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Bacterial lipopolysaccharide differently modulates steroidogenic enzymes gene expressions in the brain and testis in rats

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ABSTRACT

Bacterial lipopolysaccharide (LPS) is a major component of the cell wall of gram negative bacteria contributing to the pathogenesis of bacterial infection, in particular in those diseases affecting central nervous system and reproductive tissues. The present work is an attempt to study the regulation of steroidogenic enzymes gene expression in the brain and testis in LPS induced rats. Adult male albino rats were administered LPS (5 mg/kg BW) to induce acute inflammation. LPS administration induced severe oxidative damage in the brain and testicular tissue which was evident from decreased activities of enzymic antioxidants and increased lipid peroxidation levels. The mRNA expression of 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD) and androgen receptor corepressor-19 kDa (ARR19) in the brain and testis were determined. The mRNA expression of 3β-HSD and 17β-HSD was increased in the brain with significant decrease in the testis at 24 h and 48 h in LPS treated animals. The results also demonstrated an interesting finding that LPS treatment completely represses ARR19 in the brain, while not in the testis. These findings show ARR19 might play a crucial role in regulation of neuronal and testicular steroidogenesis in inflammatory diseases.

1. Introduction

Steroid hormones are synthesized in steroidogenic cells of the adrenal, testis, ovary and brain and are required for normal reproductive function and bodily homeostasis (Stocco et al., 2005). The brain and the peripheral nerves synthesize steroids which then act as neuromodulators in a paracrine or autocrine mode. Recent studies have demonstrated the role of sex steroids in regulation of gene expression, neuronal survival, synaptic transmission, neuronal and glial differentiation in many brain areas (Saldanha et al., 2009; Garcia-Segura, 2008; Stoffel-Wagner et al., 1999). Conversely, androgens produced in testicular Leydig cells are essential for male sexual differentiation, maintenance of spermatogenesis and expression of male secondary sex characteristics (Song et al., 2012).

Proinflammatory cytokines are important pathogenic mediators in infectious and inflammatory diseases, in particular diseases affecting central nervous system and reproductive tissues. Infection and inflammation can be reproduced *in vivo* by administration of bacterial lipopolysaccharide (LPS), and several studies have observed inhibition of testicular steroidogenesis in animals treated with LPS (O'Bryan et al., 2000; Allen et al., 2004) or with septic agents that generate LPS (Sharma et al., 1996). It has also been shown that proinflammatory cytokines generated by LPS have an inhibitory role in steroidogenesis through the production of increased reactive oxygen species (Reddy et al., 2006). A numerous studies have shown that LPS plays a pivotal role in the regulation of steroidogenic enzymes gene expression including 3β-HSD and 17β-HSD (Magata et al., 2014; Gottfried-Blackmore et al., 2008).

It has been demonstrated that proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 1 (IL-1) have an inhibitory role on gonadal functions particularly in the steroidogenesis of Leydig cells (Hong et al., 2004; Herrmann et al., 2002). Elevated levels of TNF-α and IL-1 have been observed in human patients with critical illness, burns and sepsis (Calandra et al., 1990; Cannon et al., 1990; Damas et al., 1989), who experience impaired gonadal functions with low serum testosterone levels (Vogel et al., 1985; Woolf et al., 1985). Moreover, administration of TNF-α to healthy men and rats causes a decline in serum testosterone levels (Mealy et al., 1990; van der Poll et al., 1993), while treatment with TNF-α or IL-1 causes an inhibition of steroidogenesis in cultured Leydig cells (Hales, 2002). In contrast, Ghezzi et al. (2000) have

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shown that neurosteroid levels are increased *in vivo* after LPS treatment and also negatively regulate LPS-induced TNF production. It has also been demonstrated that LPS increases the expression and activity of 17β-HSD in brain microglia (Gottfried-Blackmore et al., 2008). With this background, the present work was designed to study the mechanism behind the differential regulation of neuronal and testicular steroidogenesis in LPS administered rats.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide from *Escherichia coli* (serotype 055:B5), pregnenolone and androsten-3.17-dione were purchased from Sigma Chemicals Company, Saint Louis, MO, USA. All other chemicals used were of analytical grade and were obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and HiMedia Laboratories Pvt. Ltd., Mumbai, India.

2.2. Experimental animals

Adult male albino rats of Wistar strain weighing 200 ± 10 g (10–12 weeks old) were used in the study. The animals were maintained under standard conditions of humidity, temperature (25 ± 2 °C), and light (12 h light/12 h dark). The animals were housed in large spacious cages bedded with husk. They were fed with a standard rat pelleted diet and had free access to water throughout the experimental period. Experimental animals were handled according to the University and Institutional Legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (BDU/IAEC/2011/28/29.03.2011).

2.3. Study design

The animals were randomly divided into four groups consisting of six rats each. Saline treated animals served as controls (Group I). The animals of other groups were injected with LPS (dissolved in 0.5 ml of sterile saline) at a dose of 5 mg/kg body weight intraperitoneally (i.p.) to induce acute inflammation. The animals were sacrificed at different time intervals *i.e.*, 12 h (Group II), 24 h (Group III), 48 h (Group IV) after LPS injection. Both the testes and brain were excised immediately and used for further analyses.

2.4. Preparation of tissue homogenate

Briefly, the tissue was chopped and minced in 6–8 ml of 0.25 M sucrose/TKM buffer (50 mM Tris–HCl, 25 mM KCl and 5 mM $MgCl_2$). The tissue was then homogenized gently by using Potter–Elvehjem homogenizer. The homogenate was centrifuged at 1500 rpm for 5 min. The supernatant was transferred and used as whole tissue homogenate for further analysis.

2.5. Estimation of protein

Protein content of the tissue fractions was estimated by the method of Lowry et al. (1951). Briefly, 0.1 ml of the diluted tissue homogenate was made up to 1 ml with water. To this, 4.5 ml of alkaline copper reagent (2% Na_2CO_3 in 0.1 N NaOH was mixed with 0.5% $CuSO_4$ containing 1% sodium potassium tartrate in the ratio of 50:1) was added, mixed and allowed to stand at room temperature for 20 min. Later, 0.5 ml of Folin's Ciocalteau reagent was added and shaken well. The blank and bovine serum albumin (BSA) standards were also treated in a similar manner. The blue color complex formed was measured at 640 nm after 15 min against the blank.

2.6. Assay of enzymic antioxidants

2.6.1. Superoxide dismutase (SOD)

The enzyme was assayed according to the method of Marklund and Marklund (1974). The degree of inhibition of auto-oxidation of pyrogallol, in an alkaline pH by SOD was used as a measure of the enzyme activity. The enzyme activity is defined as units/mg protein, where one unit is equal to the amount of enzyme required to inhibit auto oxidation of pyrogallol by 50% per minute.

2.6.2. Catalase (CAT)

The activity of catalase was assayed by the method of Sinha (1972). The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus formed was measured at 610 nm. The activity of catalase was expressed as μmol of H_2O_2 consumed/min/mg protein.

2.7. Determination of reactive oxygen species (ROS) levels

Generation of ROS was determined by using 2′,7′-dichlorofluorescin diacetate (DCFH-DA) as a probe, according to the method of LeBel et al. (1992). In brief, the assay buffer contained 20 mM Tris–HCl, 130 mM KCl, 5 mM $MgCl_2$, 20 mM NaH_2PO_4, 30 mM glucose and 5 μM DCFH-DA. The assay medium was incubated at 37 °C for 15 min and 1 μmol of H_2O_2 was added to the mixture at the end of assay. Dichlorofluorescein (DCF) formation was measured in a fluorescence spectrophotometer with 488 and 525 nm as excitation and emission wavelengths, respectively.

2.8. Assay of lipid peroxidation (LPO)

Estimation of LPO was carried out following the procedure of Hogberg et al. (1974) using thiobarbituric acid (TBA). Malondialdehyde (MDA), formed as an end product of peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA reacts with TBA to generate a colored product that absorbs at 532 nm. The ferrous sulphate and ascorbate induced LPO system contained 10 mM ferrous sulphate and 0.2 mM ascorbate as inducers (Devasagayam, 1986). The level of lipid peroxides was expressed as μmol of MDA formed/mg protein.

2.9. Assay of steroidogenic enzymes

2.9.1. 3β-Hydroxysteroid dehydrogenase (3β-HSD)

The activity of 3β-HSD was measured by the method of Shivanandappa and Venkatesh (1997). The enzyme was assayed in 0.1 M Tris–HCl buffer (pH 7.8) containing 500 μM NAD^+, 0.5 ml of color reagent (1% Tween 20 containing 0.08% iodonitrotetrazolium chloride) and the substrate (100 μM pregnenolone) in a total volume of 3 ml. The reaction was started by adding the enzyme (50 μl) and incubated at 37 °C for 60 min. The reaction was stopped by the addition of 2 ml of phthalate buffer (2.55 g of potassium hydrogen phthalate dissolved in a mixture of 51 ml 0.1 N HCl and 2.5 ml Tween 20; pH was adjusted to 3.0 and the volume made up to 250 ml). The turbidity was removed by centrifugation at 3000 rpm for 20 min and the supernatant was read at 490 nm in a spectrophotometer. For standard curve, aliquots of graded concentrations of freshly prepared NADH (0–150 nM) were reacted with 0.5 ml of color reagent (1% Tween 20 containing 0.08% iodonitrotetrazolium chloride and 0.02% phenazinemethosulfate) and after color formed,