Steroids 207 (2024) 109438

Contents lists available at ScienceDirect

Steroids

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

Time-dependent dual mode of action of COX-2 inhibition on mouse serum corticosterone levels

Patrycja Pańczyszyn-Trzewik [a], Magdalena Sowa-Kućma [a], Paulina Misztak [b], Anna Tabecka-Lonczynska [c], Katarzyna Stachowicz [b,*]

[a] Medical College of Rzeszów University, Institute of Medical Science, Department of Human Physiology, 35-310 Rzeszow, Kopisto Street 2a, Poland
[b] Maj Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland
[c] Department of Biotechnology and Cell Biology, Medical College, University of Information Technology and Management in Rzeszow, Sucharskiego 2, 35-225 Rzeszow, Poland

ARTICLE INFO

Keywords:
Corticosterone
NS398
COX-2
LPS

ABSTRACT

To elucidate the effect of cyclooxygenase-2 (COX-2) inhibition on corticosterone release, mice were divided into a group receiving NS398, a selective COX-2 inhibitor at a dose of 3 mg/kg for seven days, and a group receiving NS398 for fourteen days. After this time, the mice were sacrificed, and blood serum was collected. An ELISA protocol was used to analyze serum corticosterone levels. Short-term COX-2 inhibition increased corticosterone levels, while long-term inhibition lowered them. The exact schedule of experiments was repeated after the lipopolysaccharide (LPS) *Escherichia coli* challenge in mice to check the influence of stress stimuli on the tested parameters. In this case, we observed increases in corticosterone levels, significant in a seven-day pattern. These results indicate that corticosterone levels are regulated through a COX-2-dependent mechanism in mice.

1. Introduction

Stress stimuli activate the hypothalamic–pituitary–adrenal (HPA) axis, resulting in the release of glucocorticoids (cortisol in humans and corticosterone in rodents) [1–3]. Chronic stress stimuli, evidenced by the presence of elevated circulating glucocorticoids, are associated with dysregulated HPA axis and systemic reorganization [1–3]. Adapting the body to stressors through changes to maintain homeostasis is known as allostasis [4]. Repeated stress stimuli or a one-time strong stressful event results in the inability of the body to achieve homeostasis. This condition is known as allostatic load [4]. It causes metabolic changes, alterations in the nervous system, and hippocampal atrophy [4]. Breaking the homeostatic barrier by stress is a recognized factor for depression [5,6]. Therefore, looking for regulators of coping mechanisms with stress is essential.

During physiological challenges, stress, or dysregulation of the glutamatergic system, prostaglandins are derived from arachidonic acid by phospholipase A_2 through cyclooxygenase (COX-2) [7]. COX-2 is an inducible form that has been shown to be expressed in the kidneys, and such brain structures as the cerebral cortex, limbic system and spinal cord, among others [7]. It has been observed that COX-2 inhibitors affect corticosterone secretion in rodents [8–10] and display antidepressant-like as well as pro-cognitive effectiveness [11–13]. Furthermore, it was documented that COX-2 inhibitors impairs glucocorticoid levels in rats, both in serum and renal cortex and adrenal gland [7,14,15]. A massive set of our experiments has shown that by inhibiting COX-2 with NS398, a selective COX-2 inhibitor, an effective modulation of rodent behavior is observed [16,17]. NS398 was potent in depression-like studies influencing the mGluR5 antagonist (MTEP) or imipramine and in the cognitive part of the studies [17].

The most widely used adjunctive drug for treating depression and refractory cases it is in fact a COX-2 inhibitor – celecoxib [7]. Although plenty of indirect evidence points to the involvement of COX-2 in regulating the HPA axis, no studies show the effect of COX-2 inhibition on corticosterone levels straightforwardly. Since long-term COX-2 inhibition may be associated with changes in renal metabolism [18,19], corticosterone undergoes deactivation in kidneys [19], it is essential in such a study to monitor their status by histological inspection. It has been documented that in the kidney, prostaglandins and COX-2 are involved as regulators of renal hemodynamics and salt/water homeostasis [18,19]. Furthermore, the role of adrenal cortex and adrenal gland in regulation of HPA components has been documented [7,14,20]. In

* Corresponding author at: Department of Neurobiology, Maj Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland.
E-mail address: stachow@if-pan.krakow.pl (K. Stachowicz).

https://doi.org/10.1016/j.steroids.2024.109438
Received 23 February 2024; Received in revised form 6 May 2024; Accepted 6 May 2024
Available online 7 May 2024

addition, COX inhibitors (aspirin or indomethacin) regulate the renin-angiotensin system through signals from the macula densa [18,19].

This is the first study showing that COX-2-dependent mechanisms are involved in corticosterone concentrations. Our hypothesis is based on the fact that both psychological and microbe-induced stressors, e.g., LPS, were associated with glutamate (Glu) excitotoxicity [20,21], which was showed as regulated by COX-2 [20,21]. Finally, acetylcholine (Ach) levels were examined in the prefrontal cortex and hippocampus of mice using ELISA. The literature documents the regulation of the HPA axis indirectly as a stress response through Ach [20–25].

2. Materials and methods

2.1. Animals and housing

The experiments were performed on group-housed male C57BL/6J mice. The mice were 8–10 week old. The animals were kept under recommended laboratory conditions (temperature of 21 ± 1 °C, relative humidity of 50 ± 5 %), with lightning period 12: 12 h (light: dark cycle). Each group included ten animals. Experiments were performed during the light period (8: 00–17: 00). Food and water were freely available. All procedures were conducted according to the guidelines of the National Institutes of Health Animal Care and Use Committee and were approved by the Ethics Committee of the Institute of Pharmacology, Polish Academy of Sciences in Krakow (Approval Number: 178/2017 and 158/2019).

2.2. Drug treatment

The following drugs were used: N-[2-(Cyclohexyloxy)-4-nitrophenyl] methanesulfonamide (NS398, Abcam Biochemicals, UK), LPS serotype 0127:B8 (Sigma-Aldrich); 3-[(2-methyl-1,3–tiazol-4-yl) ethynyl]-pyridine (MTEP; Tocris Cookson ltd., Bristol, UK). NS398 (3 mg/kg) was dissolved in 10 % DMSO; 10 % DMSO was used for vehicle group injections. LPS was used as an aqueous suspension. All compounds were injected *i.p.* once daily (before 11: 00), for 1, 7 or 14 consecutive days. A separate cohort of mice was designated for LPS experiments with the same experimental schedule. A control group without LPS challenge was scheduled as a positive control during these experiments and was treated in the same way as LPS – injected mice.

2.3. Blood serum and kidneys collection

Our main objective in this study was to determine the level of HPA axis changes during prolonged administration of a COX-2 inhibitor (NS398) by detecting serum corticosterone levels and analyzing renal status since COX-2 is constitutively expressed in the kidneys and metabolized there [18,19]. We chose serum corticosterone as the indicating variable 24 h after the last administration to exclude a short-term effect of the drug on the system. 24 h after the last administration of tested compounds, animals were decapitated and trunk blood (about 1 ml) and kidneys were collected. The kidneys were weighed immediately after collection. Serum obtained by centrifugation and kidneys were frozen on dry ice and stored at −80 °C until the start of biochemical analysis. Collection was scheduled between 8:00–11:00.

2.4. Endocrine assay (corticosterone)

Blood serum was centrifuged at 1800 x g for 30 min. We stored serum aliquots at −80 °C until thawed for the assay. Serum corticosterone concentration was determined by enzyme-linked immunoassay (ELISA) using Corticosterone Rat/Mouse Elisa Kit (Demeditec Diagnostics GmbH, Kiel, Germany), according to the manufacturer's protocol. Briefly, 10 μl of each standards, samples and control were dispensed into 96-wells plate. Next, 100 μl of incubation buffer and 50 μl of enzyme conjugate were added and incubated for 2 h at room temperature on a microplate mixer. After washing, 200 μl of substrate solution to each well was pipetted and color developed inversely proportional to the amount of corticosterone in the initial step. The reaction was stopped by adding 50 μl of stop solution, and the absorbance was determined at a wavelength of 450 nm within max. 15 min using the Synergy 2 multi-mode microplate reader and Gen5 Software (BioTek, Winooski, VT, USA). All assays were performed in duplicate. The standard range was: 15–2,250 ng/ml. The intra- and inter-assay coefficients of variation were < 8 % for all analyses.

2.5. Tissues collection

The mice were decapitated 24 h after the last injection. Then, the prefrontal cortex (PFC) and hippocampus (HC) were dissected according to the Mouse brain atlas [26], frozen on dry ice, and stored at −80 °C. PFC was taken by cutting the anterior part of the forebrain at the level of Bregma 2.20 mm. Olfactory bulbs and the anterior striatum were cut off. Therefore the tissue taken for analysis contains a majority of the PFC. Subsequently, the brain was cut into two hemispheres in the sagittal line. Then, the whole HC was taken out from each hemisphere.

2.6. Acetylcholine assay

Acetylcholine (ACh) levels were determined in the PFC and HC using a commercially available Amplex Red Acetylcholine/Acetylcholinesterase Assay Kit (Invitrogen Molecular Probes, Paisley, UK) according to the manufacturer's protocol. Briefly, the tissue samples (PFC and HC) were homogenized in the appropriate lysis buffer with a protease inhibitor cocktail (BioShop, Canada). After centrifugation for 5 min at 10,000 RPM (4 °C), the protein concentration in the supernatant was determined using the BCA method (Pierce, Rockford, USA). Next, all the samples were diluted with the reaction buffer. ACh measurements were performed on 96-well plates and the reaction involved: 100 μL of a sample, 50 μM of Ach, 200 μM of Amplex Red Reagent, 0.1 U/mL of choline oxidase, and 1 U/mL of HRP. After adding all reaction components, the plates were incubated at room temperature for 30 min in the dark. Fluorescence intensity was detected by Synergy2 Multi-mode microplate reader and Gene5 software (Winooski, VT, USA) with excitation and emission of 530 nm and 590 nm, respectively. Then, the results were calculated using GraphPad PRISM.

2.7. Hematoxylin and eosin (H&E) staining

The tissue samples of mouse kidney were fixed in 4 % paraformaldehyde and embedded in Paraplast Plus (Sigma-Aldrich; Darmstadt, Germany). Five-micrometer sections were stained with hematoxylin and eosin following standard procedures to evaluate the whole structure of the tissue by contrast staining of the cell cytoplasm and nuclei [27]. The microphotographs of kidney fragments in all analyzed groups were taken using the Olympus BX43 microscope equipped with the soft cellSens Dimension software.

2.8. Urine pH

Urine pH analysis was scheduled during daily injections so as not to stress the animals. A pH was measured while the animal was kept for injection (the litmus paper was placed directly under the mouse during urination). Approximate urine pH analysis was performed using litmus papers (Sigma, Che. Comp., Lot 010B164536, P-4536, pH Test Strips 4.5–10.0) [28]. Not all animals urinated during the experiment. Hence, some of time points are interpreted only as observations due to the small number of results in the group.

2.9. Statistical analysis

The results were presented as the means ± S.E.M. *t*-test, one-way

(followed by the Dunnett's test, or Tukey's) analysis of variance (ANOVA) were used for statistical analysis. GraphPad Prism software, ver. 8.0 (San Diego, CA, USA) was used. $P < 0.05$ was considered significant.

3. Results

3.1. Decrease of serum corticosterone after prolonged COX-2 inhibition

There are significant changes in serum corticosterone levels depending on the duration of COX-2 inhibition. Acute COX-2 inhibition with NS398, a selective COX-2 inhibitor at a dose of 3 mg/kg (*i.p.*), caused a significant increase in serum corticosterone levels in C57Bl/6J mice (*t*-test: t = 3.673, df = 11; P = 0.004) (Fig. 1 B). Vehicle: 89.52 ± 22.22, NS398: 172 ± 8.82 (ng/ml). However, long-term COX-2 inhibition for seven days did not change corticosterone levels compared to the control group of mice (ns) (Fig. 1 C). Vehicle: 143.0 ± 11.40, NS398: 163.2 ± 4.898 (ng/ml). Prolonged COX-2 inhibition for fourteen days using NS398 resulted in a significant reduction in serum corticosterone levels (*t*-test: t = 13.56, df = 10; P < 0.001) (Fig. 1 D). Vehicle: 238 ± 6.91, NS398: 69.89 ± 9.23 (ng/ml).

3.2. No changes in serum corticosterone levels after prolonged COX-2 inhibition in LPS-injected C57Bl/6J mice

We have studied the involvement of lipopolysaccharide (LPS) *Escherichia coli* mechanisms in action of NS398. LPS is a structural component of the outher membrane of Gram-negative bacteria with documented effects *in vitro* and *in vivo* [29]. LPS is used as a model of stress: LPS-induced depression-like model in mice [30,31]. LPS was used in this context in our study. LPS challenge exerts a biphasic effect. It induces "sickness behavior" during the first 2–6 h after the challenge, then 24 h later, it triggers "depressive behavior" [32]. We challenged mice 24 h before the last injection with the tested compounds in the seven-day schedule to combine treatment with tested compounds with the "depressive phase" of the LPS challenge. A completely different response of the HPA axis to COX-2 inhibition in mice was observed when LPS (0.83 mg/kg) was applied. Seven-day COX-2 inhibition, which was ineffective in modulating serum corticosterone (see Fig. 1), increased corticosterone if LPS challenge was applied (*t*-test: t = 4.464, df = 9; P = 0.002) (Fig. 2 B). Vehicle + LPS: 60.91 ± 9.89, NS398 + LPS: 119.6 ± 8.096 (ng/ml). Moreover, when COX-2 inhibition was extended to fourteen days, there was no effect on corticosterone levels in LPS-treated mice (ns) (Fig. 2 C). Vehicle + LPS: 85.39 ± 15.09, NS398 + LPS: 127.8 ± 15.52 (ng/ml). The observed results suggest a completely different response of the body to LPS challenge due to the duration of COX-2 inhibition.

3.3. Histological analysis of mouse kidneys after prolonged administration of NS398 did not reveal tissue abnormalities

Here, kidney weight was increased in a group of mice treated with NS398 (Suppl. 1A). Observed effects seem to be specific, because no changes in mice weight were detected (Suppl. 1B). A cross-section of mouse kidneys in the vehicle-treated group, as well as in groups treated with NS398 for 14 days, revealed regular histology with no abnormalities (Suppl. 1C). In the renal cortex some distal convoluted tubules (DC) were found. The visible oval and round glomerular corpuscles (G), which are capillary networks lined by a thin layer of endothelial cells, were observed between the tubules. Thin Bowman's capsule delimitated the G.

3.4. Changes in pH of urine mice treated with LPS

Injections of tested compounds until day thirteenth did not affect urine pH. However, injection of LPS (0.83 mg/kg) resulted in a significant decrease in the pH of urine (Suppl. 2). Administration of NS398 (3 mg/kg) did not change that trend (F (6,30) = 16.07; P = 0.007) (Suppl. 2B and 2C). At the same time, no further increase in kidney weight was observed with the simultaneous administration of LPS (Suppl. 2D). The results above suggest changes in kidney activity during manipulations. However, not all animals urinated during the experiment. Hence, some of time points are interpreted only as observations due to the small number of results in the group.

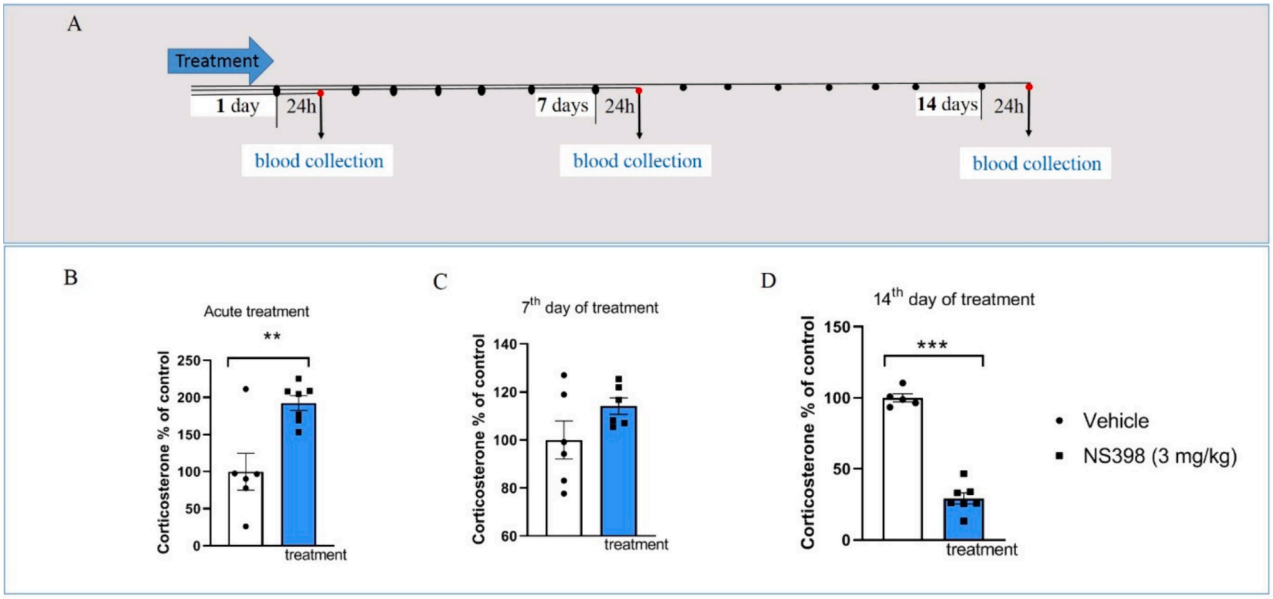

Fig. 1. The first part of the panel shows an experimental schedule (A). The second part of the panel shows the effect of treatment with NS398 (3 mg/kg) on serum corticosterone level of C57Bl/6J mice. Single injection results are shown in (B), 7-day treatment in (C), and 14-day treatment in (D). (B) Veh: 89.52 ± 22.22, NS398: 172.4 ± 8.821 ng/ml; (C) Veh: 143 ± 11.40, NS398 163.2 ± 4.898 ng/ml; (D) Veh: 238.9 ± 6.913, NS398: 69.89 ± 9.23 ng/ml. Corticosterone level was measured using ELISA kits. Values are expressed as the means ± S.E.M., and were evaluated by *t*-test, $^{**}P < 0.01$, $^{***}P < 0.0001$ vs. vehicle group (n = 5–7).

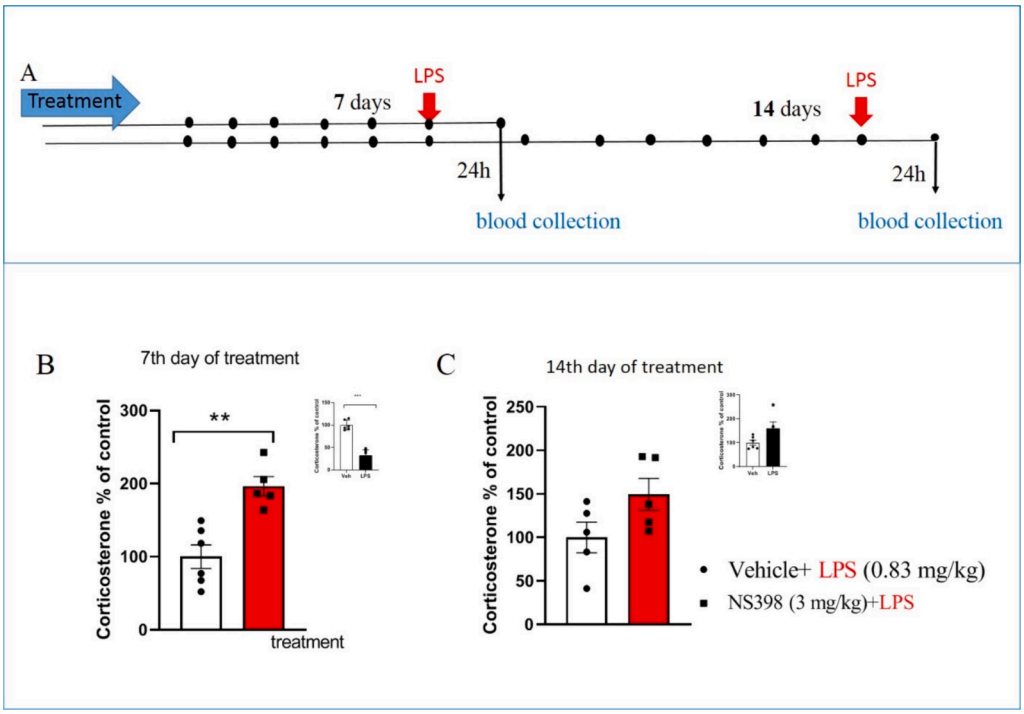

Fig. 2. The first part of the panel shows an experimental schedule (A). The effect of LPS challenge (0.83 mg/kg) on serum corticosterone level in mice treated with NS398 (3 mg/kg) for 7 days (B), and for 14 days (C). (B) Veh + LPS: 60.91 ± 9.89, NS398 + LPS: 119.6 ± 8.096 ng/ml; (C); Veh + LPS: 85.39 ± 15.09, NS398 + LPS: 127.8 ± 15.52 ng/ml. Corticosterone level was measured using ELISA kits. Inserts shows changes in vehicle group made by LPS challenge (7^{th} day: t = 9,796, df = 10, P < 0.0001; 14^{th} day: t = 2,229, df = 9, ns). Values are expressed as the means ± S.E.M., and were evaluated by *t*-test, **P < 0.01, vs. vehicle group; (n = 5–6).

3.5. Changes in corticosterone level after MTEP (1 mg/kg) and NS398 (3 mg/kg) treatment for seven days

A decrease in corticosterone level was observed in the serum of mice treated with MTEP (1 mg/kg) for seven days, while co-administration of MTEP + NS398 resulted in restoration corticosterone to the basal level. A one-way ANOVA detected significant changes ($F(3,20) = 1.931$, $P < 0.0001$) (Fig. 3). Vehicle: 143 ± 11.40, MTEP: 99.36 ± 12.07, NS398: 163.2 ± 4.898, MTEP + NS398: 176.9 ± 8.91 (ng/ml).

3.6. Changes in acetylcholine level in prefrontal cortex and hippocampus after prolonged COX-2 inhibition

One-way ANOVA showed significant changes in acetylcholine (Ach) levels in the prefrontal cortex when long-term COX-2 inhibition was used ($F(3, 23) = 4.836$; $P = 0.0094$). No such changes were detected in the hippocampus using one-way ANOVA ($F(3, 24) = 1.924$; $P = 0.1526$) (Suppl. 3). However, due to the increase in ACh levels in the control group after fourteen days of COX-2 inhibition, the observed increases should be regarded as apparent.

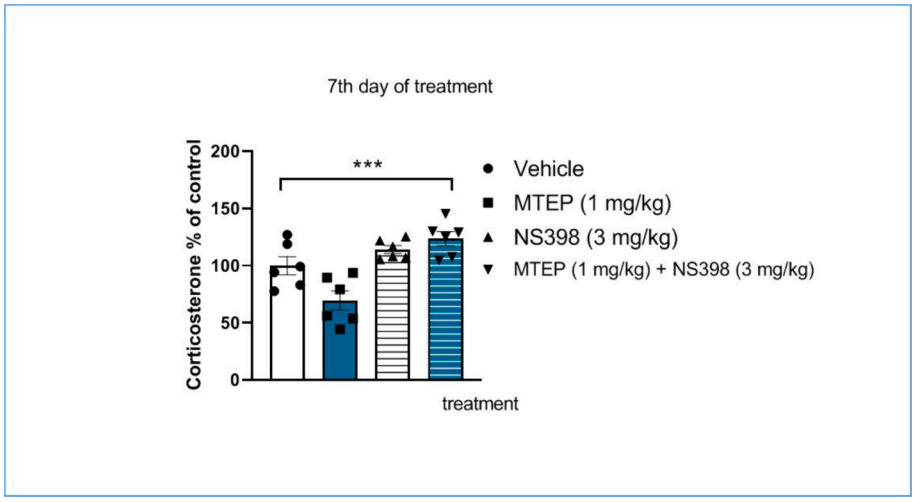

Fig. 3. The effects of seven days of administration of MTEP (1 mg/kg), NS398 (3 mg/kg) and MTEP + NS398 on serum corticosterone in C57Bl/6J mice. Corticosterone level was measured using ELISA kits. Values are expressed as the means ± S.E.M., and were evaluated by one-way ANOVA, ***$P < 0.001$ vs. vehicle group (n = 6).

4. Discussion

This study examined changes in the hypothalamic–pituitary–adrenal (HPA) axis after the treatment with NS398, a selective COX-2 inhibitor. The results show that a single injection of NS398 exerts a completely different HPA axis response than the more extended applications. We found a significant increase in serum corticosterone in mice treated acutely with NS398. Quite different changes in the HPA axis response were detected after seven days of NS398 treatment, at a time point when antidepressant-like efficacy begins [7,17] and lasts up to fourteen days with more pronounced corticosterone suppression. Detection of serum corticosterone in rodents is used to indicate the stress response [33]. The effect of a stressor on the body's response and inflammation depends on the duration and frequency of the stressor response, and repeated exposure to a stressor can potentially exacerbate inflammation [33]. Thus, a completely different body response can be observed when searching for inflammatory parameters (in this case, COX-2 levels). Indeed, this is what was found in our study. COX-2 inhibition caused an increase in corticosterone level after a single administration, and then the response was weaker (day 7) to show a decrease in the parameters studied after 14 days. Inhibition of COX-2 is associated with reduced symptoms of stress, anxiety, and depression in animals [7,17], so the decrease in corticosterone observed in our study seems to confirm the involvement of COX-2 in these mechanisms.

The results observed here are consistent with findings showing that the antidepressant effect of effective drugs is associated with corticosterone suppression [34]. The changes in serum corticosterone levels by NS398 we observe may take place at several levels: 1) at the brain at the level of the structures involved in the stress response (hypothalamus Ht, cortex Cx, hippocampus Hc, amygdala Am) via changes in the release of stress hormones, and Glu [35–37], 2) peripherally at the level of the adrenal glands [35–38], 3) and through receptors located in the kidneys, via affecting the corticosterone metabolism [38,39]. These may all influence the back hormonal signals entering the brain, informing the mind how to interpret the situation. Influencing COX-2 – changes in the brain's Glu level occur [7,17]. It was documented that the level and activity of COX-2 impact both ionotropic Glu receptors and the metabotropic ones in the brain [7,17]. Behavioral MTEP's (a ligand of metabotropic glutamate receptor 5) action was modified by NS398 via changes in Glu transporters in a synapse [20]. These changes may influence the signal reaching Hc to release hormonal signals [39]. Moreover, we should consider the impact of COX-2 inhibition on MTEP's potential to modify corticosterone levels via changes in Ca^{2+} metabolism [7,17]. Our preliminary data confirm this possibility, as while COX-2 inhibition after seven days was not effective in modeling corticosterone levels, it did block the potential of MTEP (Fig. 3). Liu et al. [40] found that LPS-mediated $[Ca^{2+}]i$ oscillations follow the same pattern as those induced by mGluR5 activation in microglia cell lines. Furthermore, the mGluR5 antagonist, MTEP, abolished the LPS-mediated $[Ca^{2+}]i$ oscillations [40]. This kind of modulation may take place here. So we decided to check the mouse HPA axis response to the LPS challenge. The results observed here indicating elevated corticosterone levels in the serum of LPS-treated mice are consistent with the results of Kelly et al. [33], who documented an increased HPA response and doubly elevated corticosterone production in mice with early priming. The authors found that the use of corticosterone in drinking water over a long period elevates the response of cultured primary microglia to LPS exposure and the secretion of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) [33]. In this case, pre-exposure to LPS caused a twofold increase in serum corticosterone in mice, suggesting similar mechanisms to those observed by Kelly et al. [33]. However, prolonged exposure to COX-2 inhibition for fourteen days potently stopped that trend (see Fig. 2). Comparing these results with our earlier ones, it should be noted that elevated corticosterone levels in mice challenged with LPS and COX-2 inhibition, which was ineffective in this case, were accompanied by a decrease in spatial, hippocampal-dependent memory in the Barnes test [20]. This indicates the coherence of the results we obtained and raises their importance.

COX-2, phospholipase A2 (PLA2), and G proteins (GPSRs) are expressed in kidney tissue [41–44]. Regulation of hormonal systems occurs in the kidney [45,46]. Experiments with COX-2 knockout mice found renal abnormalities followed by animal death [46]. Our interest was to check the influence of chronic treatment with tested compounds on kidney tissue conditions, as any kidney abnormalities could influence the results presented here. The renal diagnose with the use of COX-2 inhibitors is vital due to some side effects observed in patients with nonsteroidal anti-inflammatory drugs (NSAIDs) use [18]. It was documented peripheral edema, increased sodium reabsorption, weight gain which are observed in a first week of therapy [18]. However, following side-effects are hearth failure [18]. COX-2 is expressed in kidney cortex in macula densa [18]. Tubulointerstial injury was found when COX-2 inhibitors were used [18]. Furthermore, celecoxib was found to induce renal papillary necrosis and tubulointerstinal nephritis [18]. Taking all this into account, we performed a histological analysis of the kidney's condition using hematoxylin and eosin staining. The fourteen-day treatment has been selected for a study as more aggravating. However, in our histological analysis, we found no changes in renal tissue or renal function (when assessing pH of urine) that would suggest an impact on the observed results.

Our study shows that the COX-2 pathway is intimately linked to the HPA axis response during stress. The interplay between COX-2/HPA axis may be one of the most critical factors during depression and cognitive changes. In the future, more long-term treatment will be valuable to track changes in HPA axis parameters. It is known and accepted that the response of the HPA axis is a dynamic process, so single-point experiments are not sufficient. Therefore, we used three points, acute administration, administration for seven days and administration for fourteen days. However, longer-term observations would have been desirable. Fig. 4 – presents described mechanisms of corticosterone metabolism.

The lack of changes in brain ACh levels suggests that the changes in the HPA axis and subsequent corticosterone levels after long-term COX-2 inhibition are rather peripheral. This hypothesis is supported by our earlier study, in which we showed a decrease in COX-2 expression in the brain after seven days of COX-2 inhibition, an effect that was not observed after longer doses of NS398 [47]. This may also explain the different responses of the system after seven and fourteen days in our current study. If the changes are peripheral, a regulatory mechanism in the kidneys and adrenal glands should be considered [48]. It has been documented that the HPA axis regulates hormonal balance in response to acute and chronic stress. Glucocorticoids, the main effector, are released by the zona fasciculata of the adrenal glands under the control of ACTH and re-released by the pituitary gland [49]. Previous studies have shown that COX2 inhibitors reduce glucocorticoid levels in rats [49]. It has been reported earlier that the immune system's rapid response by releasing ACTH and corticosterone is independent of the induced PGE2 synthesis by COX-2 [50]. However, prolonged ACTH release was found under the influence of PGE2 in cerebral vascular cells [51], possibly due to weakened transcription of the CRH gene. It is, therefore, observed that the enzymes involved in the constitutive and inducible synthesis of prostaglandins may act in a complementary manner, depending on the time of action, via the neural or humoral route, activating the HPA axis in response to inflammation [50]. Subsequent research by Elander et al. [52] using mice with genetic deletion of the COX-1 gene did not show an increase in corticosterone levels 1 h after the induction of inflammation by LPS injections compared to wild-type mice. However, 6 h after LPS injection, corticosterone levels increased significantly. After using a selective COX-1 inhibitor (SC-560) in mice, inhibition of corticosterone levels was also observed. Therefore, two different cyclooxygenase isoforms probably perform separate but complementary stress hormone functions in response to the inflammatory process [52]. Moreover, we may suspect that the entire system underwent rearrangement after our manipulation to achieve

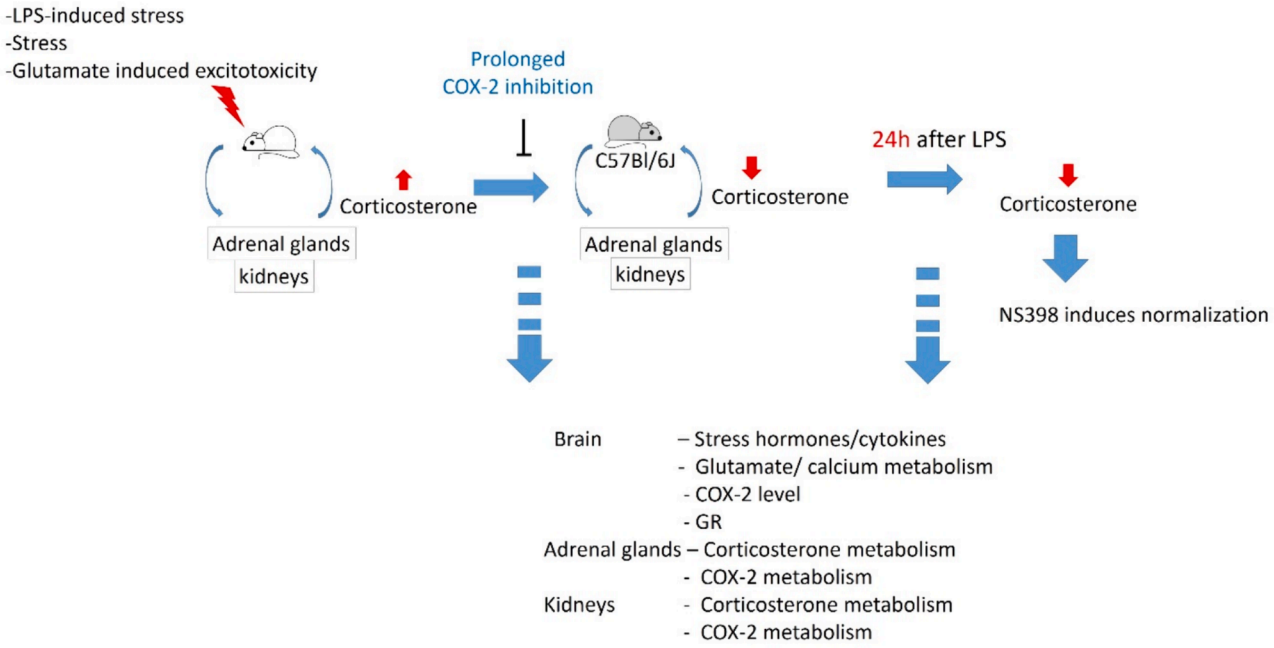

Fig. 4. Schematic assembly of mechanisms that interfere with corticosterone levels during COX-2 inhibition.

homeostasis after LPS administration. It has been shown that a dramatic increase in COX-2 expression in the kidney and adrenal glands is observed after LPS provocation [48]. Moreover, LPS provocation was associated with massive corticosterone release observed 24 h after challenge, and increased blood–brain barrier permeability [53]. Moreover, ACh affects cortisol secretion, as observed on freshly isolated zona fasciculata/reticularis (ZFR) cells isolated from the bovine adrenal cortex and primary cultures of the bovine adrenal cortex [54]. Maximum levels were observed after 48 to 72 h, and cortisol concentrations declined [54]. This may explain the different corticosterone levels in our results after introducing LPS – suggesting that central nervous system changes joined with peripheral changes in regulating the HPA axis. Of course, further studies are needed to delve deeper into this mechanism.

Undoubtedly, a limitation of this study is the lack of data on adrenal-dependent mechanisms to understand whether the changes in corticosterone levels are primary or secondary, pituitary-dependent or not. In addition, we did not study the expression of steroidogenic enzymes in the adrenal cortex, and such an approach would have allowed a more direct link between changes in corticosterone and changes in HPA axis activity. At the same time, since this study is a continuation of years of research on COX-2 inhibition, changes in the parameter by sex were not taken into account, which has recently been raised as one of the factors guiding changes. However, this work is a simple but vital experimental study that provides new data on the role of COX-2 activity and the putative mGluR5 unit in regulating corticosterone levels.

5. Conclusion

In conclusion, the data obtained in this manuscript indicate that COX-2 inhibition affects corticosterone release in mice. The effects are time-dependent, as demonstrated by seven-day and fourteen-day COX-2 inhibition. Short-term COX-2 inhibition released corticosterone, while long-term inhibition reduced its serum levels in mice. In addition, the effect of LPS challenge on corticosterone was regulated by COX-2 inhibition. This mechanism may therefore suggest that COX-2 is a vital factor modulating corticosterone synthesis/release in mice.

CRediT authorship contribution statement

Patrycja Pańczyszyn-Trzewik: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Magdalena Sowa-Kućma:** Methodology, Investigation, Formal analysis, Data curation. **Paulina Misztak:** Methodology, Investigation. **Anna Tabecka-Lonczynska:** Methodology, Investigation, Data curation. **Katarzyna Stachowicz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was partially supported by grant No.: UMO-2014/13/D/NZ7/00292, assigned by the National Science Centre, Poland, to K. Stachowicz and by the statutory fund of Maj Institute of Pharmacology, PAS, Poland.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2024.109438.

References

[1] S. Gong, Y.L. Miao, G.Z. Jiao, M.J. Sun, H. Li, J. Lin, M.J. Luo, J.H. Tan, Dynamics and correlation of serum cortisol and corticosterone under different physiological or stressful conditions in mice, PLoS One 10 (2015) e0117503.

[2] R. Ovejero, A. Novillo, M. Soto-Gamboa, M.E. Mosca-Torres, P. Cuello, P. Gregório, G. Jahn, P. Carmanchahi, Do cortisol and corticosterone play the same role in coping with stressors? Measuring glucocorticoid serum in free-ranging guanacos (Lama guanicoe), JEZ-A Eco Gen. Physiol. 319 (2013) 539–547.

[3] A. Ströhle, F. Holsboer, Stress responsive neurohormones in depression and anxiety, Pharmacopsychiatry 36 (2003) 207–214, https://doi.org/10.1055/s-2003-45132.
[4] B.S. McEwen, Sex, stress and the hippocampus: allostasis, allostatic load and the aging process, Neurobiol. Ag 23 (2002) 921–939.
[5] Y. Naisberg, Homeostatic disruption and depression, Med. Hypoth. 47 (1996) 415–422.
[6] P.W. Gold, The organization of the stress system and its dysregulation in depressive illness, Mol. Psych. 20 (2015) 32–47.
[7] K. Stachowicz, Deciphering the mechanisms of regulation of an excitatory synapse via cyclooxygenase-2. A Review, Biochem. Pharmacol. 192 (2021) 114729.
[8] J. Bugajski, A. Gądek-Michalska, Effect of cyclooxygenase inhibitors on the vasopressin induced ACTH and corticosterone response during crowding stress, J. Physiol. Pharmacol. 54 (2003) 247–256.
[9] N. Müller, M.J. Schwarz, COX-2 inhibition in schizophrenia and major depression, Curr. Pharmacol. Des. 14 (2008) 1452–1465.
[10] P. Casolini, A. Catalani, A.R. Zuena, L. Angelluci, Inhibition of COX-2 reduces the age-dependent increase of hippocampal inflammatory markers, corticosterone secretion, and behavioral impairments in the rat, J. Neurosci. Res. 68 (2002) 337–343.
[11] A. Institoris, E. Farkas, S. Berczi, Z. Sule, F. Bari, Effects of cyclooxygenase (COX) inhibition on memory impairment and hippocampal damage in the early period of cerebral hypoperfusion in rats, Eur. J. Pharmacol. 574 (2007) 29–38.
[12] A. Linda, L.A. Kotilinek, M.A. Westerman, Q. Wang, K. Panizzon, G.P. Lim, A. Simonyi, S. Lesne, A. Falinska, L.H. Younkin, S.G. Younkin, M. Rowan, J. Cleary, R.A. Wallis, G.Y. Sun, G. Cole, S. Frautschy, R. Anwyl, K.H. Ashe, Cyclooxygenase-2 inhibition improves amyloid-β-mediated suppression of memory and synaptic plasticity, Brain 131 (2008) 651–664.
[13] N. Müller, COX-2 inhibitors, aspirin, and other potential anti-inflammatory treatments for psychiatric disorders, Front. Psych. (2019).
[14] A. Gądek-Michalska, J. Bugajski, A.J. Bugajski, R. Głód, Effect of adrenergic antagonists and cyclooxygenase inhibitors on the nicotine-induced hypothalamic-pituitary-adrenocortical activity, J. Phys. Pharmacol. (2002) 275–287.
[15] M.Z. Zhang, R.C. Harris, J.A. McKanna, Regulation of cyclooxygenase-2 (COX-2) in rat renal cortex by adrenal glucocorticoids and mineralocorticoids, PNAS (1999) 15280–15285.
[16] L. Engstrom, K. Rosen, A. Angel, A. Fyrberg, L. Mackerlova, J.P. Konsman, D. Engblom, A. Blomqvist, Systemic immune challenge activates an intrinsically regulated local inflammatory circuit in the adrenal gland, Endocrinology (2007) 1436–1450.
[17] K. Stachowicz, B. Bobula, K. Tokarski, NS398, a cyclooxygenase-2 inhibitor, affects memory performance disrupted by imipramine in C57Bl/6J Mice, Brain Res. 1734 (2020) 146741.
[18] R.S. Harris, COX-2 and the kidney, Cardiovasc. Pharmacol. 47 (2006) 37–42.
[19] K. Hierholzer, I. Lichtenstein, H. Siebe, D. Tsiakiras, I. Witt, Renal metabolism of corticosteroid hormones, Klin. Wochenschr. 60 (1982) 1133–1135.
[20] K. Stachowicz, B. Bobula, M. Kusek, T. Lenda, K. Tokarski, Evidence for the interaction of COX-2 with mGluR5 in the regulation of EAAT1 and EAAT3 protein levels in the mouse hippocampus. The influence of oxidative stress mechanisms, Brain Res. 1771 (2021) 147660.
[21] C.A. Murray, B. McGahon, S. McBennett, M.A. Lynch, Interleukin-1 beta inhibits glutamate release in hippocampus of young, but not aged, rats, Neurobiol. Ag 18 (1997) 343–348.
[22] N.S. Philip, L.L. Carpenter, A.R. Tyrka, L.H. Price, Nicotinic acetylcholine receptors and depression: a review of the preclinical and clinical literature, Psychopharmacol 212 (2020) 1–12.
[23] M.A. Sundar, T.S. Shammugarajan, V. Ravichandrian, 3,4-dihydroxyphenylethanol assuages cognitive impulsivity in Alzheimer's disease by attuning HPA-axis via differential crosstalk of α7 nAChR with MicroRNA-124 and HDAC6, ACS Chem. Neurosci. 9 (12) (2018) 2904–2916.
[24] M.B. Newman, S.J. Nazian, P.R. Sanberg, D.M. Diamond, R.D. Shytle, Corticosterone-attenuating and anxiolytic properties of mecamylamine in the rat, Prog. Neuro Psychopharamac. Biol. Psych. 25 (2001) 609–620.
[25] S. Paul, W.K. Jeon, J.L. Bizon, J.S. Han, Interaction of basal forebrain cholinergic neurons with the glucocorticoid system in stress regulation and cognitive impairment, Front. Aging Neurosci. 7 (2015) 43.
[26] G. Paxinos, K.B.J. Franklin, The Mouse Brain in Stereotaxic Coordinates, 2nd ed., Academic press, San Diego, 2001.
[27] K.S. Suvarna, C. Layton, J.D. Bancroft, Theory and Practice of Histological Techniques, 7th ed., Churchill Livingstone, 2013.
[28] E. Lepowsky, F. Ghaderinezhad, S. Knowlton, S. Tasoglu, Paper-based assays for urine analysis, Biomicrofluidics 11 (2017) 051501.
[29] J. Matsuzaki, M. Kuwamara, R. Yamaji, H. Inui, Y. Nakano, Inflammatory responses to lipopolysaccharide are suppressed in 40% Energy-restricted mice, Nut Immun (2001) 2139–2144.
[30] R. Yin, K. Zhang, Y. Li, Z. Tang, R. Zheng, Y. Ma, Z. Chen, N. Lei, L. Xiong, P. Guo, G. Li, Y. Xie, Lipopolysaccharide-induced depression-like model in mice: meta-analysis and systematic evaluation, Front. Immunol. 14 (2023) 1181973.
[31] Xt. Guan, Wj. Lin, Mm. Tang, Comparison of stress-induced and LPS-induced depressive-like behaviors and the alterations of central proinflammatory cytokines mRNA in rats, Psych. J. 4 (2015) 113–122.
[32] M. Maes, M. Berk, L. Goehler, C. Song, G. Anderson, P. Gałecki, J. Leonard, Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways, BMC Med. 10 (2012).
[33] K.A. Kelly, L.T. Michalovicz, J.V. Miller, V. Castranova, D.B. Miller, J.P. G. O'Callaghan, Prior exposure to corticosterone markedly enhances and prolongs the neuroinflammatory response to systemic challenge with LPS, PLoS One 13 (2018) e0190546.
[34] C.C. Weber, G.P. Eckert, W.A. Müller, Effects of antidepressants on the brain/plasma distributionof corticosterone, Neuropsychopharmacology 31 (2006) 2443–2448.
[35] C. Romano, M.A. Sesma, C.T. Mcdonald, K. Omalley, A.N. Vandenpol, J.W. Olney, Distribution of metabotropic glutamate receptor mGlu5 immunoreactivity in rat brain, J. Comp. Neurol. 355 (1995) 455–469.
[36] A.N. Van der Pol, C. Romano, P. Ghosh, Metabotropic glutamate receptor mGluR5 subcellular distribution and developmental expression in hypothalamus, J. Comp. Neurol. 362 (1995) 134–150.
[37] M. Pokusa, B. Prokopova, N. Hlavacova, A. Makatsori, D. Jezova, Effect of blockade of mGluR5 on stress hormone release and its gene expression in the adrenal gland, Can. J. Physiol. Pharmacol. 92 (2014) 686–692.
[38] S.E. Dryer, Glutamate receptors in the kidney, Nephrol. Dial. Transplant. 30 (2015) 1630–1638.
[39] K.T. Akama, L.I. Thompson, T.A. Milner, B.S. McEwen, Post-synaptic density-95 (PSD-95) binding capacity of g-protein-coupled receptor 30 (GPR30), an estrogen receptor that can be identified in hippocampal dendritic spines, J. Biol. Chem. 288 (2013) 6438–6450.
[40] F. Liu, R. Zhou, H. Yan, H. Yin, X. Wu, Y. Tan, L. Li, Metabotropic glutamate receptor 5 modulates calcium oscillation and innate immune response induced by lipopolysaccharide in microglial cell, Neuroscience 281 (2014) 24–34.
[41] B.J. MacNeil, A.H. Jansen, A.H. Greenberg, D.M. Nance, Effect of acute adrenalectomy on sympathetic responses to peripheral lipopolysaccharide or central PGE2, Am. J. Physiol. Reg. Int. Comp. Physiol. 278 (2000) 1321–1328.
[42] A.C. Teilmann, O. Kalliokoski, D.B. Sørensen, J. Hau, K.S.P. Abelson, Manual versus automated blood sampling: impact of repeated blood sampling on stress parameters and behavior in male NMRI mice, Lab Anim. 48 (2014) 278–291.
[43] H.F. Cheng, R.C. Harris, Renal effects of non-steroidal anti-inflammatory drugs and selective cyclooxygenase-2 inhibitors, Curr. Pharm. Des. 11 (2005) 1795–1804.
[44] F. Burdan, A. Chałas, J. Szumiło, Cyclooxygenase and prostanoids – biological implications PHMD 60 (2006) 129-141. (in Polish).
[45] G. Eisenhofer, I.J. Kopin, D.S. Goldstein, Catecholamine metabolism: A contemporary view with implications for physiology and medicine, Pharmacol. Rev. 56 (2004) 331–349.
[46] J. Dlnchuk, B.D. Car, R.J. Focht, J.J. Johnston, B.D. Jaffee, M.B. Covington, et al., Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II, Nature 378 (1995) 406–409.
[47] K. Stachowicz, P. Misztak, P. Panczyszyn-Trzewik, T. Lenda, S. Rzeźniczek, M. Sowa-Kućma, Upregulation of the mGlu5 receptor and COX-2 protein in the mouse brain after imipramine and NS398, searching for mechanisms of regulation, Neurochem. Int. 150 (2021) 105193.
[48] W. Matysiak, B. Jodłowska-Jędrych, Does administration of non-steroidal anti-inflammatory drug determine morphological changes in adrenal cortex: ultrastructural studies, Protoplasma 246 (2010) 109–118.
[49] J. Bugajski, A. Gądek-Michalska, A.J. Bugajski, Effect of constitutive- and inducible-cyclooxygenase in the carbachol-induced pituitary-adrenocortical response during social stress, J. Physiol. Pharmacol. 53 (2002) 453–462.
[50] L. Elander, L. Engstrom, J. Ruud, M. Mackerlova, P.J. Jakobsson, D. Engblom, C. Nilsberth, A. Blomqvist, Inducible prostaglandin E2 synthesis Interacts in a temporally supplementary sequence with constitutive prostaglandin-synthesizing enzymes in creating the hypothalamic–pituitary–adrenal axis response to immune challenge, J. Neurosci. 29 (2009) 1404–1413.
[51] M. Ek, D. Engblom, S. Saha, A. Blomqvist, P.J. Jakobsson, A. Ericsson-Dahlstrand, Inflammatory response: pathway across the blood-brain barrier, Nature 410 (2001) 430–431.
[52] L. Elander, J. Ruuda, M. Korotkova, P.J. Jakobsson, A. Blomqvist, Cyclooxygenase-1 mediates the immediate corticosterone response to peripheral immune challenge induced by lipopolysaccharide, Neurosci. Lett. 470 (2010) 10–12.
[53] A. Solarz, I. Majcher-Maślanka, J. Kryst, A. Chocyk, Early-life stress affects peripheral, blood-brain barier, and brain responses to immune challenge in juvenile and adult rats, Brain Behav. Immun. 108 (2023) 1–15.
[54] S.W. Walker, M.W.J. Strachan, E.R.T. Lightly, B.C. Williams, L.M. Bird, Acetylcholine stimulates cortisol secretion through the M3 muscarinic receptor linked to a polyphosphoinositide-specific phospholipase C in bovine adrenal fasciculata/reticularis cells, Mol. Cell. Endocrinol. 72 (1990) 227–238.