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Effects of Abscisic Acid on Streptozotocin-Induced Changes in Hippocampal Expression of Cyclooxygenase-2 (COX-2) and Phosphorylated ERK Signaling (P-ERK) in Male Rats

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ABSTRACT

Background: Central administration of STZ (Streptozotocin) induces oxidative damage, neuroinflammation, cholinergic deficits, β-amyloid and tau protein accumulation in the brain. Abscisic acid (ABA) as a phytohormone is produced in a variety of animal tissues, including brain. Recently data show it has involved in a wide spectrum of activities in CNS including, learning and memory and pain regulation.

Objectives: Here, the alterative effects of abscisic acid and possibility of involving PKA and PPARβ/δ receptors, on Streptozotocin-induced changes in hippocampal expression of cyclooxygenase-2 (COX-2) and ERK signaling (p-ERK) in male Wister rats was investigated.

Materials and Methods: STZ was injected intracerebroventricularly (i.c.v.) (3 mg/kg), ABA was administrated alone (10 μg/rat, i.c.v.) or accompanied with PPARβ/δ receptor antagonist (GSK0660, 80 nM/rat) or selective inhibitor of PKA (H89, 80nM/rat) for 14 days. Western blot analysis was used to indicate changes in hippocampal COX-2 and p-ERK expression.

Results: The results showed that STZ produced a significant increase in hippocampal expression of COX-2 and a decrease in expression of p-ERK. ABA significantly prevented the effects of STZ. However, ABA effects were blocked by PPARβ/δ receptor antagonist (GSK0660) and selective inhibitor of PKA (H89).

Conclusions: It seems that the ABA moderates STZ-induced neuronal inflammation and ERK signaling deficiency by PPARβ/δ receptor and PKA signaling.

Keywords: Abscisic acid; Streptozotocin; COX-2; p-ERK; Rats

INTRODUCTION

Streptozocin (STZ) is a drug and chemical agent for induction of significant metabolic changes, with source of *Streptomyces acromogenes*. It is an acceptable agent for induction of diabetes and Alzheimer's disease in rodents (1). Streptozocin has also toxic potential in many cells and tissues (2). Intracerebroventricular (icv) injection of streptozotocin due to association with the development of an insulin-resistant brain state, induction of neuroinflammation changes, oxidative stress, producing learning and memory and also cognitive deficits is a acceptable method for induction of malfunction in the CNS (3-5). Regarding the STZ effects, it seems that brain cholinergic system also affected by STZ (6). Streptozotocin (STZ) is a glucosamine-

nitrosourea compound that was originally identified as an antibiotic. It is toxic to beta cells of pancreas and usually transported through glucose transporter 2 and commonly used to induce experimental diabetes in animals. Investigations show that ICV/IP (intraperitoneal) injection of STZ produces significant elevation in cerebral aggregation of Aβ fragments, total tau protein, and Aβ deposits. In other words, STZ in a rodent's brain by induction of many biochemical alterations is considered to be a valid experimental model for neurodegenerative disease. Recent data indicate that STZ can produce Alzheimer like disease pathological alteration in amyloid precursor protein (APP), glucose metabolism, insulin signaling, cholinergic deficits, oxidative stress, neuroinflammation, synaptic function, protein kinases, and apoptosis (7-9).

Neuroinflammatory responses happening in the central nervous system (CNS) are tightly linked to the pathways leading to neuronal cell death in Alzheimer's disease (AD) (10). It has been reported that inflammatory activation of glial cells results in cognitive deficit and neuronal death (11). Cyclooxygenase-2 (COX-2) is an inducible isoform of the cyclooxygenase enzyme that plays an essential role in initiation and progression of inflammation (12). COX2 is expressed in many brain diseases, for example AD, depression and global ischemia (13-15). Clinical trials have shown that long-term use of COX inhibitors can decrease the incidence of AD by affecting inflammatory response, neuronal loss and behavioral changes (16, 17).

Extracellular signal-regulated kinase (ERK), a type of MAPK, has been involved as a critical component in a large number of signaling systems implicated in hippocampal-dependent memory formation and synaptic plasticity (18). The inhibition of ERK phosphorylation leads to cognitive deficits (19) and previous reports found decreased levels of ERK protein and mRNA in AD hippocampus compared with controls (20, 21).

Recently, it has been demonstrated that physiological functions of ABA are not restricted to plants and it has a crucial role in animals (22). In addition, ABA is involved in some cell functions such as inflammatory and immune responses (23, 24), insulin sensitization and glucose homeostasis (25, 26), and regulates stem cell expansion and stimulation (27).

ABA improves spatial and passive avoidance learning and memory in rats (28). Moreover, it shows anti-anxiety effects (29) and a dose-dependency manner inhibition in spatial and passive avoidance learning and memory deficits in STZ rats (30).

Since despite the well-known anti-inflammation effects of ABA, its effect on experimental STZ-induced inflammation and ERK signaling pathways in the hippocampus of rats has not yet been clarified. In the present study we investigated the effect of central administration of ABA on modulation of STZ-induced inflammation and ERK signaling pathways in the rat's hippocampus, with emphasis on alterations in hippocampal expression of COX-2 and p-ERK.

Materials and methods

Animals

Adult male Wistar rats (230–270 gr total rat 49 and 7 per group) were used. The animals were obtained from the Shahid Bahonar University of Kerman Animal House. Food and water were available *ad libitum*. The animals were housed under a 12 h light/dark cycle in controlled conditions with a temperature of 22 ±2 °C. All experimental procedures were approved by the Animal Research Ethics Committee of the Kerman Neuroscience Research Center, Kerman, Iran (EC: 96/17) and confirmed to the standard ethical guidelines (NIH, publication no. 85-23, revised 1985).

Surgery

For central injection of drugs, anesthetized rats with ketamine and xylazine (60 and 10 mg/kg, respectively) were implanted with guide cannulas stereotaxically (Stoelting, USA). Guide cannulas (22-gauge stainless steel needle) were implanted and aimed bilaterally into right and left ventricles. The intended coordinates for the left and right ventricles were AP=1.6 mm from Bregma, ML= ±0.8 from the midline, and DV=3.4 mm from the skull surface (31). Guide cannulas were fixed to the skull through two stainless steel screws and

acrylic dental cement. Then, the animals were housed in individual cages and had a one-week recovery period before drug injection. Stereotaxic surgery was performed, and after the recovery period (1 week) drugs were injected into the lateral ventricle with 15-minute intervals. The animals were killed just after the behavioral test ,the data were published (32), the brains were removed and fixed in formalin for 2 days and the correct placement of the cannula was confirmed by histological examination. If cannula was not fixed in the accurate place, the rat's data were omitted from the analysis.

Drugs

STZ, (±)-cis,trans-ABA, H-89 dihydrochloride hydrate(PKA inhibitor), and GSK0660(PPARβ/δ receptor antagonist) were purchased from Sigma-Aldrich, USA. STZ was dissolved in the normal saline solution (0.9% w/v sodium chloride), ABA was dissolved in the saline solution and dimethyl sulfoxide (DMSO) at a ratio of 2:1 (v/v). H-89 and GSK0660 were dissolved in DMSO (0.1% in final) and diluted with saline solution.

Microinjection

Central injection of drugs was performed through a 27-gauge internal cannula connected via polyethylene tubing to a 10 µl Hamilton syringe. The needle was left in the place for 1 min before it was slowly retracted. The injection needle was inserted 1 mm beyond the tip of the guide cannula.

Experimental design

The animals were randomly divided into seven experimental groups (n = 7):

Control group which received no injection; STZ sham group received STZ vehicle; ABA sham received ABA vehicle ; STZ group that received STZ (3 mg/kg, body weight in saline, 5 µL/I.C.V injection); STZ + ABA-treated groups received STZ and 15 minutes later received ABA (10µg/rat, 2 µL/injection from days 1-14); STZ+ABA+GSK which received STZ, ABA and GSK0660 (GSK, 80 nM/rat, 2 µL/injection from days 1 to 14); STZ+ABA+H89 which received STZ, ABA and H89 (H89, 80 nM/rat, 2 µL/injection from days 1 to 14). The doses of drugs were selected according to the previous studies (29, 33-35).The experiment was performed between 9.00 a.m. to 2.00 p.m. at standard laboratory conditions, such as ambient temperature of 25±21C and sound proof room.

Western blot analysis

Rat hippocampal tissues were lysed in RIPA buffer containing 10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 Mm ethylenediaminetetraacetic acid, 0.1% sodium dodecyl sulfate, 0.1% Na-deoxycholate, 1% NP-401% NP-40 and protease inhibitors (1 mM phenylmethyl sulfonyl fluoride, 2.5 µg/ ml of leupeptin, 10 µg/ml of aprotinin) and 1 mM sodium orthovanadate. Equal amounts (40 µg) of protein were electrophoresed on 9% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to polyvinylidene difluoride (PVDF) membrane (Roche). Total protein concentration was determined by Bradford method. After blocking with 5% non-fat dried milk in Tris-buffered saline with Tween 20 (blocking buffer, TBS-T, 150mM NaCl, 20mM Tris–HCl, pH 7.5, 0.1% Tween 20), the membranes were incubated with primary COX-2 and p-ERK antibody (1:1000) overnight at 4 °C. The primary antibody was detected with goat antimouse horseradish peroxidase-conjugated secondary antibody (1:15,000, Santa Cruz Biotechnology, USA). The antibody-antigen complexes were identified using the ECL system and exposed to Lumi-Film chemiluminescent detection film (Roche, Germany). Lab Works analyzing software (UVP, UK) was used to evaluate the intensity of the blotting bands. β-actin (1:10,000) was used as the loading control. The expression values were presented as tested proteins / β-actin ratio for each rat.

Statistical analysis

Data are presented as means ± standard error of the mean. Statistical analysis comprised one-way analysis of variance followed by post-hoc Tukey's test. The p-value < 0.05 was considered statistically significant.

RESULTS

It should be noted that because the behavioral data showed that the solvents had no effect on the behavior of the animals (32) they were not evaluated at the molecular analysis.

Immunoblot analysis showed significant differences in hippocampal COX-2 protein levels among the different experimental groups. As shown in Figure 1, COX-2 induction in STZ-treated group ($p < 0.01$) was significantly increased compared with the control group; while pretreatment with ABA (10µg/rat) diminished the effect of STZ on COX-2 expression ($p < 0.05$). However, GSK and H89 could completely inhibit the positive effect of ABA ($P<0.05$) (Figure 1).

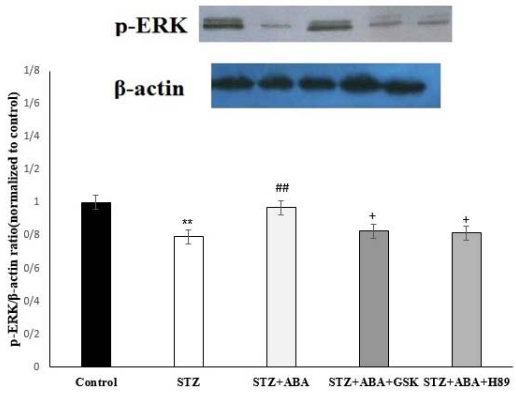

Figure 1. Cox2 protein levels in hippocampus of rats in experimental groups. One-way analysis of variance was used. Values represent mean ± SEM. **P < 0.01 versus control group, #P < 0.05 versus STZ group, +P < 0.05 versus STZ+ABA group.

Figure 2 shows that following administration of STZ, hippocampal levels of p-ERK were significantly decreased ($p < 0.01$); while pretreatment with ABA (10µg/rat) reduced the effects of STZ on p-ERK expression ($p < 0.01$). However, ABA- induced p-ERK protein up-regulation was prevented after GSK or H89 treatment ($p<0.05$) (Figure2).

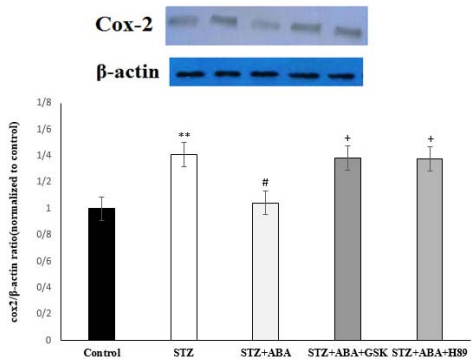

Figure 2. Phosphorylated ERK levels in hippocampus of rats in experimental groups. One-way analysis of variance was used. Values represent mean ± SEM. **P < 0.01 versus control group, ##P < 0.01 versus STZ group, +P < 0.05 versus STZ+ABA group.

DISCUSSION

The present study investigated the possible protective effect of ABA on STZ -induced alteration in hippocampal expression of COX-2 and p-ER. Here also the role of PPARβ/δ receptor antagonist (GSK0660) and selective inhibitor of PKA (H89) in the effects were assessed. Data showed that ABA in lateral ventricle of rats moderates STZ-induced neuronal inflammation and ERK signaling deficiency by PPARβ/δ receptor and PKA signaling.

Previous reports show that the Central STZ typically produces prolonged impairment of glucose/energy metabolism, neuroinflammation, and oxidative stress in the brain (8).In line with the previous data our finding also showed STZ-induced elevation in inflammation of hippocampus.

Also, high levels of COX-2 and low levels of p-ERK proteins in the hippocampus were detected following STZ-induced Alzheimer's disease model. Cognitive impairment after STZ administration is induced by the degradation of phospholipids, which results in increases in the free fatty acid arachidonic acid. COX-2 is a rate-limiting enzyme in the metabolism of arachidonic acid to prostanoids, particularly prostaglandins (PGs), which significantly contribute to neuro-inflammation with oxidative stress (36). Interestingly, central administration of ABA was able to attenuate the effects of STZ on COX-2 induction and increased the levels of p-ERK protein.

A higher level of expression in COX-2 mRNA and protein was found in the brains of AD models (37, 38). It has been demonstrated that COX-2 plays an essential role in pathological process of AD and the inhibition of COX-2 is a potential neuroprotective strategy for limiting the progression of the disease through its action on the downstream effects of the insulin signaling pathway inhibiting neuroinflammation and oxidative stress (15, 17, 39).

In vivo studies have revealed an anti-inflammatory capacity for ABA in animal models

of colitis (40) and pulmonary inflammation (41). Such beneficial effects is mediated generally by decrease in inflammatory leukocyte infiltration and modulation of adhesion molecules expression (40, 41). It has been shown that ABA attenuates TNF α expression and macrophages infiltration in obesity-related inflammation in db/db mice (42). Furthermore, ABA significantly increases the immune regulatory and anti-inflammatory cytokine IL-10 expression in mice (41).

Moreover, in this study we observed that the expressions of COX-2 were significantly increased in STZ-treated rats and decreased strongly by ABA (10μg/rat). It seems that ABA induces an anti-inflammatory role by diminishing COX-2 expression in STZ-treated rats.

In addition, our data showed that central administration of STZ reduces p-ERK protein expression in the hippocampus. This result is supported by the previous studies that showed down-expression of hippocampal ERK phosphorylation causes cognitive impairments (19).

Recent studies have shown that Aβ oligomers elevation post PTZ -treatment inhibits ERK activation and subsequently CREB in human neuroblastoma cells and primary neurons and beta-amyloid-induced cell death is accompanied by ERK suppression (43, 44). In the Tg2576 model of AD, ERK signaling is dysregulated and unable to correctly function in new memory formation (45). It has been shown that ABA can bind to a membrane G protein complex receptor, which leads to phosphorylation and activation of ADP-ribosyl cyclase (ADPRC), overproduction of the calcium mobilizer cyclic ADP-ribose (cADPR), and consequent increase of the intracellular calcium concentration (22, 23, 46).

Recently reported that ABA has precognitive and anti-anxiety effects by extracellular calcium influx through L-type calcium channels, intracellular calcium currents and extracellular signal-regulated kinase signaling (p-ERK) (35). In addition, recent studies have shown that ABA also acts as an activator of the cAMP/PKA signaling pathway (26, 47).

The data indicated that the expression of p-ERK was significantly decreased in STZ-treated rats and the effect was prevented by ABA (10μg/rat). Although ABA signaling pathway is not fully elucidated, actually ABA in animals and plants uses a wide spectrum of signaling pathways. Its pathway is highly conserved across species and it has been known as a potent agonist for lanthionine synthetase C-like 2 receptor and peroxisome proliferator-activated receptors family member (PPARs) (48). ABA stimulates Ca2+ release by activation of downstream targets including phospholipase C / protein kinase C (PLCPKC) cascade and adenylate cyclase cAMP-dependent protein kinase A (PKA) pathway (49). Moreover, the data elucidated, the ABA preventive effect on STZ-induced alteration in hippocampal expression of COX-2 and p-ERK involved PPARβ/δ receptor and p- PKA signaling systems.

In conclusion, the results provided evidence that central injection of ABA can attenuate STZ-induced neuronal inflammation and MAPK signaling deficiency in the hippocampus, mainly through attenuation of STZ-induced COX-2 and an increase of p-ERK expressions. However, further studies are needed to explain the detailed role(s) and exact mechanism(s) of ABA in this regard. As a limitation, since the relevant behavioral data have already been published, they could not be presented and discussed here.

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Conflict of interest

The authors declare that there is no conflict of interest.

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