Chemico-Biological Interactions 256 (2016) 134–141

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Hydrogen peroxide-induced oxidative damage in peripheral blood lymphocytes from rats chronically treated with corticosterone: The protective effect of oxytocin treatment

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ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form
18 June 2016
Accepted 7 July 2016
Available online 9 July 2016

Keywords:
Chronic stress
Corticosterone
Oxytocin
Genotoxicity
Comet test
Oxidative stress

ABSTRACT

Contemporary lifestyle is commonly associated with chronic stress, an environmental factor contributing to development of various psychological and somatic disorders. Increased levels of glucocorticoids, observed in the chronic stress, induce the production of reactive oxygen species leading to genotoxicity. The aim of this study was to investigate whether chronic administration of oxytocin (OXY) 10 IU/400 μL/day, *s.c.*, for 14 days, a hormone presumed to exert antioxidant effect, may prevent DNA damage in the comet assay of peripheral blood lymphocytes of Wistar rats treated chronically with corticosterone (CORT) 100 mg/L *ad libitum, per os*, for 21 days, as well as, to influence some plasma oxidative stress parameters, i.e. levels of total lipid hydroperoxide (LOOH), and malondialdehyde (MDA), and the activity of antioxidative enzyme superoxide dismutase (SOD). Even though there was no reduction in overall number of damaged cells after oxytocin treatment only, the marked increase in total comet score (TCS) after incubation with H_2O_2 in CORT group compared to controls, was absent in the CORT + OXY experimental group. Furthermore, significant decrease of highly damaged cells compared to corticosterone group was noted. Chronic oxytocin administration thus protected lymphocytes from high intensity damage that leads to cellular death. In addition, treatment with OXY along with CORT, significantly decreased concentration of LOOH in plasma, and increased SOD compared to CORT treatment only. This finding corresponds well with current reports on beneficial effects of OXY in conditions of HPA axis hyperactivity, and supports the hypothesis of OXY-mediated antioxidant action.

1. Introduction

Stress in our daily life affects the normal physiology of biological systems [1]. The primary system underlying stress physiology is the hypothalamus-pituitary-adrenal (HPA) axis. Exposure to physiological or psychological stress causes a wide range of autonomic, endocrine, and behavioral responses, necessary to reestablish homeostasis and allow the individual to cope with stress conditions [2]. However, a long-term activation of HPA axis may disturb normal responses characteristic for acute stress leading to persistent elevation of stress hormones, which may disrupt normal physiological and behavioral functions [3]. Importantly, glucocorticoids are among the most widely prescribed drugs for the treatment of immune and inflammatory diseases, as well as a component of many chemotherapy regimens [4].

Exogenous corticosterone (CORT) application to rodents is widely used as a model for chronic stress [1,5,6]. CORT administration to rats for 21 days caused adrenal gland atrophy, prevented regular weight gain of animals, and disrupted circadian rhythms of adrenocorticotropic hormone (ACTH) and CORT [6]. Harmful effects

Abbreviations: ACTH, adrenocorticotropic hormone; CORT, corticosterone; HPA, hypothalamus-pituitary-adrenal axis; IL-6, interleukin 6; LOOH, total lipid hydroperoxide; MDA, malondialdehyde; NO, nitric oxide; OXY, oxytocin; PBL, peripheral blood lymphocytes; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TCS, total comet score.

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http://dx.doi.org/10.1016/j.cbi.2016.07.006
0009-2797/

of chronically elevated glucocorticoid level, either because of prolonged stress [7–9] or because of exogenous hormone administration [1,10,11], are most probably linked to an increased formation of reactive oxygen or nitrogen species (ROS and RNS, respectively). In excess and over time, due to an imbalance in production and efficiency of anti-oxidant defense, they cause chronic oxidative stress. Therefore, it has been demonstrated that the effects of chronically administered CORT on oxidative damage are comparable to that induced by a psychological stressor, such as restraint stress [11].

Genotoxic effects of glucocorticoids [12–17] may also be related to oxidative stress [18]. Attack of DNA by ROS or RNS can lead to strand breaks, DNA–DNA, and DNA–protein cross-linking, and formation of modified bases complexes. An imbalance in the base excision repair process, the main pathway for repair of oxidative DNA lesions in mammals, can also be deleterious [19].

Oxytocin (OXY), beside its known wide spectra of central and peripheral actions, influences a variety of complex social behaviors, such as sexual and maternal behavior, affiliation, social recognition, cognition, aggression, neuromodulation and tolerance development [20–22]. This neuropeptide can significantly modulate the reaction of the organism to stress by its influence on the endocrine and paracrine function [23]. Importantly, Moosmann and Behl [24] have identified a novel class of endogenous antioxidants, short soluble tyrosine- or tryptophan-containing peptide hormones and mediators such as LHRH, [Leu] and [Met]enkephalin, angiotensin I and II, vasopressin and oxytocin. These peptide hormones can scavenge RNS derived from nitric oxide (NO) and peroxynitrite, and may constitute an important part of the antioxidant defense system, which can act as potent biochemical antioxidants under variety of conditions. During the course of the years, numerous experimental data corroborated a potent antioxidant action of OXY. Actually, the OXY's cytoprotective effects are achieved via complex anti-oxidative, anti-inflammatory, immuno-modulatory, trophic, and anti-apoptotic pathways [25,26]. Thus, it has been described that the pretreatment of rats with this peptide exerts a radioprotective action on animal survival and blood cells count [27]. OXY has a protective effect in myocardial ischemia in rats [28]. It attenuates NADPH-dependent superoxide activity and interleukin 6 (IL-6) secretion in vascular cells and macrophages [29]. In addition, it improves the antioxidative state of the colonic tissue and ameliorates oxidative colonic injury in rats via a neutrophil-dependent mechanism [30]. Furthermore, it has been shown that OXY alleviates oxidant renal injury in pyelonephritic rats by antioxidant actions and by preventing free radical damaging cascades that involves excessive infiltration of neutrophils [31], improves renal function and tissue damage in renal ischemia/reperfusion injury in rat, along with the alleviation of oxidant tissue responses [32]. Also, it decreases blood biochemical parameters of oxidative stress and protects testicular tissue in streptozotocin-induced diabetic rats [33]. The data on OXY influence on DNA are scarce. Djelić et al. [34] described that high concentrations of OXY in cultures of human peripheral blood lymphocytes (PBLs) do not express any genotoxic properties on the basis of numerical and structural chromosome aberration analysis and the *in vitro* sister chromatid exchange test. On the other side, Andrade et al. [35] reported that 21 and 65 days long treatments with different doses of OXY in rats could significantly increase frequency of hippocampal cells that presented some level of DNA damage using the alkaline comet assay.

In the light of the above-mentioned data, here we examined if the treatment with OXY may be beneficial in preventing DNA damage that occurred during exposure to chronically high levels of glucocorticoids. Therefore, using the comet assay, we evaluated the influence of OXY on the intensity of DNA damage in peripheral PBL of rats treated chronically with CORT in the absence and presence of hydrogen peroxide (H_2O_2), which is a strong oxidizing agent. In addition, we examined if OXY treatment affected plasma concentrations of lipid hydroperoxide (LOOH) and malondialdehyde (MDA) as parameters of oxidative stress, and activity of antioxidant enzyme, superoxide dismutase (SOD).

2. Materials and methods

2.1. Animals and treatments

Adult male Wistar rats (Military farm, Belgrade, Serbia), weighing 250–300 g, 8 weeks old at the onset of the experiment, were used. Animals were pair-housed with *ad libitum* access to food and water and standard vivarium conditions (temperature of 20 ± 1 °C and 12-h light/dark cycle).

After at least 4 days of adaptation to laboratory conditions, animals were randomly allocated into 4 groups (6 rats in each group): CORT-group received CORT (100 mg/L) dissolved in drinking water for 21 days, in order to ensure that animals were exposed continually to the elevated level of hormone; OXY-treated rats received 10 IU of peptide/400 μL/day *s.c.* for 14 days; CORT + OXY-group received the combination of CORT and OXY in the course of the last 14 days of CORT treatment; and the fourth group was the corresponding control group, receiving saline *s.c.* and vehicle *per os.*

All procedures in this study were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996) and approved by the Committee for Ethical Animal Care and Use of the Faculty of Pharmacy, University of Belgrade (Permit Number: 323-07-00067/2015-05).

Corticosterone (Sigma-Aldrich Co., Cat. No. C2505, USA) was dissolved in 2% solution of Tween 80 (Polysorbatum 80, Fargon, Cat. No.102254, USA) in tap water. In order to prevent degradation of hormone induced by light, bottles with CORT solution were covered with aluminium foil. Oxytocin (Sigma-Aldrich Co., Cat. No. O3251, USA) was dissolved in isotonic saline to a final concentration of 25 IU/mL. Control group of animals received vehicle *per os* (2% Tween 80) for 21 days, and *s.c.* 400 μL of saline for 14 days. Solutions, freshly prepared, were injected at the same time every day (between 9:00 and 10:00 a.m.).

2.2. Experimental procedure

Rats were sacrificed with 50 mg/kg *i.p.* thiopental-sodium (Nesdonal®, Roche, Switzerland) and trunk blood was collected via cardiac puncture and put into heparinized tubes at 4 °C until analysed. For LOOH, MDA and SOD determination, blood samples in heparinized tubes were centrifuged at 3000 rpm for 15 min at 4 °C, and stored in duplicate at −80 °C. In order to examine the possible effects of treatments on peripheral lymphocyte (PBL) DNA damage, we performed alkaline comet test that has become one of the most used test in assessing DNA damage in a wide range of cell types [36]. This *in vitro* test attracts attention by its simplicity, sensitivity and speed and is becoming one of the standard methods for quantifying DNA lesions and resistibility to damages caused by the action of oxidative agents in individual cells. The comet test effectively detects DNA strand breaks, alkali-labile sites, and occurrence of incomplete base excision repair in wide range of cell types, such as peripheral blood cells [37]. The experiment was divided into two parts. In the first part, we determined if the treatments applied affected the PBL DNA, while in the second part we tested the susceptibility of lymphocyte DNA from all groups to exogenous oxidative stress evoked by incubation with H_2O_2 (1.5 mM). Our previous pilot tests with the doses of H_2O_2 and durations of exposition showed that this high concentration of H_2O_2 during 5 min produced appropriate DNA damage and good cell viability.