

Article

The Impact of CB1 Receptor on Nuclear Receptors in Skeletal Muscle Cells

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Abstract: Cannabinoids are abundant signaling compounds; their influence predominantly arises via engagement with the principal two G-protein-coupled cannabinoid receptors, CB1 and CB2. One suggested theory is that cannabinoids regulate a variety of physiological processes within the cells of skeletal muscle. Earlier publications have indicated that expression of CB1 receptor mRNA and protein has been recognized within myotubes and tissues of skeletal muscle from both murines and humans, thus representing a potentially significant pathway which plays a role in the control of skeletal muscular activities. The part played by CB1 receptor activation or inhibition with respect to these functions and relevant to targets in the periphery, especially skeletal muscle, is not fully delineated. Thus, the aim of the current research was to explore the influence of CB1 receptor stimulation and inhibition on downstream signaling of the nuclear receptor, NR4A, which regulates the immediate impacts of arachidonyl-2'-chloroethylamide (ACEA) and/or rimonabant in the cells of skeletal muscle. Murine L6 skeletal muscle cells were used in order to clarify additional possible molecular signaling pathways which contribute to alterations in the CB1 receptor. Skeletal muscle cells have often been used; it is well-documented that they express cannabinoid receptors. Quantitative real-time probe-based polymerase chain reaction (qRT-PCR) assays are deployed in order to assess the gene expression characteristics of CB1 receptor signaling. In the current work, it is demonstrated that skeletal muscle cells exhibit functional expression of CB1 receptors. This can be deduced from the qRT-PCR assays; triggering CB1 receptors amplifies both NR4A1 and NR4A3 mRNA gene expression. The impact of ACEA is inhibited by the selective CB1 receptor antagonist, rimonabant. The present research demonstrated that 10 nM of ACEA notably amplified mRNA gene expression of NR4A1 and NR4A3; this effect was suppressed by the addition of 100 nM rimonabant. Furthermore, the CB1 receptor antagonist led to the downregulation of mRNA gene expression of NR4A1, NR4A2 and NR4A3. In conclusion, in skeletal muscle, CB1 receptors were recognized to be important moderators of NR4A1 and NR4A3 mRNA gene expression; these actions may have possible clinical benefits. Thus, in skeletal muscle cells, a possible physiological expression of CB1 receptors was identified. It is as yet unknown whether these CB1 receptors contribute to pathways underlying skeletal muscle biological function and disease processes. Further research is required to fully delineate their role(s).

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1. Introduction

Studies have shown that several physiological functions played by the majority of tissues are regulated by cannabinoids [1]. The same is the case for skeletal muscles [2,3]. It has been established that the endocannabinoid system (ECS) works as a complicated endogenous signaling system that is comprised of at least two cannabinoid receptors together with their endogenous ligands as well as enzymes that bring about biosynthesis and degradation of ligands [4]. Receptors of cannabinoids are members of the G-protein coupled receptors superfamily and they have been grouped as CB1 and CB2 re-

ceptors that participate in adenylate cyclase regulation [5]. A number of cells and tissues such as skeletal muscles express cannabinoid receptors [6–8]. The role of CB1 receptor as the main receptor bringing about the outcomes of the endocannabinoid system in metabolic processes. CB1 receptors have proven to be the most commonly expressed GPCRs in case of brain tissue [9], though they are also found in peripheral tissues like skeletal muscle, pancreas, liver and adipose tissue [10,11]. Conversely, CB2 receptors are mostly found to be expressed by constituents of immune system. In particular, they are expressed inside thymus, tonsils and spleen [12,13].

One of the largest tissues of human body is the skeletal muscle. In the case of rats, it constitutes majority of the body weight [14–17]. In general, it has been recognized as the main site of metabolism of fatty acids and glucose [18–23]. The role of skeletal muscle in sustaining body glucose homeostasis has proven to be very important [24]. Moreover, skeletal muscle serves as site of insulin resistance as well. As this tissue is capable of oxidizing fatty acid and glucose, its role in metabolic disorders is also important [25]. A number of scientists sometimes refer skeletal muscle as an endocrine organ considering discharge of inflammatory mediators by this tissue [17]. Taken together, skeletal muscle is essentially involved in glycemic control, sustaining glucose homeostasis and in regulating metabolic reactions of human system [24]. The skeletal muscle may perform the abovementioned functions through the endocannabinoid system; yet this possibility has not been investigated thoroughly.

NR4A is a subfamily of the orphan nuclear receptor superfamily consisting of three members, Nur77 (NR4A1), Nurr1 (NR4A2) and NOR1 (NR4A3) [26]. Several recent studies have demonstrated that NR4A receptors are key transcriptional regulators implicated in various biological processes, such as inflammation, lipid and glucose metabolism, insulin sensitivity, energy balance, and cell proliferation and differentiation [27,28]. These studies have focused on NR4A mainly in the liver, adipose, and skeletal muscle [29,30]. There is growing evidence to suggest that the activation of NR4A leads to an increase in the gene expression of intracellular downstream signaling pathways that potentially participate in the regulation of glucose and fatty acid metabolism and cell growth in skeletal muscle [31,32]. The NR4A family is also reported to play metabolic roles in all major insulin-sensitive target tissues. For example, NR4A1 is reported to be a factor in regulating glucose and lipid metabolism in muscle [33,34]. Cross-talk between the cannabinoid CB1 receptor and NR4A signaling may potentially represent an important yet unknown mechanism contributing to the regulation of skeletal muscle functions. Thus, further studies using L6 skeletal muscle cells as a cell model to understand the molecular signaling involved in the cross-talk are highly significant.

The CB1 receptor was found to be expressed in skeletal muscle [2], cannabinoid receptor ligands were shown to produce a CB1 receptor-dependent reduction in cAMP levels in transfected CHO cells [35] and different regions of the rat brain [36], and cAMP was found to be involved in an increased expression of NR4A in skeletal muscle [7,20,36,37]. Therefore, CB1 receptors can potentially affect the nuclear receptor subfamily 4, group A (NR4A), through the cAMP or G β γ pathway in rat L6 skeletal muscle cell myotubes. Consequently, CB1 receptors may potentially modulate glucose and fatty acid metabolism and inflammation in skeletal muscle tissue. To address this issue, an investigation of the signaling events (NR4A family) underlying the activation and inhibition of the CB1 receptor in rat L6 skeletal muscle cells took place. The purpose of this work was to explain the potential signaling underlying the cannabinoid CB1 receptor modulation on NR4A mRNA gene expression.

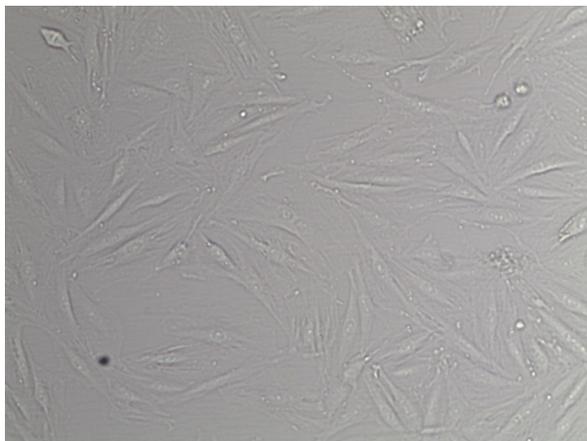
2. Materials and Methods

Tocris Bioscience (Bristol, UK) supplied the insulin, arachidonyl-2'-chloroethylamide (ACEA), and rimonabant, while Santa Cruz (Dallas, TX, USA) provided the dimethylsulphoxide reagent. Thermo Scientific Company (Waltham, MA, USA) supplied the Maxima Probe qPCR Master Mix (2X) and Thermo Scientific RevertAid

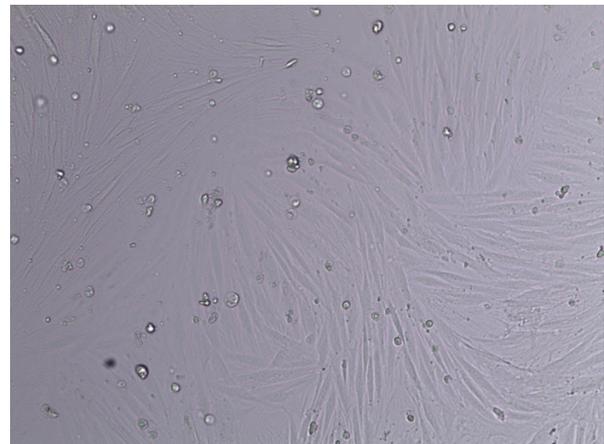
First Strand cDNA Synthesis, while Qiagen (Hilden, Germany) was the provider of the RNeasy Mini Total RNA Purification kits and RNase-Free DNase Set. Applied Biosystem (Waltham, MA, USA) provided Trizol and charcoal stripped serum, while FBS (fetal bovine serum) was obtained from Capricorn Scientific (Ebsdorfergrund, Germany). Sigma Company (Saint Louis, MO, USA) supplied horse serum, and Ham-F 10 was obtained from PAA Company (Cambridge, UK). Dulbecco's modified essential medium (DMEM) was supplied by Caisson (Denver, CO, USA).

2.1. Cell Culture

The American Type Culture Collection (Manassas, VA, USA) provided the L6 skeletal muscle cell line from rats along with the myoblast cell line, originating from cells which had been maintained in the form of an attached monolayer culture in DMEM which had a high glucose level (4500 mg/L) with L-glutamate which had been supplemented by 10% (*v/v*) heat-inactivated FBS and 100 µg/mL of penicillin-streptomycin. Incubation of the cells took place at a temperature of 37 °C using a 5% carbon dioxide atmosphere at 90% humidity. Passaging of the cells occurred at around 60–70% confluency, while changing of the medium was carried out thrice weekly, as shown in Figure 1. The confluent cells underwent 14 days of further culturing in 25 cm² flasks in order to allow myotubes to form, in alignment with the protocols outlined in my previous publications [15–17], albeit with minor modifications (Figure 1). After around two weeks of culturing, the 70–90% confluent myotubes were then exposed to 2% (*v/v*) delipidated serum for a period of 5 h before undergoing starvation for a further 19 h. Figure 1 then shows that the cells were treated for varying periods of time (1, 3, 5, and 24 h) with vehicle (0.1% DMSO), rimonabant 100 nM, ACEA 10 nM, and insulin 100 nM. The ACEA and rimonabant cells had undergone pre-treatment using rimonabant for 10 min before adding ACEA. After the treatment, the cells were washed using ice-cold PBS, before lysing using Trizol (2mL per flask).



(a)



(b)

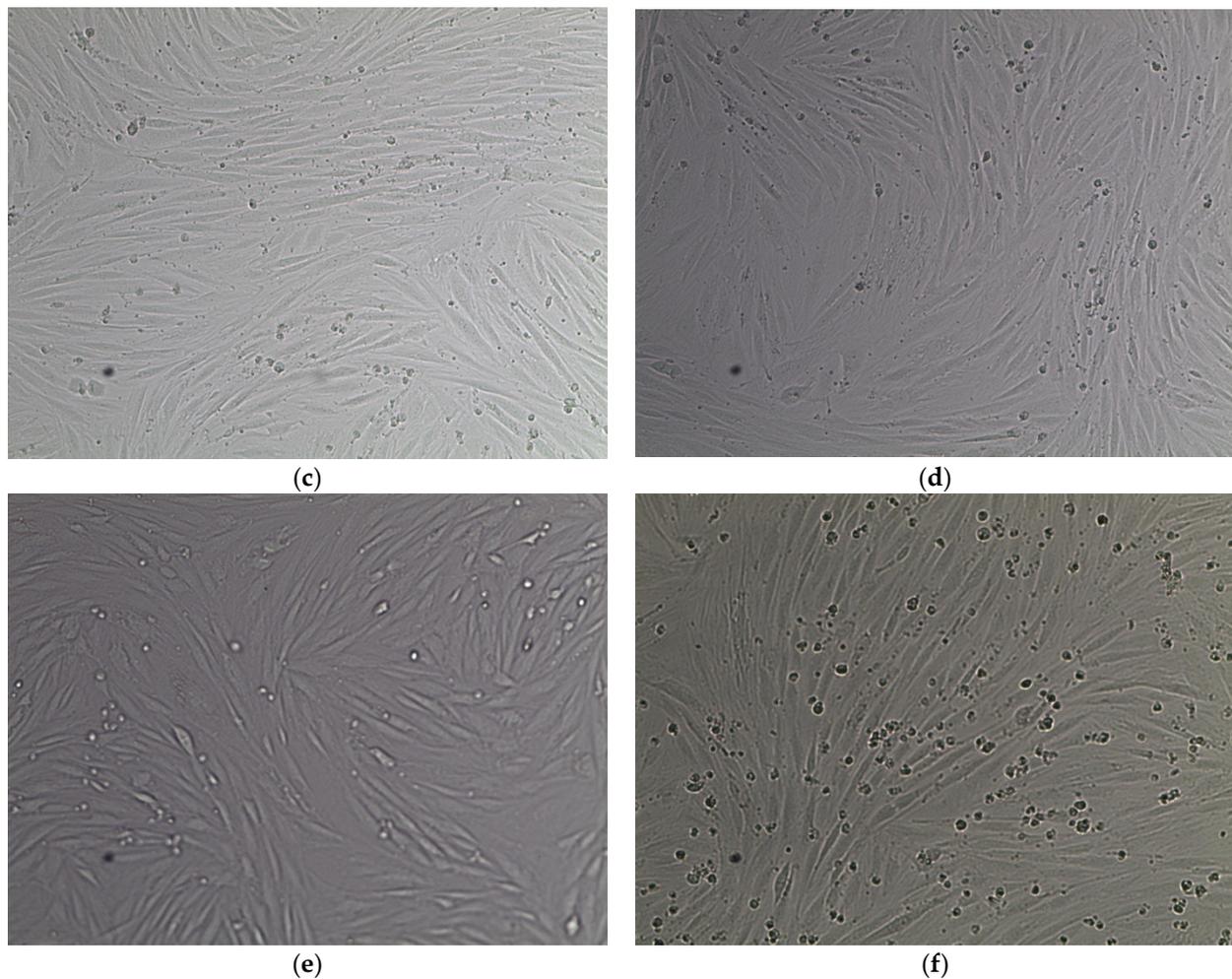


Figure 1. Images depicting L6 skeletal muscle myoblasts from rats, differentiated into L6 skeletal muscle myotubes (passage 7). (a) Myoblasts obtained on day 3 of the tissue culture in 10% FBS Ham F-10 media (10×). (b) Cells obtained on day 4 of the tissue culture in 6% horse serum Ham F-10 media (10×). (c) Myotubes obtained on day 5 of the tissue culture in 2% horse serum Ham F-10 media (10×). (d) Myotubes obtained after 4 h of tissue culture in 2% delipidated serum Ham F-10 media (10×). (e) Myotubes obtained after one hour of cell starvation in only Ham F-10 media (10×). (f) Myotubes obtained after 19 h of cell starvation in only Ham F-10 media (10×).

2.2. Extraction of RNA and Synthesis of cDNA

The L6 skeletal muscle cells of rats were placed in 25 cm² flasks and scraped in 2 mL of ice-cold Trizol, whereupon the RNA was separated and isolated in line with the guidelines of the manufacturer. RNeasy purification columns (Qiagen, Hilden, Germany) were then used to carry out the RNA clean-up and on-column DNase digestion. A spectrophotometer (JENWAY Genova Nano, Stafford, UK) was then used to assess the concentration and purity of the RNA. In order to carry out the cDNA synthesis, a quantity of 500 ng of total RNA underwent reverse transcription via RevertAid First Strand cDNA Synthesis in a process taking 5 min using a total volume of 20 µL at a temperature of 25 °C, before the temperature was increased to 42 °C for the subsequent one-hour period. Finally, termination of the reaction took place for 5 min at 70 °C. Gene expression was then quantified using the relative standard curve approach on the basis of the TaqMan quantitative real-time PCR (qRT-PCR). For this process, the preparation of the samples was carried out using a total reaction volume of 25 µL (comprising 13 µL Maxima Probe qPCR Master Mix 2× reagent, 1.5 µL of forward primer (10 µM), 2.5 µL Probe (2 µM), 1.5 µL of reverse primer (10 mM), 5 µL of water, and 5 µL of cDNA). A 7500 fast real-time PCR system (Applied Biosystems, Waltham, MA, USA) was employed to complete the qRT-PCR analysis, while the determination of the gene expression was made

by considering the relationship to the reference gene, TATA. Primer Express software (Applied Biosystems, USA) was used in the case of probes and primers for all genes, as shown in Table 1, with the design and synthesis performed by Integrated DNA Technologies, Inc. (Coralville, IA, USA). The standard curve approach was employed, using a slope ranging from -3.2 to -3.6 with R^2 values exceeding 99%, reflecting efficiency of amplification approaching 100%.

Table 1. List of gene primer and probe sequences.

Gene	Sequences (5' → 3')	Amplicon Size (bp)
NR4A1	Probe 5'-CTTTATCCTCCGCCTGGCCTACCGA-3'	95
	Forward primer 5'-TGTTGCTAGAGTCCGCCTTTC-3'	
	Reverse primer 5'-CAGGCCTGAGCAGAAGATGAG-3'	
NR4A2	Probe 5'-TACGCTTAGCATAACAGGTCCAACCCAGTG-3' For-	116
	ward Primer 5'-CCAAAGCCGATCAGGACCT-3'	
	Reverse primer 5'-GACCACCCATTGCAAAAGAT-3'	
NR4A3	Probe: 5'-ACTGTCCCACCGACCAGGCCACT-3'	92
	Forward Primer: 5'-GACGCAACGCCAGAGAC-3'	
	Reverse primer 5'-TAGAACTGCTGCACGTGCTCA-3'	
TATA-BOX	Probe 5'-TCCCAAGCGGTTTGCTGCAGTCA-3'	73
	Forward Primer 5'-TTCGTGCCAGAAATGCTGAA-3'	
	Reverse Primer 5'-GTTCGTGGCTCTCTTATTCTCATG-3	

2.3. Data Analysis

Data are presented in the form of mean \pm SEM following the generation of triplicate or quadruplicate wells from no fewer than three experimental groups. Data analysis of mRNA data employed one-way ANOVA and a Tukey test. The GraphPad Prism, version 5.03 (GraphPad Software Inc, San Diego, CA, USA) was used for all analyses, and the statistical significance level was determined to be $p < 0.05$.

3. Results

3.1. Effects of ACEA, Rimonabant, and Insulin on NR4R1 mRNA Gene Expression

Using delipidated serum, treating the cells with ACEA (10 nM) for 5 h significantly up-regulated NR4A1 mRNA gene expression ($p < 0.01$). However, these responses were blocked by rimonabant (100 nM). The influence of ACEA on NR4A1 is therefore CB1 dependent. Interestingly, rimonabant significantly down-regulated NR4A1 mRNA gene expression ($p < 0.001$). Notably, using the delipidated serum, treating the cells with insulin (100 nM) for 5 or 24 h significantly down-regulated NR4A1 mRNA gene expression ($p < 0.001$) (Figure 2).

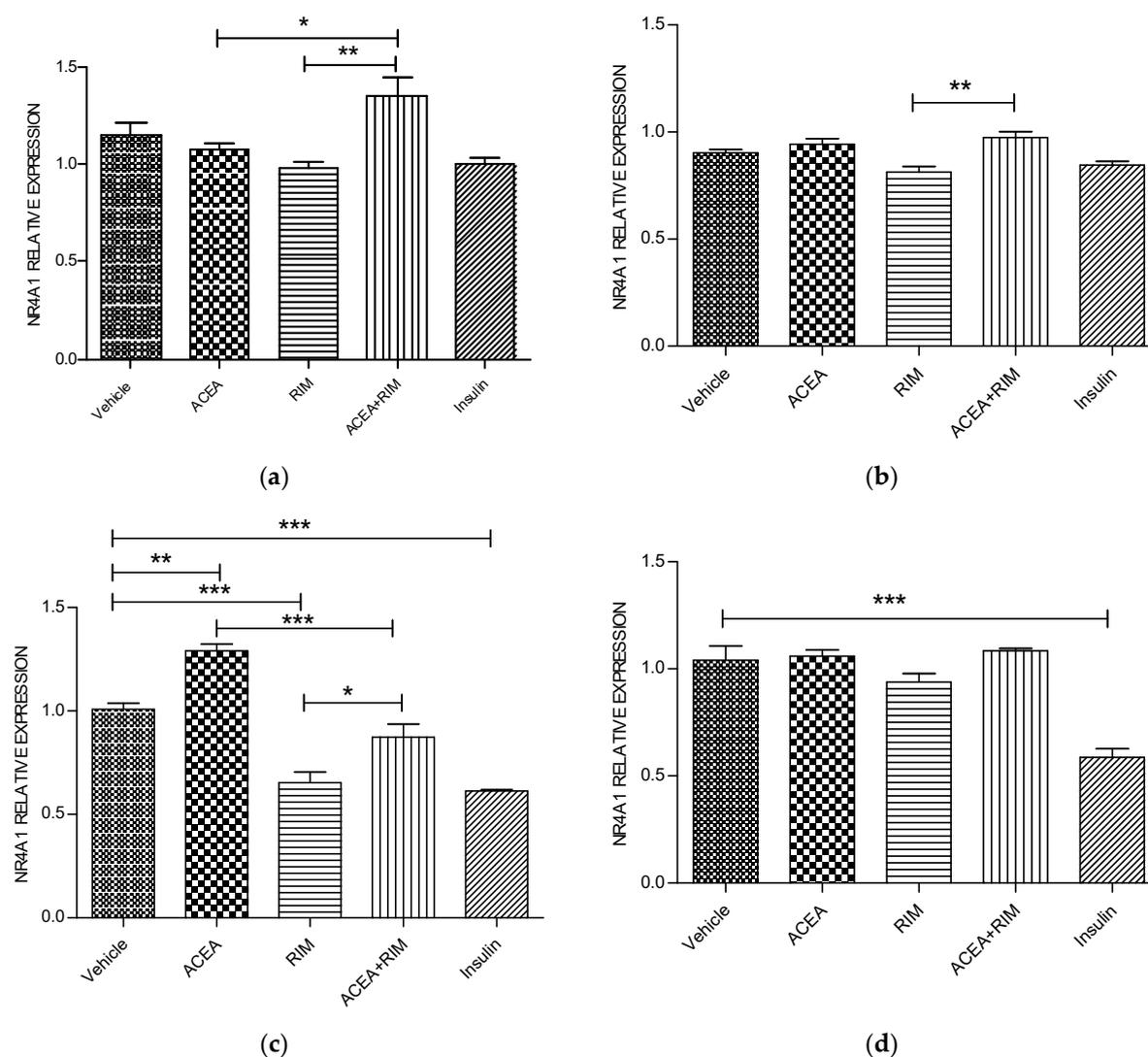


Figure 2. NR4A1 muscle myotubes—ACEA, rimonabant, ACEA and rimonabant, and insulin affect NR4A1 gene expression; myotubes fed with delipidated serum. The stimulation time covered 3 to 24 h, and NR4A1 mRNA levels, relative to TATA-Box, was evaluated by quantitative real-time PCR (qRT-PCR) (100 nM). The following scenarios explain the stimulation process conducted: (a) Stimulation was done for 1 h. (b) Stimulation was done for 3 h. (c) Stimulation was applied for up to 5 h. (d) Stimulation was applied for up to 24 h. The data were reported as the mean \pm SEM of three separate groups. (n = 3; *denotes $p < 0.05$, **denotes $p < 0.01$, and ***denotes $p < 0.001$). Data were investigated by conducting one-way ANOVA test and Tukey test. NR4A1; nuclear receptor subfamily 4, group A, member 1.

3.2. Effects of ACEA, Rimonabant, and Insulin on NR4R2 mRNA Gene Expression

Using delipidated serum, treating the cells with rimonabant (100 nM) for 24 h significantly down-regulated NR4A2 gene expression ($p < 0.05$). By contrast, treating the cells with insulin for 1 or 3 h significantly down-regulated NR4A2 mRNA expression (Figure 3).

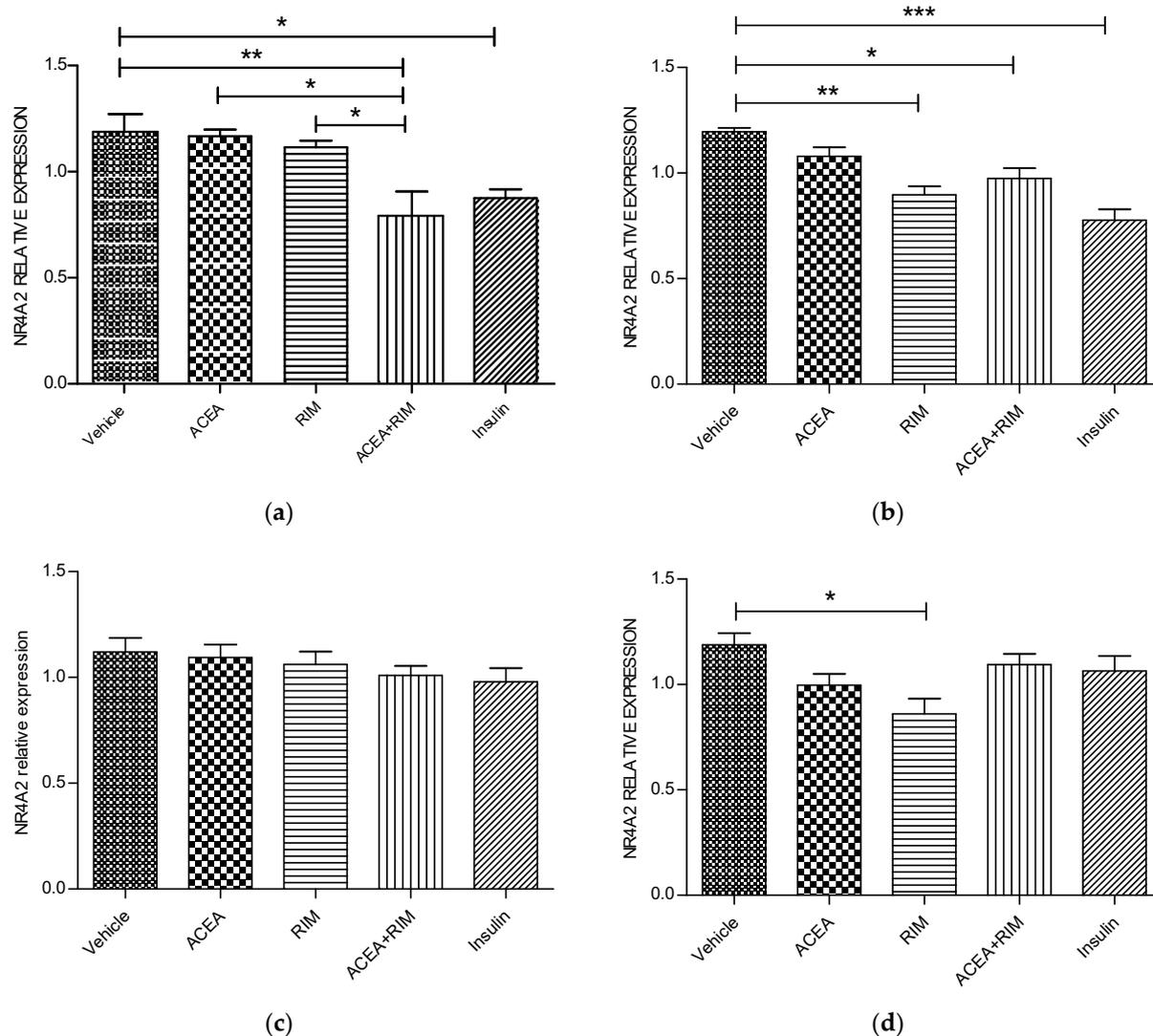


Figure 3. NR4A2 muscle myotubes—ACEA, rimonabant, ACEA and rimonabant, and insulin affect NR4A2 gene expression; myotubes fed with delipidated serum. The stimulation time covered 3 to 24 h, and NR4A2 mRNA levels, relative to TATA-Box, was evaluated by quantitative real-time PCR (qRT-PCR) (100 nM). The following scenarios explain the stimulation process conducted: (a) Stimulation was done for 1 h. (b) Stimulation was done for 3 h. (c) Stimulation was applied for up to 5 h. (d) Stimulation was applied for up to 24 h. The data were reported as the mean \pm SEM of three separate groups. ($n = 3$; *denotes $p < 0.05$, **denotes $p < 0.01$, and ***denotes $p < 0.001$). Data were investigated by conducting one-way ANOVA test and Tukey test. NR4A2; nuclear receptor subfamily 4, group A, member 2.

3.3. Effects of ACEA, Rimonabant and insulin on NR4A3 mRNA Gene Expression

Using delipidated serum, treating the cells with ACEA (10 nM) for 5 h significantly up-regulated NR4A3 mRNA gene expression ($p < 0.05$). However, these responses were blocked by rimonabant (100 nM). The influence of ACEA on NR4A3 is therefore CB1 dependent. Interestingly, treating the cells with rimonabant (100 nM) for 1 h and 3 h significantly down-regulated NR4A3 gene expression ($p < 0.05$ and $p < 0.05$, respectively). Notably, using the delipidated serum, treating the cells with insulin (100 nM) for 1, 3 or 5 h significantly down-regulated NR4A3 mRNA gene expression (Figure 4).

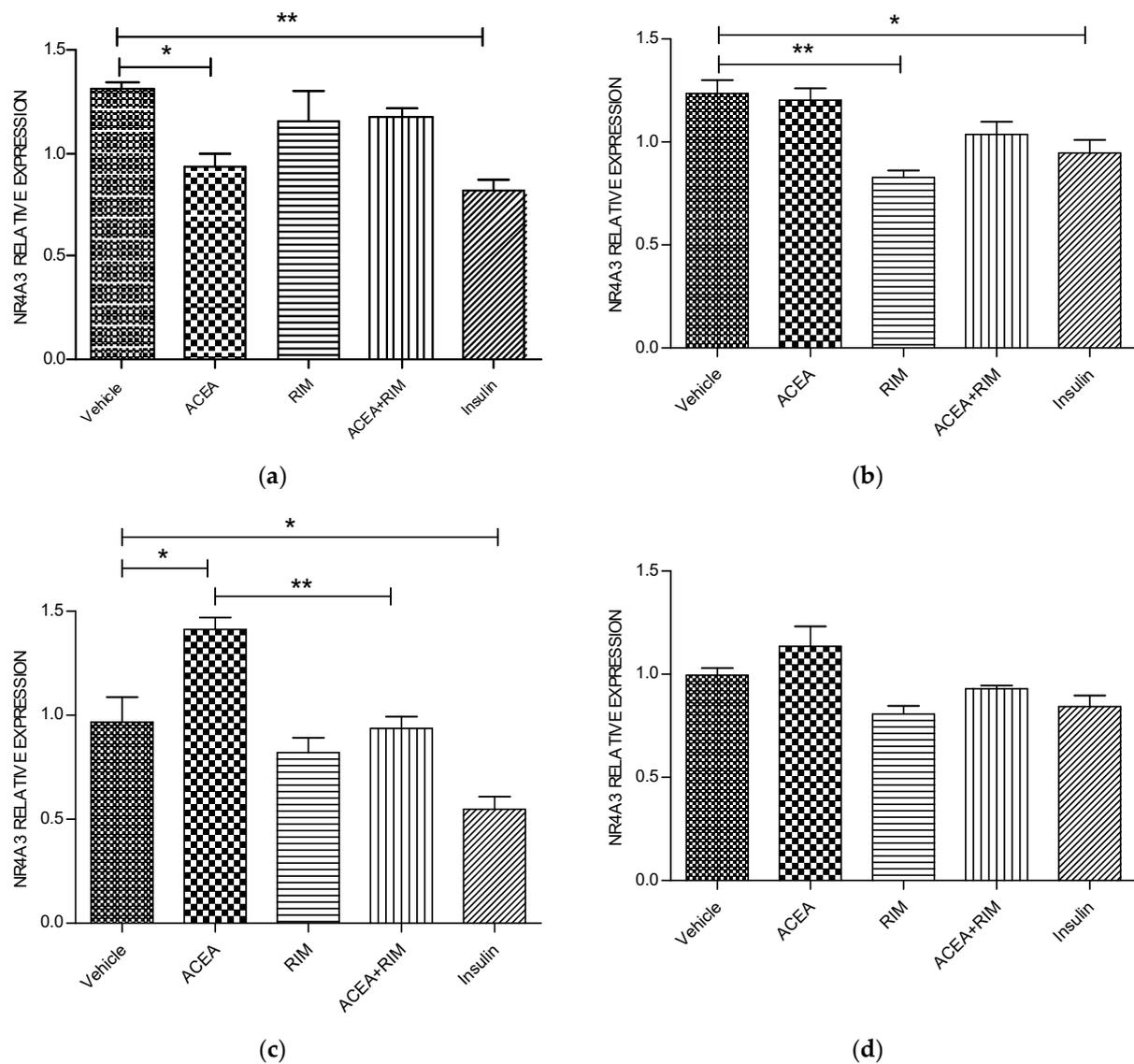


Figure 4. NR4A3 muscle myotubes—ACEA, rimonabant, ACEA and rimonabant, and insulin affect NR4A3 gene expression; myotubes fed with delipidated serum. The stimulation time covered 3 to 24 h, and NR4A3 mRNA levels, relative to TATA-Box, was evaluated by quantitative real-time PCR (qRT-PCR) (100 nM). The following scenarios explain the stimulation process conducted: (a) Stimulation was done for 1 h. (b) Stimulation was done for 3 h. (c) Stimulation was applied for up to 5 h. (d) Stimulation was applied for up to 24 h. The data were reported as the mean \pm SEM of three separate groups. ($n = 3$; *denotes $p < 0.05$, and **denotes $p < 0.01$). Data were investigated by conducting one-way ANOVA test and Tukey test. NR4A3; nuclear receptor subfamily 4, group A, member 1.

4. Discussion

According to the novel findings of the present study, endocannabinoid analogue ACEA raises the gene expression of mRNA related to NR4A1 and NR4A3 in skeletal muscles. It has an effect through ACEA facilitated by the subtype cannabinoid CB1 receptor. To our knowledge, it is the first research about the effect of ACEA and its cannabinoid CB1 receptor subtypes on nuclear receptors (NR4A) in skeletal muscles, and it characterizes a novel mechanism of signaling for the cannabinoid CB1 receptor's role in the cells of skeletal muscles. In the current research, the functionality of CB1 receptor was examined by evaluating the effect of CB1 receptor agonism (ACEA) or antagonism (rimonabant) on the activation of significant genes (nuclear receptors; NR4A1, NR4A2 and NR4A3) that might be involved in mitogenic, inflammatory and metabolic functions in rat skeletal muscle cells. Therefore, this study might also represent evidence that the CB1 receptor is potentially functionally active regarding inflammation and possibly in mitogenic and metabolic functions and for glucose and fatty acid metabolism.

Interestingly, in the current study, insulin 10 nM downregulated NR4A1, NR4A2 and NR4A3 expression in skeletal muscle cells using these time frame. Those NR4A expression was similarly inhibited by rimonabant. Rimonabant, a selective cannabinoid CB1 receptor antagonist/inverse agonist, was proven to inhibit the gene expression engaged in glycolysis proteins, glucose oxidation, insulin resistance and metabolism, transportation of fatty acids and their oxidation and regulation of energy and its metabolism, proliferation, differentiation and myogenesis (NR4A1, NR4A2 and NR4A3) in this study. Those NR4A expression was likewise reduced by insulin. In terms of the effect of ACEA, the expressions of genes (NR4A1 and NR4A3) have been substantially contrasted to those of insulin and rimonabant. The current pharmacological investigation makes use of rimonabant, a selective cannabinoid CB1 receptor antagonist, and ACEA, a specific cannabinoid CB1 receptor agonist. As a result, rimonabant's impact provides convincing evidence for the participation of cannabinoid CB1 receptors at the very least. The selective cannabinoid receptor antagonist rimonabant was shown to enhance the gene expression involved in insulin sensitivity, glucose uptake, myogenesis and other metabolic processes which is in line with the same effect of insulin for those genes. This agrees with previous literature on skeletal muscle [38–40] that found the activation of CB1R in skeletal muscle cells is associated with insulin resistance, and impaired metabolic function, owing to increased energy intake and storage, impaired glucose and lipid utilization, and enhanced oxidative stress. This conclusion does not conflict with a previous researcher [27], who found that—in adipose cells (3T3-L1 cells), but not in skeletal muscle—the activation of NR4A receptors is known to promote glucose utilization by enhancing the activity of insulin to stimulate glucose transport since this research study used a different cell line culture.

ACEA was also employed, as it is 2000-times more specific for CB1 receptors than CB2 receptors [35]. In this current work, ACEA was observed to boost NR4A1 and NR4A3 mRNA expression in rat skeletal muscle myotubes. Rimonabant, a selective CB1 receptor antagonist/inverse agonist, was shown to prevent this ACEA-induced action. Because ACEA is a selective CB1 receptor agonist at the dose utilized in this current study, this data implies that NR4A1 and NR4A3 activation was mediated through CB1 receptor activation. In skeletal muscles, the CB1 receptor is an active receptor based on functions, according to this research. The following are the outcomes of our research. (I) The selective cannabinoid CB1 receptor agonist ACEA boosts NR4A1 and NR4A3 expression. The purpose of testing the action of this agonist at a concentration of 10 nM [35] was to test this hypothesis. (II) Rimonabant, a selective antagonist of the cannabinoid CB1 receptor subtype and CB1 inverse agonists and antagonists [38,41], blocked these responses. These data, taken together, offer significant evidence for the participation of cannabinoid CB1 receptors in ACEA-induced upregulation of NR4A expression. (III) Rimonabant suppresses the expression of the NR4A mRNA gene.

In this current study, the activation of cannabinoid CB1 receptor has been found to enhance expression of NR4A in skeletal muscles. So, CB1 receptors can moderate metabolism of glucose and fat in skeletal muscles. It is reinforced by the circumstance that (1) NR4A was found to be decreased in the skeletal muscles among diabetic animals [27], (2) NR4A is linked with genes linked with fatty acid and glucose utilization via mRNA up-regulated expression of FOXO1, PDK4, lipin-1 α , and PGC-1 α [42], (3) after feeding the high-fat diet, NR4A null mice was compared with wild-type animals that presented reduced mRNA expression of PDK4, Lipin 1 α , and GLUT4 along with impaired insulin resistance and phosphorylation of insulin receptor substrate 1 (IRS-1) in skeletal muscles and reduced clearance of blood glucose, higher body weight and low energy consumption [28], (4) C2C12 siRNA-NR4A cells were presented to reduce the mRNA expression in C2C12 cells of fatty acid translocase (CD36/fat), uncoupling GLUT4 and protein3 (UCP3) than that of wild-type native C2C12 cells [43,44] and (5) non-insulin glucose consumption was revealed to raise significantly NR4A expression mediated by adenovirus in C2C12 cells than those of normal C2C12 cells [45,46]. Based on this, the CB1 receptor modulation through ligands may affect utilization of fatty acid and glucose in skeletal muscles. As a result, CB1 receptor agonists/antagonists could be explored as a potential therapeutic option in patients with diabetes or adiposity. Additional research will be conducted in the future to elucidate this point.

NR4A activation has been linked to enhanced gene expression of various metabolic genes in a variety of tissues [47], particularly in skeletal muscles. Activation of NR4A has been linked to muscle growth and development, glucose metabolism, and oxidation of fatty acid [34,43]. As a result, it is probable that ACEA exerts at least the effects listed earlier in skeletal muscle, and that these actions are facilitated via activation of cannabinoid CB1 receptors. Cannabinoid receptors, namely cannabinoid CB1 receptors, have been shown to regulate a variety of cellular responses implicated in obesity and glucose homeostasis formerly [1,39,40]. Because cannabinoid CB1 receptors increased NR4A mRNA gene expression in skeletal muscles and NR4A1 restrains inflammation, glucose transport, and insulin action [45,48], it is possible that cannabinoid CB1 receptors modulate skeletal muscle physiological roles including glucose and fatty acid metabolism. The function of these genes in muscle tissue are little understood in the research.

Using rimonabant, a unique therapeutic intervention in the treatment of hyperglycemia and obesity might occur through the antagonism of the endocannabinoid system. In fact, studies from animals and humans showed an increase in the levels of endocannabinoids in the obese state. In addition, obese animal models showed that the levels of endocannabinoids were increased in the peripheral and hypothalamus tissues [49–51]. Furthermore, previous studies demonstrated that circulating levels of endocannabinoids including anandamide (AEA) and 2-Arachidonoylglycerol (2-AG) were raised in visceral adipose tissue in hyperglycaemic type 2 diabetic and obese patients [49,52,53]. Moreover, CB₁ knock-out mice were found to be resistant to diet-induced obesity [50,54]. Originally, CB₁ receptor antagonism was also realized to potentially enhance metabolic parameters [55–57].

CB1 receptor expression has been reported in rodent and human skeletal muscle in past studies [2]. CB1 receptor protein expression was observed to be considerably lower in obese Zucker rats' soleus muscle compared to lean Zucker rats [40]. CB1 receptor mRNA expression in the soleus muscle of C56BL/6 mice was likewise observed to be higher following high fat eating [1,49]. The G proteins of the Gi/o family are involved in CB1 receptor signaling [58]. Pertussis toxin, in fact, decreased the effect of CB1 receptor activation [59]. As a result, Gi/o inhibits adenylyl cyclase and, as a result, cAMP buildup. Ion channels can also be regulated by Gi/o, with calcium channels being inhibited [60–62] and activating potassium channels through G $\beta\gamma$ [63–65]. Further studies are required to be performed to examine the mechanism beyond the NR4A signaling in response to CB1 receptor activation/inhibition.

Rimonabant may also act as an agonist through other receptors like GPR55, according to some research [66]. It is also worth noting that rimonabant can also work as an inverse agonist. So, the explanation of rimonabant response is very difficult to describe. On the basis of current data, it is also impossible to say if the effects of rimonabant are depending on CB1 receptor inhibition (a CB1 receptor dependent manner). More studies, such as employing charcoal stripped serum, must be done to get a precise, comprehensive picture of these concerns. To better understand these effects, more research is needed, for example utilizing GPR55 antagonist or CB1 receptor siRNA. Of note, it can be suggested that there is a need for more studies assessing protein expression level. Additionally, more work is also suggested to assess the direct effect of cannabinoid CB1 receptor on NR4A using knock out of CB1 receptor in L6 skeletal muscle cells or to mimic the muscle physiology using knock out of CB1 receptor in primary myoblast isolated from mice or rats. This could provide more evidence with regards to this signaling. Other areas that could benefit from more studies include assessing more important genes connected to this signaling in skeletal muscle such as PDK4 and CPT1B and assessing functional assays such as glucose uptake and fatty acid oxidation.

5. Conclusions

On the basis of these outcomes, it has been established that ACEA raises the mRNA expression of NR4A1 and NR4A3 frequently through the CB1 cannabinoid receptor signaling pathway. In addition, the expression of NR4A mRNA was found to be down-regulated by rimonabant. In the skeletal muscles, the cannabinoid CB1 receptors are expressed efficiently and often signals via NR4A pathways. Cannabinoid CB1 receptor antagonists/agonists/inverse agonists can be a useful mediator to assist the skeletal muscles in functional activities. In fact, the cannabinoid CB1 receptors are novel and highly significant drug targets in the curing and therapeutic management of inflammatory and metabolic diseases. Overall, it was found that the CB1 receptor is significantly efficient in skeletal muscles of rats. More research is necessary to validate the detailed part of endocannabinoids in the gene expression regulation in skeletal muscles and its significance in the development of glucose and fatty acid metabolism, inflammation, proliferation, myogenesis, obesity, and insulin resistance. This might also support evidence for possible potential roles of the CB1 receptor in skeletal muscle, and this may have general implications for diabetes mellitus, obesity, inflammation and wound healing.

6. Significance

This study describes a previously undiscovered signalling system in skeletal muscle cells that involves cannabinoid CB1 receptors. Furthermore, it explains a previously unknown process of skeletal muscle nuclear signalling to reveal another possible therapeutic target for at least metabolic diseases.

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Conflicts of Interest: The author declares that has no conflict of interest.

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