Heliyon 8 (2022) e11336

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The involvement of peroxisome proliferator-activated receptor gamma (PPARγ) in anti-inflammatory activity of N-stearoylethanolamine

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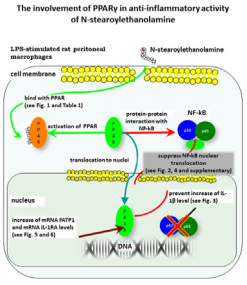
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
N-Stearoylethanolamine
PPAR
NF-kB
IL-1β
Inflammation
Insulin resistance

ABSTRACT

Background: N-stearoylethanolamine (NSE) is a bioactive lipid amine with a wide range of biological activities. Anti-inflammatory properties of NSE were previously confirmed on multiple animal models. However, the molecular mechanisms of anti-inflammatory action of NSE remain unclear. In the current study, we examined the involvement of nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) in the NF-kB –dependent pathway of anti-inflammatory action of NSE using different methodological approaches.

Methods: Molecular modeling calculated the possibility of NSE binding PPAR. *Ex vivo* experiment, using selective agonist of PPARα/γ - LY-171883 and antagonist of PPARγ - GW9662, examined the role of PPARα/γ in the NSE's effect on nuclear NF-kB translocation in LPS-activated rat peritoneal macrophages. Finally, the NSE's action on mRNA level of PPARγ-dependent genes was studied in the liver of insulin-resistant rats.

Results: The results of molecular docking showed that NSE could bind to PPARγ and compete for the binding site with antagonist GW9662 and agonist LY-171883. These data was supported by *in vitro* study where pre-treatment with NSE prevented further LPS-induced NF-kB translocation into the nuclei of rat peritoneal macrophages. NSE treatment before GW9662 and LPS addition normalized the level of NF-kB translocation and IL-1β content. This finding confirmed a competitive binding of NSE with GW9662 for the ligand-binding domain of PPARγ. Additional *in vivo* study showed that NSE administration changed the mRNA expression of several PPARγ target genes, including SLC27A1 encoding fatty acid transport protein-1 and IL1RN - interleukin-1 receptor antagonist in insulin resistant rats.

Conclusion: NSE suppressed nuclear translocation of NF-κB in LPS-stimulated peritoneal macrophages via PPARγ and changed hepatic mRNA expression of PPARγ target genes (SLC27A1, IL1RN) in insulin resistant rats.

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https://doi.org/10.1016/j.heliyon.2022.e11336
Received 23 February 2022; Received in revised form 18 April 2022; Accepted 24 October 2022

1. Introduction

N-stearoylethanolamide (NSE) is a lipid-signaling molecule from N-acylethanolamines (NAEs) family. NAEs are produced "on demand" from their precursors N-acylphosphatidylethanolamines (NAPE) in response to tissue injury and stress to restore homeostatic balance and prevent further damage [1]. NAEs are the derivatives of ethanolamine and fatty acids with different length and saturation of acyl chain, which determine their biological activity. Unsaturated NAEs (anandamide) belong to endocannabinoid system and mainly exert their biological action by activating cannabinoid receptors (CB) [2]. NAEs with saturated chains (N-palmitoylethanolamine and N-stearoylethanolamine) are CB inactive, however, they show cannabimimetic/cannabinoid-like properties [3]. Various biological and pharmacological effects of NSE have been reported under different pathological conditions. NSE has been shown to protect cells from oxidative stress [4], exhibiting membranoprotective [5], neuroprotective [6] and adaptogenic properties [7], as well as anorexic [8] and anti-inflammatory action [9].

In our previous studies we have reported that NSE normalizes liver and pancreas lipid composition, increases insulin sensitivity and restores the ratio of low-density lipoprotein (LDL) cholesterol/high-density lipoprotein (HDL) cholesterol in blood plasma of rats with obesity-induced insulin resistance (IR) [10].

Over the past 2 decades, different research revealed that obesity and insulin resistance are associated with chronic low-grade inflammation. In case of adaptive cellular response and inflammation, NF-kB is one of the major transcription factors that affects key genes involved in the immune, acute-phase, inflammatory response. Particularly, IL-10, TNFα, IL-1β, IL-2, IL-6, IL-12, IL-8 and other cytokines, inducible enzymes (iNOS, Cox-2), adhesion molecules (ICAM-1, VCAM-1, E selectin), the major histocompatibility complex (MHC-I, MHC-II), complementary proteins (B, C3, C4), contiguous cell cycle factors (p53, cyclin D1, etc.), inhibitors and activators of apoptosis (IAP1, c-IAP2, FasL, Bcl-2, TRAF-2, etc.) [11, 12, 13, 14]. Earlier, we have reported that NSE exert the anti-inflammatory action by inhibiting NF-kB translocation into the nuclei of LPS-activated rat peritoneal macrophages [15].

PPARs may control inflammation and dyslipidemia through multiple mechanisms unique to a given cell type. Some of these mechanisms occur in the cytoplasm by interfering with the NF-κB activating machinery. The activation of NF-κB requires the inactivation by phosphorylation of its cytoplasmic inhibitor, IκBα, by the inhibitor of NF-κB kinase complex. Thus, previous studies provided an evidence that stimulation of PPARγ with rosiglitazone significantly attenuated biliary damage and inflammation by up-regulating IκBα in cystic fibrosis transmembrane conductance regulator (Cftr) knockout mice exposed to lipopolysaccharides [16]. Meanwhile, another study showed that troglitazone selectively blocked TNFα-induced and NF-κB-dependent repression of multiple adipocyte-specific genes and improve insulin sensitivity involving p65 subunit (RELA) of NF-κB. PPARγ activation blocked p65-mediated gene transcription, and the inhibition was also independent of the presence of any PPARγ-response elements in the promoter region of NF-κB-activated genes. Thus, p65 and PPARγ antagonized the transcriptional activity of each other. The *in vivo* significance of the functional antagonism between p65 and PPARγ was that these two major transcription factors lied in a common pathway that integrates multiple signals regulating adipocyte gene expression and function. The balance between the activities of p65, PPARγ, and possibly other DNA-binding proteins therefore is important for adipocyte function and response to insulin [17].

Therefore, we suggest that particular action of NSE could be realized via interaction with PPARγ. Considering this, the aim of this study was to reveal the involvement of PPARγ in the mechanisms of anti-inflammatory action of NSE.

2. Materials and methods

2.1. Reagents

NSE was synthesized in the Department of Lipid Biochemistry at Palladin Institute of Biochemistry as described previously [18, 19]. Primers were purchased from Metabion (Litva).

2.2. Peritoneal macrophages isolation procedure

Male Sprague-Dawley rats (220 ± 20 g) were used in the study. All procedures were conducted in accordance with the rules of Commission on Bioethics of Institute of Biochemistry, National Academy of Science and "General ethics principles on experiments with animals" of the 1st National Congress on Bioethics (Kyiv, 2001). Rat peritoneal macrophages were collected by lavaging peritoneal cavity with RPMI-1640 and cultivated directly on the glass cover-slips in a 35 mm dish (1.6×10^6 cell in each sample) during 60 min with 10 μL lipopolysaccharide (LPS) (from Escherichia coli 0127:B8, Sigma-Aldrich) solution (1 mg/mL PBS). 15 min before LPS stimulation the water suspension of NSE (10^{-7}M) and/or selective agonist of PPAR-α/γ LY-171883 (Sigma-Aldrich, cat. #L5408) 125 μM, selective antagonist of PPARγ GW9662 (Sigma-Aldrich, cat. #M6191) 1.25 μM were added to the medium.

2.3. Immunofluorescence confocal microscopy

The NF-κB activation was determined by immunofluorescence assay, evaluating the nuclear translocation of NF-κB p-65 subunits. Cells were fixed with 3.5 % paraformaldehyde in PBS for 10 min at room temperature and permeabilized with 0.2% Triton X-100 (in PBS) for 5 min. To investigate the cellular localization of NF-κB, cells were treated with a rabbit polyclonal antibody against NF-κB p65 (Abcam, cat. #ab16502) diluted at 1:600 in 1% albumin on PBS for 1.5 h. After extensive washing with PBS, cells were further incubated with a goat secondary FITC-conjugated donkey anti-rabbit IgG antibody (Abcam, cat. #ab6717) diluted at 1:500 in PBS containing 0.1% albumin for 1h at a room temperature. Nuclei were stained with 0.5 g/mL of DAPI (Sigma-Aldrich, cat. #D9542), and then were analyzed with Zeiss LSM 510 Meta confocal microscope. Randomly, 30–150 cells from each confocal image (1024 × 1024 pixels) were counted with independently developed software based on ImageEn component suite (Xequte Software, New Zealand) that calculated the percentage of green pixels in the nucleus zone. The percentage of FITC particles (p65 subunit of NF- κB) was equal to the amount of green pixels.

2.4. Cytokine level measurement

The IL-1β level in supernatants of rat peritoneal macrophages was measured using ELISA kit (eBioscience, Austria, cat. #BMS630).

2.5. Animal model

The study was carried out on male Sprague-Dawley rats (188 ± 38 g). All procedures were conducted in accordance with the rules of Commission on Bioethics of Institute of Biochemistry, National Academy of Science and "General ethics principles on experiments with animals" of the 1st National Congress on Bioethics (Kyiv, 2001). Rats were housed in standard cages with free access to food and water. Obesity-induced insulin resistance was attained in rats by prolonged high-fat diet (58% fat: 23% proteins:10 % carbohydrates) as described earlier [10]. The amount of lipids in the diet was increased by addition of lard to the pellet chow. The high-fat diet (HFD) contained 55% of saturated (24% palmitic and 28% stearic acid) and 45% of unsaturated fatty acid. Control rats during the experiment were on normal pellet diet (4% fat: 23% proteins: 65% carbohydrates) with saturated/unsaturated fatty acid ratio 38%/62%,

respectively. Six months after HFD period, the oral glucose tolerance test was conducted [10]. Obtained results showed that after glucose administration (1 mL of 50% glucose solution) to HFD rats, blood glucose level was 8.5 mM within 90 min (in controls –5.1mM), and above 5mM within 150 min (in controls –decreased to normal levels – 3.8 mM). Rats that showed impaired glucose tolerance were selected and divided into 2 groups: IR and IR + NSE. Rats from IR + NSE group were treated with water suspension of NSE, per os, at a dose of 50 mg per kg of body weight, during 14 days.

Control rats with normal glucose tolerance were further subdivided into control (n = 10) and NSE (n = 7) groups. Animals in NSE and IR + NSE groups were orally received the water suspension of NSE for 2 weeks at the dose of 50 mg/kg of body weight. This particular dose of NSE has been chosen as an optimal reacting dose for the research. Schmid H. H et al. earlier reported that this concentration of NSE was found during dog coronary artery occlusion experiment [20]. The IR was confirmed based on the results of fasting plasma insulin levels (measured by ELISA kit, DRG Germany) and HOMA-IR (homeostatic model assessment – insulin resistance) value, calculated by fasting insulin (nM) × Fasting glucose (mM)/22.5.

At the end of the experiment, rats were scarified under Nembutal anesthesia (50 mg/kg body weight). The liver was immediately removed and frozen at-80 °C until further analysis.

2.6. RT-PCR analysis

RT-PCR analysis was used for evaluating the hepatic mRNA level of PPAR-target genes, fatty acid transporter protein-1 (FATP1) encoded by SLC27A1 and interleukin-1 receptor antagonist (IR1-RA) encoded by IL1RN in rats.

RNA was isolated from rat liver tissue using TRI Reagent (Sigma-Aldrich) according to the manufacturer's instructions. The concentration of the isolated RNA was determined using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). Reverse transcription was performed using First Strand cDNA Synthesis Kit (K1612, Thermo Scientific™, USA) and 1.5 μg of total RNA, as well as hexamer primer. Obtained complementary DNA was used as a template for gene-specific PCR amplification. To evaluate the mRNA expression, primers of the following sequence were used for *SLC27A1:* forward 5'-GACTTCT-CACTCTGAGCCTGGT-3', and reverse 5'-GTGTGCATAGTGGGTTGTAG GA-3'; for *IL1RN*: forward 5'-CAAGAACAAAGAAGAAGACAAGCG-3', and reverse 5'-GCAAGTGATTCGAAGCTGGTG-3'. Expression of genes was analyzed against reference gene - 18S ribosomal RNA, the primers of which had the following sequences: forward 5'-CTTAGAGGGA-CAAGTGGCG-3' and reverse 5'-GGACATCTAAGGGCATCACA-3'.

PCR amplification of genes was performed in 10 μl of SYBR™ Green PCR Master Mix (Catalog number: 4309155, ThermoFisher, USA) containing 40 pmoles of each primer. The volume was brought to 20 μl with deionized water. The amplification was performed in the "7500 Fast Real-Time PCR System" thermocycler. The amplification program began with the preliminary activation of AmpliTag Gold® DNA polymerase for 10 min at t = 95.0 °C and included 45 cycles each consisting of denaturation at t = 95.0 °C (19 s), primer attachment and elongation at t = 58.0 °C (1 min). To control specificity, a dissociation step was added - a consistent increase in temperature from 58.0 to 95.0 °C, with the recording of a decrease in the fluorescence intensity of double-stranded DNA complexes with SYBR Green. The relative level of gene expression was determined using a commonly used technique (expression level = 2-ΔCt, where Ct is the threshold amplification cycle). We calculated the expression of the target gene relative to the 18S rRNA as the difference between the threshold values of both genes.

2.7. Molecular modeling

The interaction of NSE, LY-171883 (PPARα/γ agonist), GW6471 (synthetic PPARα antagonist), and GW9662 (synthetic PPARγ antagonist) with PPARα/γ subtypes was performed by molecular docking. Spatial structures of α- (1kkq) ligand-binding domain and PPAR subtypes of γ- (3b0r) were used from the online resource RCSB Protein DataBank, NSE, GW6471 and GW9662 from ChemSpider. The spatial structures of PPAR ligand-binding domain were prepared for docking in the AutoDockTools 1.5.6 program. The same program was used for the visualization of interaction between investigated ligands and amino acid residues of the PPARα/γ molecule. The lowest level of free binding energy between the ligand and macromolecule corresponded to the positioning of the ligand in the ligand-binding domain of the receptor that was calculated using the program AutoDock Vina 1.1.2.

2.8. Statistical analysis

The data presented as mean values ± standard errors of the means (SEM) from different studied groups were compared by one-way analysis of variance (ANOVA). The correlation coefficient (r) was calculated using function CORREL from Microsoft EXCEL 365. The statistical significance was determined at the level of $p < 0.05$.

3. Results

3.1. Effect of GW6992, LY-171883 and NSE on the nuclear NF-kB translocation in LPS-activated peritoneal macrophages

The LPS stimulation of peritoneal macrophages exerted an increase in the percentage of nuclear FITC -labeled particles compare to control untreated cells (Figure 1, Supplement 1). These findings indicated an increase in the number of NF-κB dimer molecules in the nucleus after LPS treatment, followed by enhanced content of IL-1β in the culture supernatant (Figure 2).

The NSE administration to control macrophages did not affect the NF-kB translocation (Figure 1, Supplement 1). While the pre-incubation with

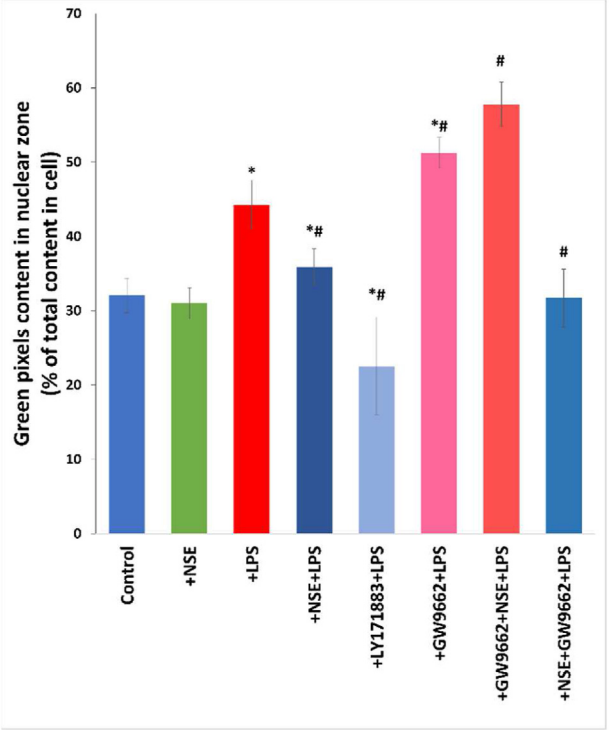

Figure 1. The number of FITC-IgG particles in the nuclei of peritoneal macrophages from control rats. Values are presented as mean ± SEM (n = 8–42). * - $P < 0.05$ compare to the control macrophages; # - $P < 0.05$ compare to the LPS-activated macrophages.

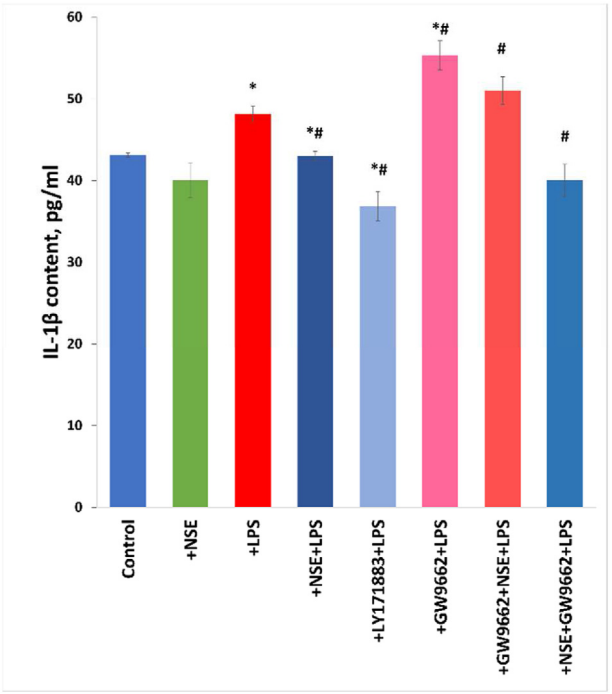

Figure 2. IL-1β content in culture supernatant. Values are presented as mean ± SEM (n = 4). * - P < 0,05 compare to the control macrophages; # - P < 0,05 compare to the LPS-activated macrophages.

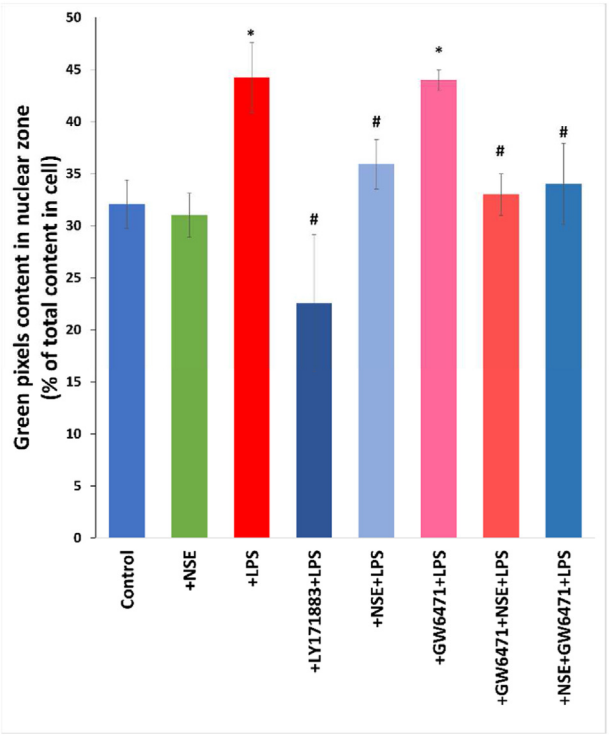

Figure 3. The number of FITC-IgG particles in the nuclei of peritoneal macrophages from control rats. Values are presented as mean ± SEM (n = 8–42). * - P < 0.05 compare to control macrophages; # - P < 0.05 compare to LPS-activated macrophages.

NSE before LPS treatment resulted in reduced FITC-IgG nuclear content and normalized level of IL-1β (Figure 2) compare to LPS-treated cells. The incubation of macrophages with LY-171883 (agonist of PPARα/γ) before the LPS stimulation resulted in significant decrease of FITC-IgG nuclear content compared to control and LPS-activated macrophages (Figure 1), followed by significant reduction of IL-1β level (Figure 2). Meanwhile, pre-incubation of LPS stimulated macrophages with GW9662 (PPARγ antagonist) triggered enhanced nuclear number of FITC-IgG particles (Figure 1) as well as increased content of IL-1β (Figure 2). The similar data was obtained during pre-incubation with GW9662, followed by NSE treatment and LPS activation (Figures 1 and 2). However, the pre-incubation of macrophages with NSE, followed by GW9662 treatment and LPS activation, prevented the increase of nuclear content of FITC-IgG particles (Figure 1) and increased level of IL-1β in culture supernatant (Figure 2).

Summarizing, there was a positive correlation between IL-1β culture supernatant level and nuclear content of FITC-IgG particles in peritoneal macrophages ($r = 0.87$; $P < 0.001$).

3.2. Effect of GW6471, LY-171883 and NSE on the nuclear NF-kB translocation in LPS-activated peritoneal macrophages

We did not observe any increase in nuclear content of FITC-IgG in peritoneal macrophages treated with PPARα antagonist - GW6471 before LPS stimulation (Figure 3, Supplement 1). This finding indicated that PPARα was less involved in the anti-inflammatory action compared to PPARγ. Meanwhile, incubation with NSE before and after GW6471 treatment, followed by LPS stimulation, did not change the nuclear level of FITC-IgG (Figure 3).

3.3. The effect of NSE on hepatic gene expression levels of FATP1 and IL-1RA

In the liver of obesity–induced insulin resistant rats, a slight increase of FATP1 mRNA level was detected. Meanwhile NSE administration to IR rats caused a 2-fold increase of FATP1 gene expression compare to controls (Figure 4). Administration of NSE to control rats did not cause any significant changes in FATP1 mRNA level.

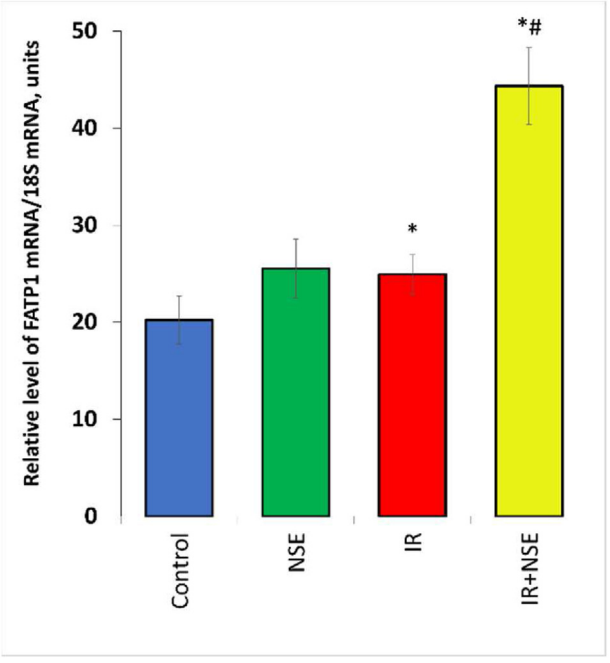

Figure 4. FATP1 mRNA level in rat liver. Expression was calculated as relative level between the difference in the threshold value of amplification cycles of target gene and relative gene. Values are presented as mean ± SEM (n = 10). * - P < 0.05 compare to control macrophages; # - P < 0.05 compare to LPS-activated macrophages.

In addition, NSE treatment triggered more than 2-fold increase in hepatic IL-1RA mRNA level in IR rats, whereas in control rats it caused a decrease in IL-1RA gene expression (Figure 5). The level of hepatic IL-1RA mRNA level in IR rats was not significantly higher compare to control rats (Figure 5).

3.4. Molecular modeling of NSE, GW9662 and LY-171883 binding to PPARγ

The results of the molecular docking have shown that all studied ligands bind to the same region of the PPARγ ligand-binding domain in the position corresponding to the lowest binding energy (Figure 6). Therefore, they all can compete for the binding site in PPARγ active center.

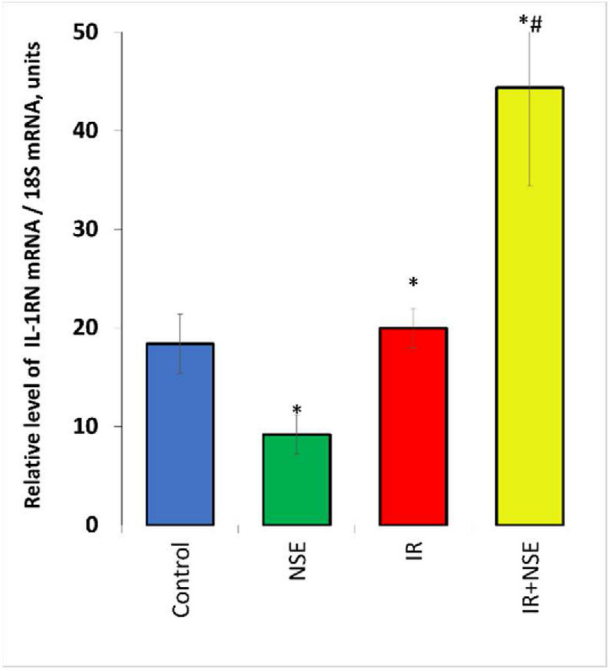

Figure 5. IL-1RA mRNA expression in rat liver. Expression was calculated as relative level between the difference in the threshold value of amplification cycles of target gene and relative gene. Values are presented as mean ± SEM (n = 10). * - $P < 0.05$ compare to control macrophages; # - $P < 0.05$ compare to LPS-activated macrophages.

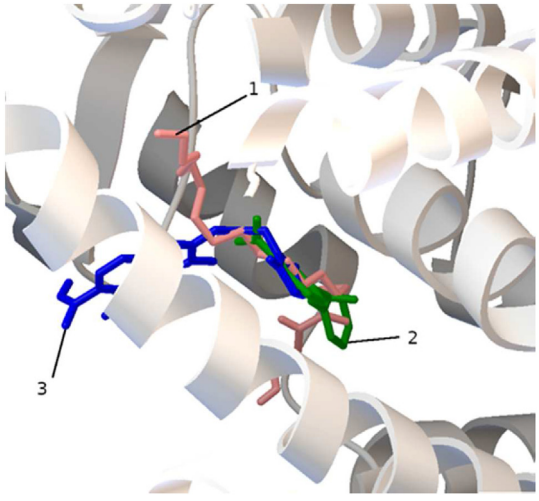

Figure 6. Results of independent docking for NSE, GW6992 and LY-171883 in the PPARγ ligand binding domain (3b0r). 1 – NSE. 2 – GW9662. 3 – LY-171883.

The list of amino acid residues involved in the interaction of investigated ligands and PPARγ molecule in a position with a minimum binding energy presented in Table 1.

The individual docking data of NSE, GW9662 and LY-171883 showed that all studied compounds have close binding energy (Gibbs free energy) and common sites (amino acid residues) in the active center of PPARγ molecule (3b0r).

4. . Discussion

The involvement of endocannabinoid system in regulation of inflammatory processes during various pathological conditions has been well studied. Endocannabinoid compounds with different chemical structure can activate cannabinoid receptors or exert anti-inflammatory properties via different pathways. Primarily, by direct influence on immune cells, or by changing the local concentrations of endocannabinoids. It was confirmed that anandamide exhibits its anti-inflammatory action via cannabinoid receptor–independent pathway, by inhibiting NF-κB activation [21]. N-palmitoylethanolamine (NPE), an endocannabionoid-like compound that is present in natural products and food as well as endogenously synthesized, acts as a balancer in inflammatory processes. NPE activates the PPARα receptor thus exerting its anti-inflammatory activity [22]. NSE is a congener of NPE and there is not enough information about its anti-inflammatory activity. Earlier study, using *ex vivo* and *in vivo* experimental models, showed that NSE decreased production of inflammatory cytokines (IL-1β, TNF-α) [15]. Further experiments reported that particular NSE's effect was associated with the inhibition of nuclear NF-κB translocation in LPS-activated rat peritoneal macrophages [23]. It is worth to note that LPS-induced inflammatory response could be inhibited by activation of either PPAR-γ or LXRα [24, 25].

The current study results suggested the involvement of PPARγ in the mechanism of anti-inflammatory action of NSE. Using the method of molecular modeling, we showed that NSE could bind to PPARγ and compete for the receptor-binding site with antagonist GW9662 and agonist LY-171883. In *ex vivo* study we used selective agonist of PPARα/γ - LY-171883, selective antagonist of PPARγ - GW9662 and PPARα - GW6471 to study the role of PPARα/PPARγ in the NSE's effect on nuclear NF-kB translocation in LPS-activated rat peritoneal macrophages. In addition, we studied the influence of NSE treatment on hepatic mRNA level of PPARγ-related genes - SLC27A1 and IL1RN in insulin resistant rats.

The incubation of peritoneal macrophages with PPAR α/γ selective agonist LY-171883 before LPS activation resulted in inhibition of

Table 1. The individual docking data of GW9662, NSE, and LY-171883 to the ligand-binding domain of PPARγ.

PPAR-active compounds	GW9662	NSE	LY 171883
Gibbs free energy of binding (kcal/mol)	-7,9	-6,6	-7,7
Amino acid residues involved in the binding		Leu228	
		Ile281	
			Gly284
			Cys285
	Arg288	Arg288	Arg288
			Ser289
	Ala292		
	Ile326	Ile326	
	Met329		
	Leu330	Leu330	Leu330
			Ile341
			Ser342
		Met364	
		Phe380	

nuclear NF-kB translocation followed by a decrease of media IL-1β content. Similar data have been confirmed earlier [26, 27]. Furthermore, there was no changes in nuclear NF-kB translocation during pre-incubation with selective PPARα antagonist GW6471 before LPS stimulation. This result suggested that PPARγ, not PPARα is involved in the anti-inflammatory response during LPS-stimulation, at least in the condition of our experiment. Administration of PPARγ selective antagonist – GW 9662 resulted in intensification of NF-kB translocation and increased level of media IL-1β. Previously described data supports the correlation between inhibition of PPARγ and increased transcriptional activity of NF-κB [28]. Administration of NSE before LPS-treatment inhibited NF-kB translocation. At the same time, NSE administration after GW 9662 treatment, followed by LPS activation did not prevent the increase in the nuclear level of FITC-IgG particles in peritoneal macrophages. Therefore, NSE did not affect the NF-kB translocation during PPARγ inhibition caused by selective antagonist binding. However, when NSE was added before GW9662 treatment, followed by LPS stimulation, the level of NF-kB translocation and IL-1β content significantly reduced, showing control cells' values. These data indicated a competitive binding of NSE with GW9662 for the ligand-binding domain of PPARγ. It is worth to admit that NSE treatment did not affect the content of FITC-IgG particles in the nuclei of control peritoneal macrophages (unstimulated with LPS).

The role of PPARs in the expression of genes that are involved in lipid metabolism, inflammation response, cell differentiation and multiple metabolic processes, especially lipid and glucose homeostasis is well studied [29]. Particular proteins, FATP1 and IL1-RA, are encoded by PPARγ-target genes SLC27A1 and IL1RN, respectfully [30, 31]. In our study, we found that NSE administration to obesity-induced IR rats significantly enhanced the liver expression of FATP1 and IL1-RA. These data together with molecular docking and NF-kB translocation findings indicated the interaction of NSE with PPARγ that resulted in increase of FATP1 and IL1-RA mRNA expression in IR rats. FATP expression is up-regulated in mouse liver and adipose tissue by PPARα and PPARγ activators. Earlier data about the down-regulation of FATP by insulin, seems counterintuitive, since fatty acid uptake would be expected to rise in response to insulin stimulation. It is important to note, however, that regulation of FATP at the transcriptional level is unlikely to be the result of the transient postprandial insulin peak, but rather a more chronic hyperinsulinemia during type 2 diabetes mellitus. Furthermore, the majority of type 2 diabetics are obese where a PPARγ expression is down-regulated [32]. According to this, FATP is likely to be responsible in part for the increased fatty acids import necessary to sustain this increased β oxidation. Therefore, NSE as an activator of PPARγ following by up-regulation of FATP1 triggers its anti-inflammatory response to obesity-induced elevated level of lipids in IR rats. Moreover, PPARγ exerts anti-inflammatory activities by inducing the production of IL-1RA that inhibits IL-1 action by competing with IL-1 for binding to the IL-1 receptor (IL-1R). The role of IL-1RA as an anti-inflammatory protein has been reported both *in vitro* and *in vivo*, including protection of cultured human islets from glucose-induced IL-1β-mediated apoptosis and reduction of systemic inflammation markers [33, 34]. These results are in agreement with our data, where NSE increases expression of IL-1RA by activation of PPARγ, exerting anti-inflammation action in IR rats. Meanwhile, it is important to note that NSE administration to control rats did not cause any changes in FATP1 mRNA level, however, reduced the IL1-RA mRNA level. This finding is in agreement with earlier studies where only high concentrations of PPAR ligands triggered anti-inflammatory action by inhibiting the secretion of IL-1, IL-6 and TNFα in stimulated monocytes [35]. Additionally, endogenous concentrations of N-acylethanolamines are normally at a very low rate (10^{-9} M), but their level dramatically increases during pathological conditions [36]. Therefore, we can suggest that exogenous administration of NSE to IR rats mediated an increase in its endogenous level that is required for further PPARγ activation. However, further studies should be conducted to confirm this hypothesis.

5. Conclusion

In conclusion, our results demonstrated the involvement of PPARγ in anti-inflammatory action of NSE. Mainly, NSE suppressed nuclear translocation of NF-κB in LPS-stimulated peritoneal macrophages via PPARγ. In addition, NSE treatment changed hepatic mRNA expression of PPARγ target genes (SLC27A1, IL1RN) in insulin resistant rats. Obtained findings suggested a promising therapeutic potential of NSE.

Declarations

Author contribution statement

Halyna Kosiakova: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Andrii Berdyshev: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Victor Dosenko: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tetyana Drevytska: Performed the experiments.

Oleksandra Herasymenko: Performed the experiments; Wrote the paper.

Nadiya Hula: Conceived and designed the experiments; Wrote the paper.

Funding statement

Halyna Kosiakova was supported by National Academy of Sciences of Ukraine (0117U004344).

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2022.e11336.

Acknowledgements

This research was fully financed by National Academy of Science of Ukraine, grant number 0117U004344. Equipment, drugs, or supplies were provided by Palladin Institute of Biochemistry National Academy of Sciences of Ukraine.

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