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Ethanol and nitric oxide modulate expression of glucocorticoid receptor in the rat adrenal cortex

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Abstract:
Background: This study was performed to investigate expression and distribution of glucocorticoid receptor (GR) in the rat adrenal cortex, acute effect of ethanol on its expression and possible role of endogenous nitric oxide (NO) in this phenomenon.
Methods: Adult female Wistar rats showing diestrus day 1 were treated with: a) ethanol (2 or 4 g/kg body weight (b.w.), *ip*), b) N^{ω}-nitro-L-arginine methyl ester (L-NAME), well-known competitive inhibitor of all isoforms of NO synthase (NOS), (30 mg/kg b.w., *sc*) followed by ethanol (4 g/kg, *ip*) 3 h later and c) L-NAME (30 mg/kg b.w., *sc*) followed by saline (*ip*) 3 h later. Untreated rats were used as controls. Adrenocortical expression of GR was estimated by immunohistochemistry.
Results: Strong nuclear GR staining was observed throughout the cortex of control rats. Acute ethanol treatment significantly decreased the expression of GR in the zona fasciculata and zona reticularis. Blockade of NO formation had no influence on this effect of ethanol, whereas L-NAME itself induced significant decline in GR immunoreactivity.
Conclusions: Obtained findings are the first to demonstrate localization and distribution of the GR throughout the rat adrenal cortex and to suggest that ethanol as well as endogenous NO may modulate adrenocortical expression of this steroid receptor.

Key words:
ethanol, L-NAME, glucocorticoid receptor, adrenal cortex

Introduction

Glucocorticoids (GC), steroid hormones synthesized and secreted at high levels by the adrenal cortex, influence the activity of almost every cell in the body [6]. They exert pleiotropic actions which are essential for the maintenance of homeostasis and responses to stressors. Because of their antiinflammatory and immunosuppressive effects, synthetic glucocorticoid agonists are widely used in the treatment of autoimmune diseases, inflammatory disorders [29], and malignancies of the lymphoid system [18].

At the cellular level, the physiological and pharmacological actions of GC are predominantly mediated through intracellular glucocorticoid receptor (GR) that belongs to the nuclear receptor family of ligand-dependent transcription factors. In the absence of the ligand, GR is located in the cytoplasm within a protein complex that includes various heat-shock proteins. After activation, GR is released from the multiprotein complex, dimerizes, and translocates to the nucleus, where it binds to specific DNA sequences called glucocorticoid response elements [33]. The importance of GR is emphasized by the finding that inactivation of the GR gene is incompatible with life [10].

In addition to other actions, GC through GR-mediated negative feedback loop at the hypothalamic and pituitary levels terminate the HPA (hypothalamic-pituitary-adrenal) axis response to stress [14]. Similarly, the expression of GR within the adrenal cortex in humans [27] and ovine fetuses [31] imply that glucocorticoid feedback may occur within the gland itself. However, very little information is available about the expression and distribution of the GR in the rat adrenal gland [17].

Although ethanol is known as a stressor [2, 25, 30], data regarding its acute effect on GR expression are missing. Also, there is evidence that nitric oxide (NO) may be involved in some effects of alcohol [1, 35] as well as that this signal molecule affects the activity of HPA axis [9, 22, 23]. In a view of these observations, the present study was designed to investigate (a) the expression and distribution of GR in the rat adrenal cortex, (b) whether acute ethanol treatment alters its adrenocortical expression and (c) possible influence of endogenous NO on this effect of alcohol.

Materials and Methods

Animals

Adult female Wistar rats (obtained from the Breeding Colony of the Medical Military Academy, Belgrade), 10–12 weeks of age and 200–250 g of weight, were used in this study. They were housed five per cage in temperature-controlled room with light on at 6:00 a.m. and off at 18:00 p.m. The animals were provided with standard laboratory diet (Veterinarski zavod, Subotica) and water *ad libitum.* The stages of estrous cycle were monitored every day by vaginal smears, and only the rats showing the diestrus day 1 were used in this study.

Chemicals

L-NAME (Sigma Co.) was dissolved in apyrogenic saline just before use. The choice of dose of L-NAME, regimen of administration and route of injection were based on previous studies [8, 35]. Ethanol (SUPERLAB, Belgrade, Serbia) was diluted with sterile saline up to 35% (v/v) and the animals were treated with different volumes to reach concentration of 2 or 4 g/kg.

Experiment

In order to avoid circadian oscillations, the experiments were carried out between 8:00 and 12:00 a.m. The animals were weighed and injected with: a) ethanol (2 or 4 g/kg b.w., *ip*), b) N^{ω}-nitro-L-arginine methyl ester (L-NAME), well-known competitive inhibitor of all isoforms of NO synthase (NOS), (30 mg/kg b.w., *sc*) followed by ethanol (4 g/kg, *ip*) 3 h later and c) L-NAME (30 mg/kg b.w., *sc*) followed by saline (*ip*) 3 h later. Untreated rats were used as controls.

The rats were sacrificed by decapitation 30 min after last injection. Blood samples were collected and left adrenal glands were promptly removed, fixed in 10% buffered formalin for 24 h, and embedded in paraffin.

The protocol was in accordance with local institutional guidelines for the care and use of laboratory animals. The investigation also conformed to the principles and guidelines of Conseil de l'Europe (published in the Official Daily N. L358/1-358/6, 18th December 1986), the U.S. National Institutes of Health (Guide for the Care and Use of Laboratory Animals, NIH publication no. 85-23) and the Canadian Council on Animal Care (CCAC).

Corticosterone determination

Serum levels of corticosterone were measured by Amersham Biotrak rat corticosterone [125I] assay system using magnetic separation with CV = 5%, per run. The groups for hormone determination consisted of five to seven rats.

Immunohistochemistry

Immunohistochemical staining was performed on 6 μm thick paraffin sections of adrenal gland. After deparaffinization in xylene followed by dehydration with descending series of alcohols and rehydration in distilled water, slides were heated for 2 min under high pressure in 10 mmol/l citrate buffer, pH 6 for epitope retrieval. The endogenous peroxidase was inactivated by incubation with 3% hydrogen peroxide in distilled water for 10 min. Rabbit polyclonal anti-mouse GR antibody (M-20): sc-1004, Santa Cruz Biotechnology Inc. diluted (1:200) in DAKO Antibody Diluent (S0809) was applied and the sections were incubated in a humidity chamber for 3 h at the room temperature. After washing with phosphate buffered saline (PBS), a refined avidin-biotin tech-