Neurotransmitters’ Changes in Bacterial Infected Treated and Untreated Rats

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Abstract: Neurotransmitters are endogenous chemicals that transmit signals across a synapse from one neuron (brain cell) to another ‘target’ neuron. Neurotransmitters are packaged into synaptic vesicles clustered beneath the membrane in the axon terminal. Neurotransmitters are released into and diffuse across the synaptic cleft, where they bind to specific receptors in the membrane on the postsynaptic side of the synapse. Male Sprague-Dawley rats received (20mg/kg) dexamethasone I.P. for three days. The animals were intraperitoneally injected 48 hours before slaughtering with 200 µl of E.coli 24 hour’s culture in nutrient broth containing approximately 1.8 x 10^6 CFU / ml. Animals were divided into four groups: - (1) Control group, (2) Escherichia coli infected group, (3) dexamethasone treated group, (4) dexamethasone and E.coli treated group. The present study was conducted to investigate the effect of dexamethasone on neurotransmitters and some other related parameters in bacterially infected and non-infected rats. Also, immunological parameters: Tumor necrosis factor α (TNF-α) in brain tissue, neurotransmitter parameters: serotonin (5-HT), dopamine (DA) and norepinephrine in brain tissue were evaluated. Oxidative stress parameters: lipid peroxide (MDA) and glutathione content (GSH) as well as total and differential leucocytic counts (WBCs) were measured and histopathological examination of the brain was carried out.

Key words: Escherichia coli · Neurotransmitters · Dexamethasone · Tumor necrosis factor α (TNF-α)

INTRODUCTION

The most general known effects of dexamethasone are to inhibit the synthesis, release and/or efficacy of cytokines and other mediators that promote immune and inflammatory reactions [1]. Gamal et al. [2] showed that dexamethasone significantly modulated the inflammatory changes and the oxidative/nitrosative stress associated with acute liver injury (ALI). Dexamethasone could modulate inflammatory and oxidative changes observed in brain. Besides, dexamethasone prevents lipid peroxidation and reduces mitochondrial injury thus suggests neuroprotective effects in fetal rat brain in intrauterine ischemia-reperfusion (I/R) injury [3].

According to Feng et al. [4] and Siqueira et al. [5], it might be necessary to combine neuroprotective agents with common therapy to treat and protect optic nerve and ganglion cells from their secondary injury. Dexamethasone increases retinal neuronal survival in the steroid-treated groups compared with the controls.

LPS induced peripheral infection activates the immune system, which conveys a message to the brain causing the production of inflammatory cytokines. Excessive expression of pro-inflammatory cytokines in the brain may cause behavioral deficits [6, 7]. Regulating the inflammatory response in the brain following a peripheral infection may be important in protection against behavioral disorders [8]. Moreover, an LPS induced inflammatory response is characterized by an increased expression of pro-inflammatory cytokines, which include interleukin (IL) 1β and tumor necrosis factor α (TNF-α).

The study aims at estimating the neurotransmitters and neuroimmunological changes associated with bacterial infection. Also, the possible neuroprotective effects of dexamethasone are going to be evaluated.

MATERIALS AND METHODS

Animals: Animals weighing 120-150 gm were used in these experiments. Rats were allowed free access to standard diet and tap water at controlled room
temperature [25±2°C] and light controlled condition with 12h-light and dark cycles. Animal handling and experimental protocols were approved by the Research Ethical Committee of the National Organization for Drug Control and Research (NODCAR, Cairo, Egypt).

**Drugs:** Corticosteroid (Dexamethasone) ampoules were brought from Sigma-Tec pharmaceutical Industries - Egypt – S.A.E. Rats were received Dexamethasone dose 20 mg/kg, I.P for 3 days according to Al-Shorbagy et al. [9].

**Bacterial Strain:** *Escherichia coli* (*E.coli*) NCTC 9001 strain was obtained from the microbiology control lab in NODCAR. Twenty four hours growth in nutrient broth was used in this study, where in the infected animal groups; each animal was injected intraperitoneal with 200 µl of *E.coli* 24 hours’ culture in nutrient broth at 37°C containing approximately 1.8 x 10^8 CFU / ml [10-12].

**Experimental Design:** Normal healthy selected male rats were used. Rats were randomly allocated into 4 experimental groups (n=10), the animals were treated according to the following scheme:

**First Group:** Animals received 0.2ml distilled water Intraperitoneal and served as control group.

**Second Group:** Rats were infected with *Escherichia coli*; animals were injected I.P. with 200 µl of *E.coli* 24 hours culture in nutrient broth at 37°C containing approximately1.8 x 10^8 CFU / ml for one day and served as infected group.

**Third Group:** Rats treated with dexamethasone (20mg/kg, I.P.) for 3 days.

**Fourth Group:** Rats received dexamethasone (20mg/kg,I.P) for 3 days. Animals were infected with *Escherichia coli*; animals were injected I.P. with 200 µl of *E.coli* 24 hours culture in nutrient broth at 37°C containing approximately1.8 x 10^8 CFU / ml for one day.

**Evaluated Parameters**

**Physiological Parameters:** Rats were then killed by decapitation 24 hours after the administration of last test dose of drugs then liver; spleen and thymus were carefully removed to measure their relative weights. Relative weight was calculated according to the formula [13]:

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\text{Relative weight} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100
\]

**Hematological Parameters Included:** Total and Differential leucocytic count:

Determination of total leucocytes was scored by the method of Hayhoe and Flemans.[14].

**Homogenate of Brain Tissue:** Brain tissue was homogenized in 5 – 10 ml cold buffer per gram tissue, using tissue homogenizer and then using cooling centrifuge at 4000 r.p.m. for 15 minutes at 4 °C to get the supernatant. The supernatant was removed for assay and stored on ice.

**Determination of Neurotransmitters:** The method is based on a fluorometric assay in which a fluorescent product results from reaction with ortho-phthaldehyde solution in case of serotonin and reaction with a mixture of alkaline sulfite and iodine solution in case of nor-epinephrine and dopamine.

**Estimation of Lipid Peroxidation (MDA):** Determination of Lipid peroxidation (MDA) was carried out using a Bio diagnostic Kit according to the method of Satoh[15] and Ohkawa et al. [16].

**Estimation of Reduced Glutathione (GSH):** Determination of reduced glutathione (GSH) was done using a Biodiagnostic Kit according to the method of Beutler et al. [17].

**Estimation of Tumor Necrosis Factor α (TNF-α):** Determination of brain tissue TNF-α concentration, the enzyme-linked-immunosorbert- assay (ELISA) method was used using Usen, Inc. [18].

**Histological Examination of the Brain:** Autopsy samples were taken from brain of rats in the different experimental groups then fixed in 10% formalin prepared in saline for 12 hours. Washing was done in tap water. Alcohol (methyl, ethyl and absolute ethyl) were used for dehydration then serial dilutions. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin wax tissue blocks were prepared for sectioning at 4 micron thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by Hematoxyline and Eosin stain [19] for histopathological examination.
RESULTS

Effect of Dexamethasone on Some Neurotransmitters

**Dopamine:** *Escherichia coli* infection significantly decreased dopamine level in brain tissue when compared with control group. Administration of dexamethasone alone (20 mg/kg, I.P.) for 3 days significantly increased dopamine levels in brain tissue and also increased significantly with *E.coli* infection when compared with infected group, p<0.05 (Fig. 1, A).

**Serotonin:** *Escherichia coli* infection significantly decreased level of serotonin in brain tissue (ng/g tissue) when compared with control group. No significant changes of the levels of serotonin in brain tissue were observed after administration of dexamethasone for 3 days and also before induction of *E.coli* when compared with infected group, p>0.05 (Fig. 1, B).

**Norepinephrine:** *Escherichia coli* infection significantly decreased norepinephrine level in brain tissue (ng/gm tissue) when compared with control group. Administration of dexamethasone before induction of *E.coli* significantly increased norepinephrine level in brain tissue (ng/gm tissue) when compared with infected group, p<0.05. (Fig.1, C).

**Immunological Parameters**

**Level of TNF-α in Brain Homogenate:** *Escherichia coli* infection significantly increased the level of TNF-α in brain homogenates (pg/mg) when compared with control group at p<0.05. Administration of dexamethasone alone significantly decreased the level of TNFα in brain homogenates (pg/mg) and on the contrary administration of dexamethasone before induction of *E.coli* infection in rats significantly increased the level of TNFα when compared with control group and dexamethasone treated group, p<0.05. (Fig. 1, D).

Fig. 1: Level of some neurotransmitters in brain homogenates of control and treated animal groups.  
(A) Level of dopamine in brain homogenates of control and treated animal groups.  
(B) Level of serotonin in brain homogenates of control and infected animal groups.  
(C) Level of norepinephrine in brain homogenates of control and treated animal groups.  
(D) Level of TNF-α in brain homogenates of control and treated animal groups.
Fig. 2: Level of MDA and GSH in brain homogenates of control and treated animal groups. (A) Level of MDA in brain homogenates of control and treated animal groups. (B) Level of GSH in brain homogenates of control and treated animal groups.

Evaluation of Oxidative Stress Parameters in Brain Homogenate
Effect of Dexamethasone on Malondialdehyde (MDA)
Level in Brain Tissue Homogenates in Escherichia Coli Induced Brain Sickness in Rats: Escherichia coli infection significantly increased the level of MDA in brain homogenates (n mol/g) when compared with control group. Administration of dexamethasone only significantly increased the level of MDA in brain homogenates (n mol/g) and significantly decreased the level of MDA in brain homogenates when administrated in infected group when compared with all positive control groups, p<0.05 (Fig. 2, A).

Effect of Dexamethasone on Glutathione (GSH) Level in Brain Tissue Homogenates: Escherichia coli infection significantly decreased the level of GSH in brain homogenates (µ mol/g) when compared with control group. Administration of dexamethasone significantly decreased the level of GSH in brain homogenates (µ mol/g) and infected brain homogenate when compared with control group, p<0.05 (Fig. 2, B).

Hematological Parameters
Total Leucocytes Count: Fig. 3 shows a significant increase of T.L.C of all treated and infected groups when compared with control group.

Histological Examination: Histological section of control rat brain showed the typical layered appearance of the cerebral cortex and the underlying structures including the hippocampus and subcortical areas. No histological abnormalities were seen (Fig. 4, A).

Brain histopathological sections of rats infected with E.coli revealed severe lesions including damage in the cortex, perivascular edema, dilatation of the vessels and necrosis of neurons (Fig. 4, B). Bilateral ventricular enlargement, cell necrosis at the sub- and peri-ventricular areas and hemorrhage were observed mainly in the edematous layer. In comparison to control group E.coli infection induced endotoxemia that was manifested by inflammatory cellular infiltration in the meningeal and ventricular spaces. Hippocampus showed severe inflammation and apoptosis of neurons. Acute hemorrhage, most prominent in the white matter of the brain, was observed. Cortex showed increased cellularity attributable to inflammatory exudates.

Sections of animals treated with Dexamethasone (20 mg/kg, I.P.) for 3 days showed no lesions in the cortex and no significant damage in underlying structures and looked almost like the control brain (Fig. 4,C).
Histopathological sections obtained from animals administered Dexamethasone before induction of *E. coli* infection revealed mild to moderate degrees of lesions in the cortex without significant damage in other subcortical areas. Some changes were comparatively less severe in this group; Dexamethasone treatment reduced histological brain damage. There was less edema in the Dexamethasone group than in *E. coli* infected group. Dexamethasone suppressed LPS-induced PMN infiltration. Treatment preserved the histological picture of the brain with reduced inflammatory cellular infiltration and intracellular edema (Fig. 4, D).

**DISCUSSION**

The human brain is an extremely complex organ that functions as the information-processing unit of the central nervous system. The major building blocks of this unit are neurons and glial cells [20, 21].

In the present study bacterial challenge animal model was used in order to assess the effect of peripheral bacterial infection on brain neurotransmitters, brain inflammatory cytokines and brain oxidative stress parameters.

Kubera et al. [22] mentioned that there is a strong association between signs of bacterial translocation and chronicity of depression and oxidative and nitrosative stress (O&NS), but not pro-inflammatory cytokines. Their results suggested that repeated intermittent LPS injections to rats may be a useful model of chronic depression and in particular for the depressogenic effects of long standing activation of the toll-like receptor IV complex. Lipopolysaccharide induces inflammation in the substantia nigra leading to death of tyrosine hydroxylase-positive cells in which p38 and inducible nitric oxide synthase were responsible for *in vivo* dopaminergic cells’ degeneration [23, 24]. These studies demonstrated that neuroinflammation can be induced by chronic lipopolysaccharide (LPS) infusion into the 4th ventricle of the rat resulting in region-selective microglia activation and impaired hippocampal-dependent memory. Furthermore, this treatment results in altered behaviorally-induced expression of the immediate early gene Arc, indicating altered network activity. LPS is known to activate microglia directly, leading to increased glutamate release and in enhanced N-methyl-d-aspartate (NMDA) -dependent signaling. Taken together, the foregoing suggests that decreasing NMDA receptor
activation during early stages of chronic neuro inflammation should reduce microglia activation causing spatial memory impairments produced by LPS.

The present study demonstrated significant changes in the level of brain neurotransmitters in response to bacterial challenge. The level of serotonin (5-HT), dopamine (DA) and noradrenaline (NE) were significantly decreased. These results agree with Fenli et al. [25] who determined the metabolism of monoamine neurotransmitters in brain tissues of rat model of depression. They have determined 5-HT, NE, DA and their metabolites, i.e., 5-hydroxyindole-3-acetic acid (5-HIAA), 4-hydroxy-3-methoxynylglycol (MHPG) sulfate, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in rat brain tissues. Rat model of depression showed decreased brain 5-HT and NE levels.

Also the present study noticed changes in the level of serotonin in response to intraperitoneal injection of E.coli which could be explained by Hollis et al. [26] who demonstrated that Lipopolysaccharide has indomethacin-sensitive actions on Fos expression in topographically organized subpopulations of serotonergic neurons.

On the other hand, the present study recognized drop in the level of brain tissue dopamine as a result of systemic bacterial infection that could be explained by Machado et al. [27] who reported the degeneration of dopaminergic neurons in an animal model based on the intranigral injection of lipopolysaccharide (LPS). They demonstrated that mild to moderate peripheral inflammation can exacerbate the degeneration of dopaminergic neurons caused by a harmful stimulus.

The present study detected a drop in the level of norepinephrine (NE) in brain tissue in response to systemic bacterial infection. Similarly, Huang et al.[28] monitored the levels of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in the culture medium of neural stem cells (NSCs) in which LPS-induced inflammatory process was conducted in NSCs. They recognized suppressed production of serotonin and noradrenaline via the modulation of Bcl-2 expression, as confirmed by the siRNA method.

The present results evidence suggests that neuroimmunoinflammatory reactions play a role in the pathophysiology of sickness behavior. Brain tissue homogenates obtained from E.coli infected animals showed significantly elevated TNF-α and significantly increased the level of NO in brain homogenates. These observations are in accordance with Engler et al. [29] who found that endotoxin administration induced a strong time-dependent increase in IL-1β, IL-6 and TNF-α mRNA levels indicating that these cytokines are de novo synthesized in the amygdala in response to peripheral immune activation. The changes in amygdaloid activity were timely related to an increase in anxiety-like behavior and decreased locomotor activity and exploration in the open-field. Acute amygdaloid response during experimental inflammation provides further evidence that the amygdala integrates immune-derived information to coordinate behavioral and autonomic responses.

Significant changes in oxidative stress parameters in brain tissue homogenates in response to in vivo E.coli bacterial challenge in this study were observed. E.coli infection significantly increased the level of MDA and NO associated with significantly decreased level of GSH in brain homogenates. Chronic LPS administration significantly decreased thymus weight, proliferative activity of splenocytes, production of interferon (IFN)γ and interleukin-(IL)-10 and increased superoxide and corticosterone production. They postulated that increased IgA responses directed against LPS of Gram-negative bacteria, indicating increased bacterial translocation, may be one of the drivers underpinning these pathways [22]. Bacterial lipopolysaccharide (LPS) causes lipid peroxidation (LPO). It has been found that LPS induces LPO in vitro, in tissue homogenates in a concentration-dependent manner [30].

In this study a significant increase in Dopamine level in brain tissue homogenates in response to dexamethasone treatment was observed; No significant changes of Serotonin and Norepinephrine levels in brain tissue were monitored after the injection administration of dexamethasone. On the other hand, injection of dexamethasone before induction of E.coli infection in rats significantly increased Dopamine and Norepinephrine levels in brain tissue when compared with E.coli infected group; rats showed non- significantly increased Serotonin level in brain tissue which is supported by finding of Tsai et al. [31].

Significantly elevated level of Dopamine in brain tissue in response to dexamethasone treatment in E.coli infected rats was observed and this finding is supported by Hendebrink et al. [32].

Also significantly increased Noradrenaline level in brain tissue in infected animals treated with dexamethasone was obtained. This could be explained by Takahashi et al. [33] who found that administration of dexamethasone induces regional and neurotransmitter-specific changes of phosphoinositide metabolism in rat brain.
Dexamethasone markedly reduced the noradrenaline-stimulated phosphoinositide metabolism in the rat hippocampus. In the rat frontal cortex, the noradrenaline-stimulated phosphoinositide metabolism was less depressed by the administration of dexamethasone. Lipopolysaccharide (LPS) invoked vascular hypo responsiveness to norepinephrine (NE) manifested by hypotension which was attenuated by dexamethasone (DE). The NE-induced presser effects were significantly attenuated 1, 4 and 5 hr post LPS. Pretreatment with DEX significantly attenuated the LPS-induced NE hypo responsiveness post LPS. LPS-induced iNOS mRNA and protein expression was demonstrated in the liver, lung, spleen, heart, kidney and brain. Low levels of neuronal constitutive NOS mRNA and endothelial cell constitutive NOS mRNA were only detected in brain or myocardial tissue, respectively [34].

The present study showed that administration of dexamethasone before induction of E. coli infection in rats significantly decreased the level of TNFα, MDA and GSH in brain homogenates when compared with E. coli infected group. These results agree with Tsao et al. [35] who found that the combined treatment with propofol plus dexamethasone reduced mortality rate and attenuated organ injury in conscious rats treated with E. coli lipopolysaccharide. These protective effects may be associated with their anti-inflammatory capacity and antioxidant activity. The increases in serum tumor necrosis factor-alpha, tissue nitric oxide and superoxide anion levels were attenuated by propofol plus dexamethasone in lipopolysaccharide rats.

The immunological findings of the present study could be explained by Di et al. [36] who demonstrated that glucocorticoids activate divergent G protein signaling pathways, via the Gq and Gia subunits, respectively, to produce different retrograde messengers, endocannabinoids and NO, which each acts in a synapse-specific manner to suppress excitatory synaptic inputs and facilitates inhibitory synaptic inputs, respectively.

Brain cells histological changes in response to E. coli bacterial infection were observed in this study. These findings agree with Cui et al. [37] who recorded lipopolysaccharide (LPS)-induced selective loss of dopaminergic neurons mentioning that lipopolysaccharide transformed cells into an amoeboid shape.

Murray et al. [38] suggested that LPS can directly activate the brain endothelium even at relatively low doses, obviating the need for systemic cytokine stimulation to transduce systemic inflammatory signals into the brain or to exacerbate existing pathology.

The present study showed that brain cells exposed to systemic E. coli bacterial infection exerted histological recovery when treated with dexamethasone. These findings are in agreement with Lee et al. [39] who mentioned that DEX treatment modulated the expression of a variety of cell types. Dexamethasone reduces brain cell apoptosis and inhibits inflammatory response in rats with intracerebral hemorrhage.

REFERENCES


