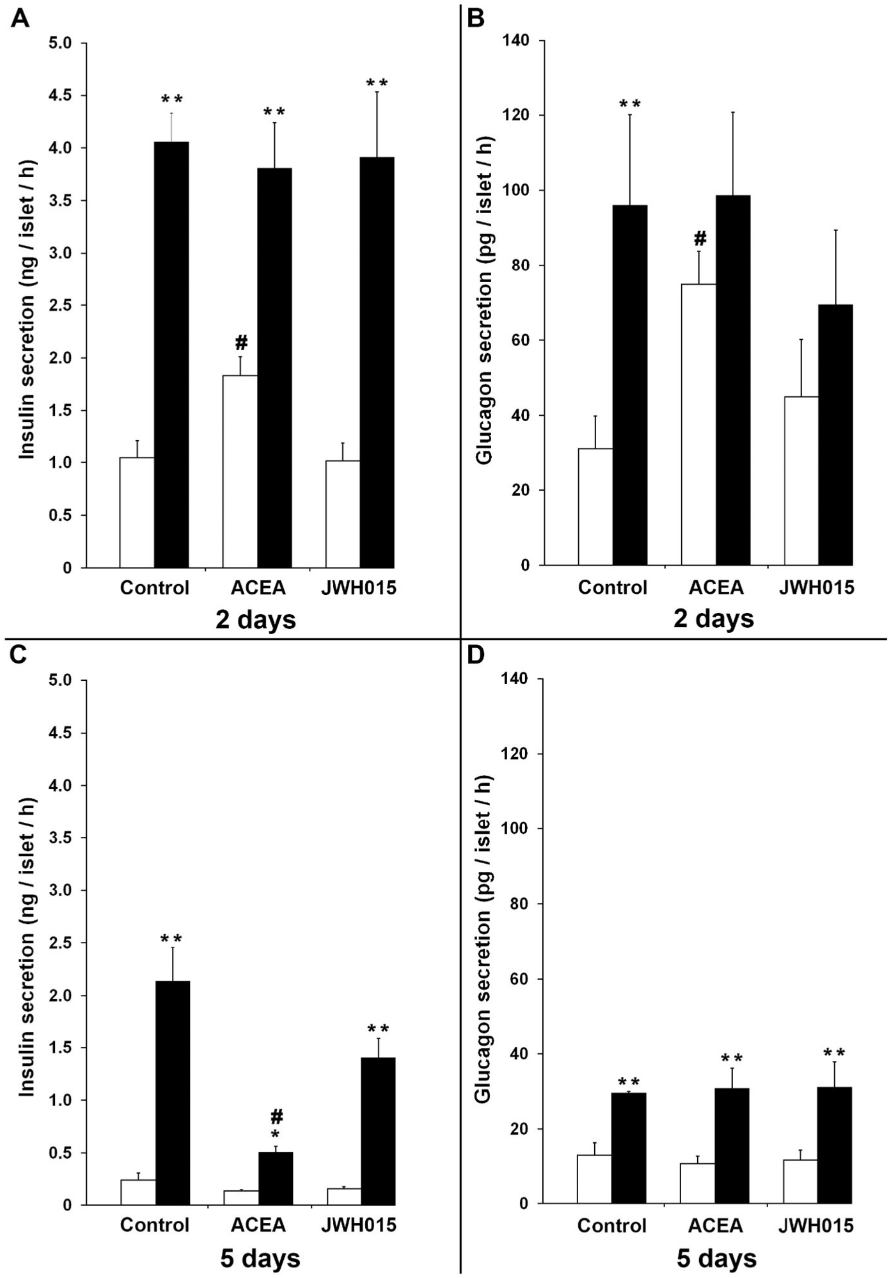


Fig. 3. Acute, one hour insulin (panels A and C) and glucagon (panels B and D) secretion from human islets after maintenance for 2 (upper panels) or 5 (lower panels) days in DMEM containing 5.5 mM glucose alone (control), or supplemented with 100 nM ACEA or 100 nM JWH015. Basal levels of insulin and glucagon secretion at 3 mM glucose are shown by the open bars and the black bars show insulin secretion at 16 mM glucose (panels A and C) and glucagon secretion at 10 mM arginine panels B and D); means + SEM, n = 4 experiments. Data were analysed by two-way ANOVA; *$p < 0.05$, **$p < 0.01$ versus the appropriate basal secretion, $^{\#}p < 0.05$ versus the appropriate control.

3.2. Effects of prolonged exposure to ACEA and JWH015 on ECS element gene expression in human islets

Quantitative RT-PCR demonstrated that human islets exposed to a CB1r (100 nM ACEA) or CB2r (100 nM JWH015) agonist for 2 or 5 days affected expression of ECS elements. Thus, as can be seen from Fig. 2A, stimulation of CB1r with ACEA (grey bars) induced significant decreases in NAPE-PLD, FAAH and MAGL mRNA levels at 2 days. While exposure of islets to JWH015 for 2 days (black bars) also significantly decreased NAPE-PLD mRNA it did not affect FAAH or MAGL expression, but it did induce significant up-regulation of mRNAs encoding CB1r and CB2r. Exposure of islets to ACEA for 5 days induced a 50% increase in CB1r mRNA levels and a significant reduction in DAGLα mRNA expression (Fig. 2B), while 100 nM JWH015 caused at least 50% reductions in mRNAs encoding CB1r, FAAH and MAGL after 5 days. These gene expression studies indicate that relatively short-term activation of CB1r by ACEA caused down-regulation of mRNAs encoding enzymes involved in endocannabinoid synthesis and degradation in human islets within 2 days, while chronic activation of CB2 receptors over 5 days decreased mRNAs encoding cannabinoid degradation.

3.3. Effects of prolonged cannabinoid receptor activation on insulin and glucagon secretion from human islets

Static incubation secretion experiments were performed to evaluate whether chronic exposure to the cannabinoid agonists affected acute glucose-stimulated insulin secretion (Fig. 3A and C) or acute arginine-induced glucagon secretion (Fig. 3B and D) from human islets

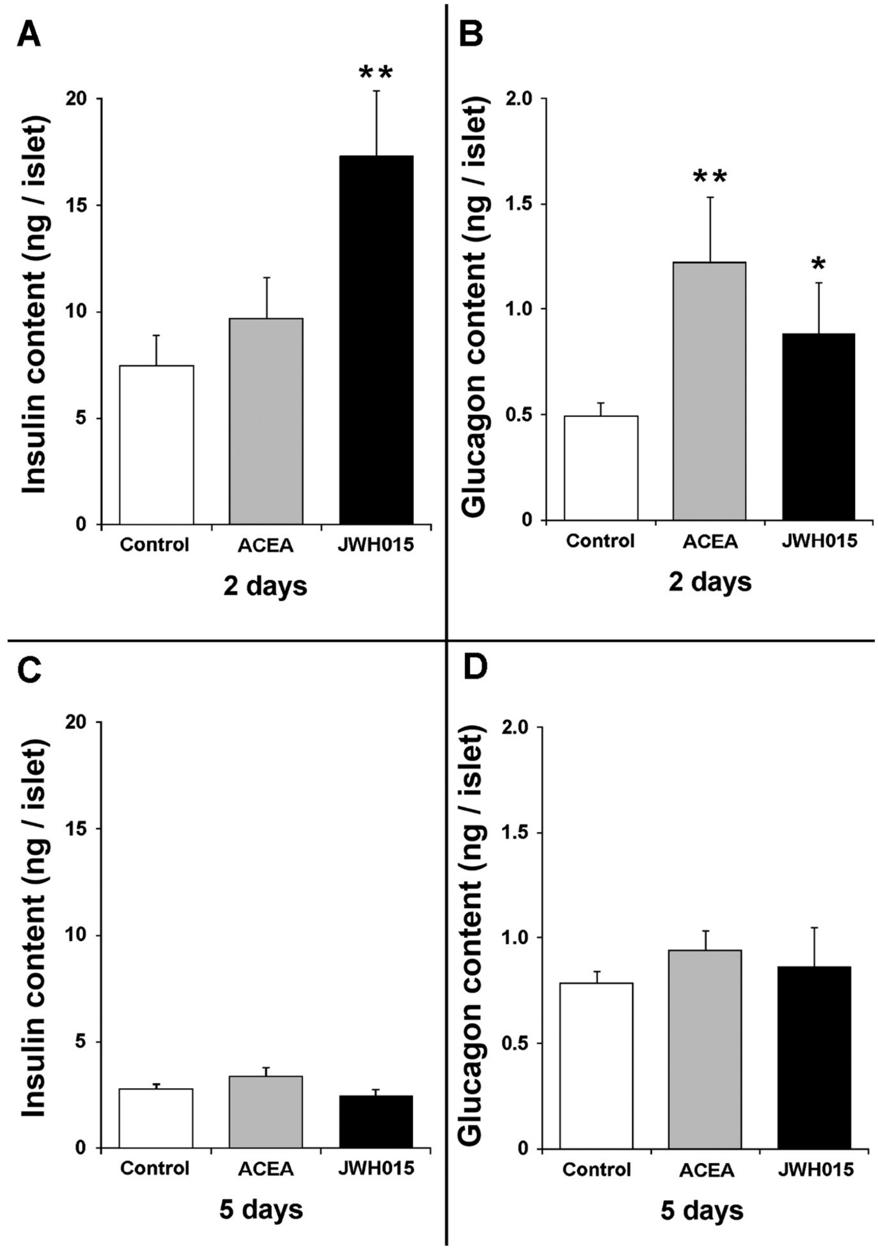


Fig. 4. Insulin (panels A and C) and glucagon (panels B and D) hormone contents of human islets maintained for 2 (upper panels) or 5 (lower panels) days in the absence of cannabinoid receptor agonists (open bars) or with 100 nM ACEA (grey bars) or 100 nM JWH015 (black bars). Data are expressed as ng/islet (means + SEM, n = 4). Data were analysed by one-way ANOVA; *p < 0.05, **p < 0.01 versus the appropriate control.

previously maintained in culture in the absence or presence of 100 nM ACEA or JWH015 for 2 (upper panels) or 5 days (lower panels).

Islets that had been maintained in culture for 2 days and 5 days showed significant elevations in insulin secretion in response to 16 mM glucose (Fig. 3A and C, control), although basal and glucose-stimulated insulin secretions were significantly reduced after 5 days in culture, compared to culture for 2 days. Basal insulin secretion at 3 mM glucose was significantly increased in human islets after incubation for 2 days with 100 nM ACEA, but the magnitude of insulin secretion at 16 mM glucose was not affected by 48 h pre-exposure to ACEA (Fig. 3A). Islets that had been treated with ACEA for 5 days showed a reduced secretory response to 16 mM glucose, but there was still a significant elevation in insulin release over the low basal insulin output (Fig. 3C). Treatment of human islets with 100 nM JWH015 for 2 days and 5 days had no significant effect on basal or glucose-stimulated insulin secretion (Fig. 3A and C).

Islets responded to acute exposure to 10 mM arginine after 2 and 5 days in culture with significant elevations in glucagon secretion (Fig. 3B and D, control), although the extent of glucagon release was significantly reduced after 5 days. Pre-exposure of human islets to 100 nM ACEA for 2 days significantly elevated basal glucagon secretion without modifying the amplitude of the glucagon secretory response to 10 mM arginine (Fig. 3B), while islets incubated with ACEA for 5 days did not show any alterations in basal or arginine-stimulated glucagon output (Fig. 3D). Islets that had been exposed to JWH015 did not show a significant glucagon secretory response to 10 mM arginine (Fig. 3B), but they responded appropriately to arginine after they had been maintained in the presence of JWH015 for 5 days (Fig. 3D).

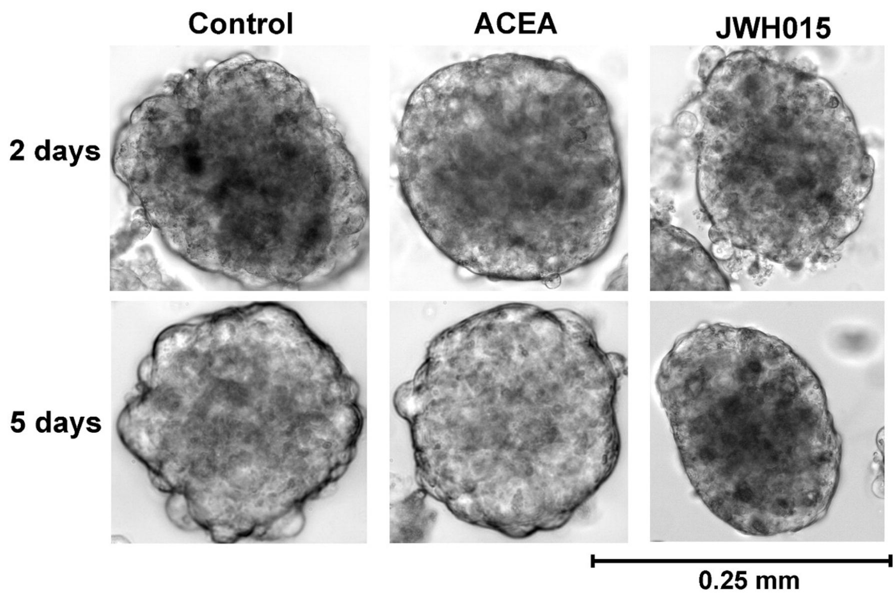


Fig. 5. Micrographs of human islets maintained in culture for 2 (upper panels) or 5 (lower panels) days in DMEM containing 5.5 mM glucose (left) or supplemented with 100 nM ACEA (middle) or 100 nM JWH015 (right). Micrographs are representative of 3 experiments using islets obtained from three different donors.

3.4. Effects of chronic exposure to cannabinoid receptors agonists on insulin and glucagon content in human islets

Since prolonged culture of human islets resulted in changes in insulin and glucagon secretion, as shown in Fig. 3, further experiments were carried out to quantify hormone content after 2 days and 5 days culture in the absence and presence of ACEA and JWH015. It can be seen from Fig. 4C (white bars) that maintenance of human islets in culture for 5 days resulted in significant reduction in insulin content compared to islets after 2 days in culture (Fig. 4A), but there was no significant change in glucagon content of the islets under the same conditions (Fig. 4B and D, white bars). Exposure of human islets to the CB1r agonist ACEA for 2 and 5 days had no effect on insulin content (Fig. 4A and C, grey bars). However, ACEA induced a significant increase in glucagon content after 2 days (Fig. 4B, grey bars), and this could be responsible for the elevated basal glucagon secretory response seen after maintenance of islets in the presence of 100 nM ACEA for 2 days (Fig. 3B). The CB2r agonist JWH015 caused a marked increase in insulin content at 2 days (Fig. 4A, black bars). This increase was not sustained after 5 days of treatment with JWH015, and islet insulin levels under this condition were not significantly different from the reduced content seen in the control islets. JWH015 also increased islet glucagon content after 2 days, but not at 5 days (Fig. 4B, black bars).

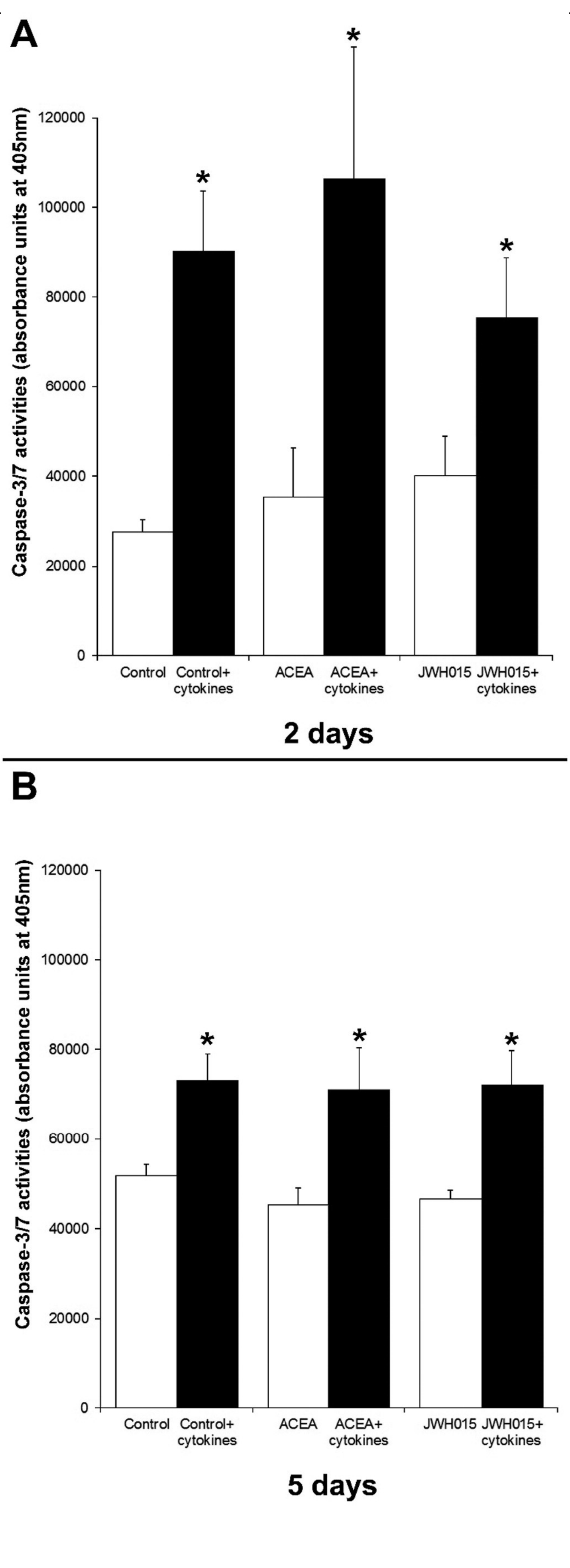
3.5. Effects of chronic exposure to cannabinoid receptor agonists on human islet morphology and apoptosis

The photomicrographs of human islets (Fig. 5) indicate that maintenance for 2 days (upper panels) or 5 days (lower panels) in the absence (left) or presence of 100 nM ACEA (middle) or JWH015 (right) did not cause any major deleterious effects on integrity, although after 5 days in culture all islets were generally less compact, with the loss of some cells. This was associated with increased basal apoptosis after 5 days compared to that seen at 2 days (Fig. 6A control, white bars versus Fig. 6B). Evaluation of the effects of chronic exposure to cannabinoid agonists on human islet cell caspase 3/7 activities was carried out in the absence and presence of mixed cytokines (black bars) and these studies indicated that pre-exposure to ACEA and JWH015 for 2 (Fig. 6A) or 5 days (Fig. 6B) had no effects on human islet cell apoptosis.

4. Discussion

We, and others, have previously identified components of a local ECS in human islets by PCR and immunohistochemistry [10,17,20,21,30], but the relative expression of these elements has not been fully investigated. Our observations indicate a relatively high mRNA expression of FAAH, NAPE-PLD and MAGL in human islets, while DAGLα and both cannabinoid receptors show lower expression levels. The high levels of FAAH mRNA that we detected in human islets is consistent with our earlier observations that this is the most abundant ECS mRNA in mouse islets [24], as are our observations of low levels of CB1r, CB2r and DAGLα. It has previously been reported that CB1 expression is considerably higher than CB2 in human islets [20], but this differential expression of the cannabinoid receptors was not confirmed in the current study. We did find here that human islets express substantially more MAGL mRNA than mouse islets do [17,24], suggesting that 2-AG levels are tightly controlled in human islets through MAGL-dependent degradation. Since whole islets were used for mRNA purification in these studies and our earlier work with mouse islets [24], it was not possible to determine the distribution and specific expression of the ECS elements in each islet cell type, but immunofluorescence staining of human pancreas has indicated the presence of MAGL, DAGLα, NAPE-PLD and FAAH in β-cells [10]. It is therefore likely that the endogenous production, accumulation and degradation of endocannabinoids are important in the modulation of islet functions.

Previous studies have demonstrated that expression of ECS components is a dynamic process that is modified in response to high fat diet, and high glucose concentrations are reported to increase AEA and 2-AG synthesis in rodent and human islets [10,20]. In the current study the effects of 100 nM of CB1r (ACEA) and CB2r (JWH015) agonists were investigated, to provide a direct comparison with an earlier study in which we had investigated the effects of chronic activation of cannabinoid receptors in mouse islets [24]. The changes that we observed in mRNA levels in isolated human islets of the enzymes involved in the synthesis and degradation of endocannabinoids suggest a negative feedback mechanism in the presence of exogenous agonists, most likely to limit endogenous endocannabinoid levels to minimise over-stimulation of the CB1 and CB2 receptors. However, it is interesting that while chronic activation of human islet cannabinoid receptors led to depletion of mRNAs responsible for regulation of endocannabinoid levels there was a simultaneous increase in the mRNAs encoding CB1r and CB2r, which may have been a



mechanism for sustained responses to these ligands. Generation of AEA and 2-AG in the human islets following exposure to ACEA and JWH015 was not quantified in the current study, but it is possible that the up-regulation of CB1r as well as CB2r mRNA following exposure to JWH015 for 2 days was a consequence of altered synthesis of the CB1r ligand AEA by the islets. The role played by endogenous human islet endocannabinoids in the patterns of ECS gene expression observed here will be the subject of future work.

These changes in the ECS mRNAs in human islets in vitro support a model of dynamic regulation of islet endocannabinoid content to maintain the "on-demand" responses of islets to physiological and pathological stimuli [3,6,19,24,26,31,32]. However, the current study does not provide direct information that islets in situ would necessarily respond in the same manner as islets in vitro. Moreover, the use of whole islets, while providing an appropriate physiological model, does not allow identification of the cells in which the alterations in ECS mRNAs occurs.

Although exposure of human islets to ACEA and JWH015 for 2 days increased insulin and glucagon content, there were no marked effects on acute secretion of these hormones, suggesting that islet stores of insulin and glucagon were enhanced, rather than β- or α-cell secretory capacity. Overall, maintenance of human islets with the cannabinoid receptor agonists was without deleterious effects, either on the capacity of islets to secrete insulin and glucagon in response to glucose and arginine respectively, or in terms of islet cell viability. Our observations that apoptosis was not enhanced by exposure of islets to CB1r or CB2r agonists is in contrast to observations that acute and chronic exposure to cannabinoids induces apoptosis in a number of cell types [5,41–43], including islet β-cells [22]. However, there is also evidence supporting a protective role of 2-AG and AEA in cell survival [44–47], and we have previously reported that pre-exposure of mouse islets to 100 nM ACEA or JWH015 for 7 days protects against cytokine-induced apoptosis [24]. A similar protective effect was not observed in the current studies, which may reflect the 5 day time-course of exposure here and 7 days with mouse islets, or it may indicate species-dependent differences in apoptotic signalling downstream of cannabinoid receptor activation in mouse and human islets. Nonetheless, our data clearly demonstrate that chronic treatment of human islets with 100 nM ACEA or JWH015 does not induce apoptosis. This apparent discrepancy with an earlier study in islets [22] may be due to the use in that study of 25 μM 2-AG and AEA to maximally reduce islet cell viability over 48 h, a concentration that is several orders of magnitude higher than detected in diabetes [3], and considerably higher than those used in the current study.

5. Conclusion

In conclusion, we report that prolonged maintenance of isolated human islets in the presence of cannabinoid CB1 and CB2 receptor agonists modulates the gene expression of ECS components and promotes short-term increases in islet insulin and glucagon content, but does not exert deleterious effects on islet viability or function. Our results suggest that over-activation of islet endocannabinoid receptors induces an adaptation process through down-regulation of endogenous islet cannabinoid levels, but the maintenance of islet viability and function suggests that islet cell death and dysfunction do not play a role in the metabolic defects arising from over-activation of the ECS in obesity and diabetes. Thus, these data are consistent with the impairments in glucose homeostasis observed following increased production of endocannabinoids being a consequence of insulin resistance rather than β-cell dysfunction or α-cell hypersecretion.

Fig. 6. Caspase-3/7 activities in human islets after maintenance for 2 (panel A) or 5 (panel B) days in DMEM containing 5.5 mM glucose alone (control) or supplemented with 100 nM ACEA or 100 nM JWH015. Apoptosis was induced by adding a cytokine cocktail (0.5 U/ml IL1β, 5 U/ml TNFα, and 5 U/ml IFNγ) for the final 20 h of incubation, shown by the black bars. Data are expressed in luminescence units (means + SEM, n = 6 for panel A, n = 4 for panel B). Data were analysed by two-way ANOVA; *$p < 0.05$ with respect to basal apoptosis in the absence of cytokines.

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