Protective role of indomethacin on lipopolysaccharide-stimulated fever induction and cerebral catecholamine biosynthesis in Wistar rat

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Submitted: 2012-10-12 Accepted: 2012-11-04 Published online: 2012-12-01

Key words: **lipopolysachharide; indomethacin; norepinephrine; dopamine; rectal temperature; cerebral cortex; thalamus-hypothalamus; midbrain; cerebellum; pons; medulla**

Neuroendocrinol Lett 2012; **33**(7):713–721 **PMID:** 23391884 NEL331012A05

Abstract

OBJECTIVES: The antipyretic and neuroprotective potential of the nonsteroidal anti-inflammatory drug "indomethacin" was tested against lipopolysaccharide-produced hyperthermia and biosynthesis of norepinephrine and dopamine, in six brain regions of male rat.

METHODS: Observations were based on a single intraperitoneal injection of each of lipopolysaccharide (250 μg Kg^{-1} body wt) and indomethacin (20 mg Kg^{-1} body wt) followed by sampling and assaying of brain specimens after 2, 8, 12 and 24 hrs. lipopolysaccharide induced a general hyperthermia (8–24 hr) that was completely abolished by pretreatment with indomethacin.

RESULTS: In virtually all brain regions tested, lipopolysaccharide stimulated the biosynthesis of norepinephrine and dopamine. Yet, pretreatment with indomethacin provoked substantial mitigation predominately after 24 hrs. A time-based manner attended by a regionally nonselective manner characterized lipopolysaccharide-induced monoamine biosynthesis; whereas, indomethacin alleviation seems to proceed in a time-dependent and regionally-selective pathway since the pons proved the fastest and/or most responsive brain region to indomethacin action. A role of prostaglandin synthesis in the development of lipopolysaccharide-induced fever and catecholamine biosynthesis was suggested, given that both responses were abolished by the cyclooxygenase-inhibitor indomethacin.

CONCLUSION: Accordingly, our data verified the potent therapy potential of indomethacin in protecting cerebral noradrenergic and dopaminergic systems against lipopolysaccharide-induced acute phase reactions.

ORIGINAL ARTICLE

INTRODUCTION

The endotoxin lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria and contributes greatly to the structural integrity of bacteria. LPS is an extremely potent toxin that can stimulate activation of different mediator cascades (Raetz & Whitfield 2002) including proinflammatory cytokine release, increased expression of adhesion molecules, chemotactic recruitment of lymphoid cells, increased phagocytotic activity of macrophages, release of reactive oxygen species, and expression of acute phase proteins (Brandtzaeg 1996). This cascade of reactions is associated with a disturbance of a variety of homeostatic mechanisms leading to a clinical syndrome in human beings known as septic shock or sepsis, which is responsible for multiple organ failure (Parrillo *et al.* 1990). Administration of small doses of LPS endotoxin to humans activates important inflammatory mediators and induces a variety of acute phase responses which are qualitatively similar to those that occur during the early stages of septic shock. Large doses, however, precipitate life-threatening circulatory collapse and multiple organ failure (Brandtzaeg 1996).

Catecholamines (biogenic amines or monoamines) are a class of neurotransmitters, which are released during the body's stress response. The major catecholamines are dopamine (DA), norepinephrine (NE), and epinephrine, which act as hormones and neurotransmitters in the peripheral and central nervous system (Dunn 1992). Earlier studies indicated that the monoamines NE and DA play a key role in numerous aspects of brain function (Glowinski & Iversen 1966). It is now accepted that immune activation triggers the sympathetic nervous system to release the neurotransmitters NE, epinephrine, and DA (Shimizu *et al.* 1994). During acute-phase reactions, the central nervous system and the immune system communicate (Sternberg 2006). This is shown in the production, by brain cells, of both cytokines and neurotransmitters (Szelényi & Vizi 2007). LPS administration was reported to have the potential to regulate the biophase level of catecholamine production in the brain and to activate the production of cytokines, both in the immune system and the central nervous system (Szelényi & Vizi 2007).

Neurochemical studies evidenced that peripheral inflammation, induced by intraperitoneal injection of LPS, resulted in a highly differentiated *in vivo* noradrenergic neurotransmission in the brain (Linthorst & Reul 1998) and dopaminergic neurotransmission in the frontal cortex, nucleus accumbens, striatum, amygdala, hippocampus, and hypothalamus (Wang *et al.* 2009). However, earlier literature on endotoxemia and related catecholaminergic neurotransmission revealed quite incompatible data regarding dose-dependent responses to LPS in different brain regions. Contradictory data were reported concerning the effects of different LPS dosages in inducing changes in catecholamine concentration in the paraventicular nucleus (Francis *et al.* 2000), in locus coeruleous and tractus solitarii (Molina-Holgado & Guaza 1996), in preoptic area (Linthorst & Reul 1998) and in the medial prefrontal cortex (Lavicky & Dunn 1995).

In inflammatory conditions, prostaglandin E2 (PGE2) and the enzyme cyclooxygenase (COX) have key roles. COX produces prostaglandins that promote inflammation, pain, and fever (Coceani *et al.* 1989). The enzyme COX-2 is regarded as the one primarily responsible for PGE2 production in acute and chronic inflammatory conditions (Ajmone-Cat *et al.* 2010). However, a role for the isoform COX-1 in inflammation was suggested (Langenbach *et al.* 1995). Indomethacin was suggested as the only dual COX-1/COX-2 inhibitor that is able to completely abolish PGE2 levels at sites of brain inflammation, and not the selective COX-2 inhibitors (Ajmone-Cat *et al.* 2010). Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) and is the agent of choice with anti-inflammatory, analgesic, and antipyretic effects (Clark & Cumby 1975). It was reported that LPS-induced neurochemical changes were either completely blocked (Masana *et al.* 1990) or attenuated (Linthorst *et al.* 1996) by pretreatment with the cyclooxygenase inhibitor indomethacin, indicating that prostaglandins may mediate the neurochemical effects of LPS. Treatment of LPS-injected animals with anti-inflammatory drugs pointed out the implications of dopaminergic (Castaño *et al.* 2002) and noradrenergic (Linthorst *et al.* 1996) neurotransmission in the regulation of inflammation.

During the past few decades, a considerable body of evidence was established in literature on LPS-induced acute phase and inflammatory challenge (Raetz & Whitfield 2002; Fan & Cook 2004; Pirnes-Karhu *et al.* 2012). However, none paid regard to studying effects on various regions of the fore-, mid- and hindbrain and very few turned to LPS effects on brain catecholamines (Dunn 1992; Lavicky & Dunn 1995; Linthorst *et al.* 1996; Molina-Holgado & Guaza 1996; Linthorst & Reul 1998; Francis *et al.* 2000; Castaño *et al.* 2002; Beishuizen & Thijs 2003) with an association to the mitigating role of indomethacin (Masana *et al.* 1990; Wang *et al.* 2004; Mohamed *et al.* 2005; Guth 2012). As such, this study was designed to bring together LPS-induced acute phase challenges in body temperature and cerebral monoamine biosynthesis with the antipyretic and neuroprotective effects of indomethacin. To achieve this, six brain regions (cerebral cortex, thalamus-hypothalamus, midbrain, cerebellum, pons and medulla), encompassing fore- mid- and hindbrain, were tested for the stimulation and alleviation of the monoamine neurotransmitters NE and DA in albino rat.

MATERIALS AND METHODS

Experimental animal and group allocation

A total number of 72 adult male Wistar rat, *Rattus norvegicus,* was used for the present assays. Rats weighing

120–150 g were obtained from the animal house facility, King Saud University. Animals were housed under standard laboratory conditions (temperature, of 24 ± 3 °C; humidity, 40–60%) with 12 hour light-dark cycles. Food and water were supplied *ad libitum* prior to the start of the experiment. Animal experiments were conducted in accordance with the policy and standard guidelines for animal experiments of the Institutional Ethics Committee.

Animals were randomly allocated into 3 experimental groups (24 animals each) according to the type of injection they received. Doses for all 3 groups were given via single i.p. injections. Animals of the first group (control, C) received sterile pyrogen-free saline (0.9% NaCl); whereas those of the LPS-intoxicated group (LPS) received LPS (250 μg Kg^{-1} body wt). Animals pretreated with indomethacin prior to LPS application (LPS-IND) received a single i.p. injection of indomethacin (20 mg Kg^{-1} body wt) followed by a single i.p. injection of LPS (250 μg Kg^{-1} body wt.), 10 minutes later. Rectal temperature was monitored using a rat thermometer (Philips AVENT Digital Thermometer Set OKM263, UK) inserted 5 cm beyond the anus. Temperature measurements were made with the same thermometer the day before the experiment and after 2, 8, 12 and 24 hrs of injection.

Decapitation and tissue sampling

Batches of 6 animals from all groups were sacrificed by decapitation after 2, 8, 12 and 24 hrs following injection. From each animal, the brain was rapidly and aseptically excised and transferred to a dry-ice-cold glass plate and dissected into 6 regions (cerebral cortex, thalamus-hypothalamus, midbrain, cerebellum, pons and medulla). All brain regions were blotted dry and weighed. Tissue samples were stored at –20 °C for later analysis.

Chemicals

Bacterial endotoxin (LPS) from *Escherichia coli*, sterotype 055: B5 was purchased as a lyophilized powder (Sigma-Aldrich Chemie Gmbh, Munich, Germany), dissolved in pyrogen-free 0.9% saline to a concentration of 2 mg mL^{-1} and kept frozen at –20 °C as a stock solution. On the day of the experiment, the stock was preheated to 37 °C, vortexed and diluted to the desired concentration. Indomethacin was purchased as a lyophilized powder (Sigma-Aldrich Chemie Gmbh, Munich, Germany) and diluted with 0.9% pyrogen- free saline to a concentration of 5 mg mL^{-1}. All chemicals used were analytical grade. Reagents were stored in hard glass bottles with glass stoppers to avoid leaching of fluorescent contaminants.

Amine measurements

Each tissue of the selected brain areas (weighing less than 300 mg) were homogenized in 10 volumes of acidified n-butanol using a Potter-Elvejhem homogenizer (Braun, Apparatebau, Melsungen, Germany). Duplicate internal standard tubes were made in parallel with the tissue homogenates. Internal standard was prepared by adding 0.4 ml standard mixture (0.1 ml of each amine) to 9.6 ml of 0.2 N acetic acid. Aliquots of 0.2 ml of this solution were diluted to 0.3 ml with 0.2 N acetic acid then received 3ml of acidified n-butanol. The homogenates and internal standard tubes were centrifuged (Labofuge GL. Heraeus sepatech, Germany) at 2000 × g for 5 minutes then 2.5 ml of the supernatant fluid were transferred to tubes containing 1.6 ml 0.2 N acetic acid and 5 ml heptane. All tubes were placed on a vortex mixer for 30 seconds and the phases were separated by centrifugation at 2000 xg for 5 minutes. NE and DA were assayed in the aqueous phase. The estimation of DA and NE levels in the selected rat tissues were carried out fluorometrically according to the method of Chang (1964) modified by Ciarlone (1978), which is based on estimating the DA and NE fluorophors formed after oxidation by iodine. For DA, fluorescence was measured using a fluorometer (Perkin Elmer LS 50B, Perkin Elmer UK Ltd, Bucks, UK) at excitation and emission wavelengths of 320 nm and 375 nm, respectively. For NE, fluorescence was measured at excitation and emission wavelengths of 380 nm and 480 nm, respectively.

Statistical analysis

Statistical procedures were performed with SPSS 17 (SPSS Inc, Chicago, IL). All data are reported as means ± SEM. One-way analysis of variance (ANOVA) was used for comparing two or more group means (control, LPS, IND and LPS-IND) for each variable. Once differences were determined, a post hoc range test (Tukey's test) was used as a follow-up test to ANOVA for pair wise multiple comparisons to determine which means differ. A probability level of less than 0.05 was accepted as significant difference.

RESULTS

Changes in rectal temperature

Results showed that following injection of LPS alone (LPS group), rectal temperature of *R. norvegicus* (Figure 1) was significantly ($p<0.05$) reduced (37.41 °C) after 2 hrs of exposure compared to control mean value (38.01 °C). This is followed by a general increase ($p<0.05$) after 8, 12 and 24 hrs (38.51 °C, 38.6 °C, 38.7 °C, respectively) compared to control values (37.52 °C, 37.62 °C, 37.77 °C, respectively). Co-administration of indomethacin and LPS (LPS-IND group) resulted in a significant decrease ($p<0.05$) in rectal temperature starting with a sharp drop after 2 hours (37.65 °C) and an increase turning levels back close to normal control values after 8, 12 and 24 hrs (38.04, 38.04 and 38.16 °C, respectively).

Effects of LPS and indomethacin on NE concentrations

Control values of NE concentrations were quite dissimilar in different brain regions, yet, there was some consistency between averages of means of all test times in cerebral cortex and cerebellum (0.21 and 0.24 μg g^{-1}, respectively), on one hand, and thalamus-hypothalamus, midbrain, pons and medulla (0.63, 0.55, 0.7 and 0.58 μg g^{-1}, respectively) on the other (Figure 2).

LPS-injected group showed an almost similar NE fluctuation scheme, over time, in all brain regions studied (Figure 2). All LPS-injected groups started with an abrupt rise ($p<0.05$) over the control values after 2 hrs in cerebral cortex (0.37 μg g^{-1}), thalamus-hypothalamus (1.47 μg g^{-1}), cerebellum (0.92 μg g^{-1}), midbrain (0.39 μg g^{-1}), pons (1.2 μg g^{-1}) and medulla (0.79 μg g^{-1}). This rise is kept up through 8 hrs of injection until a peak rise was achieved in cerebral cortex (0.43 μg g^{-1}) and thalamus-hypothalamus (1.47 μg g^{-1}), cerebellum (0.61 μg g^{-1}), midbrain (1.24 μg g^{-1}), pons (1.7 μg g^{-1}) and medulla (1.03 μg g^{-1}) after 12 hrs. As a general rule, there was a drop in NE levels after 24 hrs versus 12 hrs in all studied brain regions, namely, cerebral cortex, thalamus-hypothalamus, midbrain, cerebellum, pons and medulla (0.29, 0.79, 0.6, 0.32, 0.79 and 0.65 μg g^{-1}, respectively).

In indomethacin-pretreated *R. norvegicus*, the joint injection of LPS-IND could attenuate the increase in

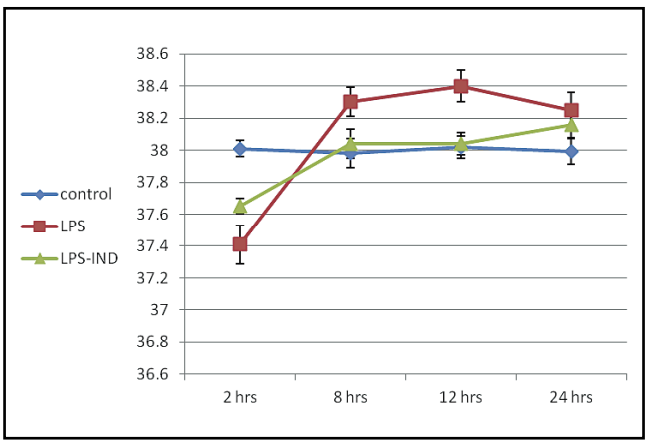

Fig. 1. Mean ± SEM Changes in the rectal temperature (°C) of male albino rats 2, 8, 12 and 24 hrs post-administration of the bacterial endotoxin (LPS) and co-administration of LPS with indomethacin (LPS-IND) versus control levels.

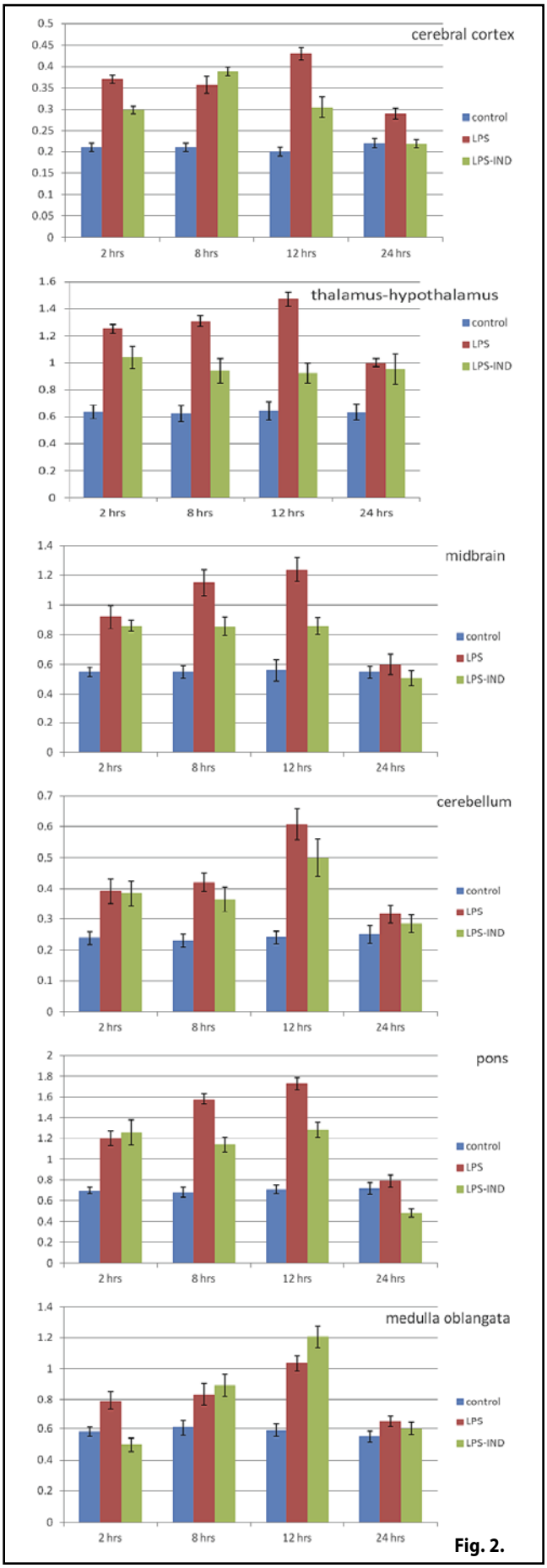

Fig. 2.

Fig. 2. Time- related changes (2–24 hrs) in NE concentrations (μg g^{-1} body wt) in 6 brain regions of albino Wistar rat, *Rattus norvegicus,* post-administration of the bacterial endotoxin "LPS" (250 μg g^{-1} body wt) and co-administration of LPS with indmethacin (20 mg Kg^{-1} body wt) (LPS-IND) versus control levels. Values are given as mean ± SEM.

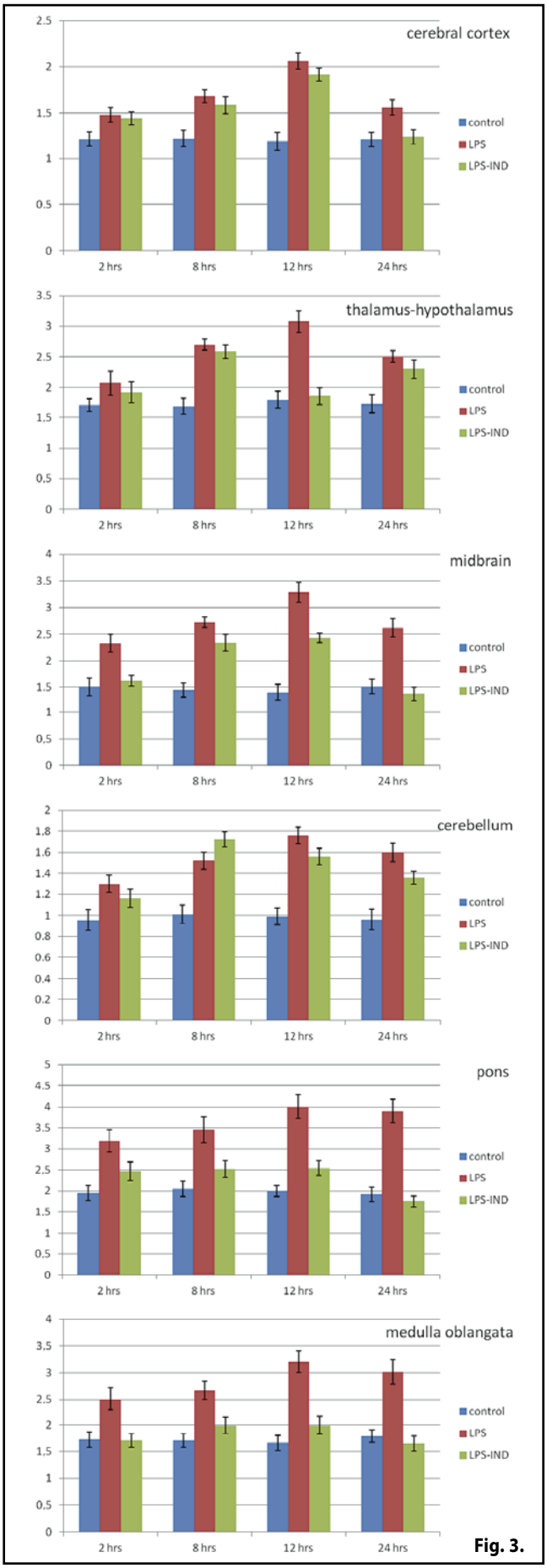

Fig. 3.

NE levels caused after 24 hrs of LPS injection in cerebral cortex (0.22 μg g^{-1}), midbrain (0.51 μg g^{-1}), cerebellum (0.29 μg g^{-1}) and medulla oblongata (0.65 μg g^{-1}). However, 24 hrs NE levels were still higher ($p<0.05$) in thalamus-hypothalamus (0.95 μg g^{-1}) and lower ($p<0.05$) in pons (0.48 μg g^{-1}) than control. These results indicate delayed mitigation of indomethacin in brain regions of thalamus-hypothalamus and pons compared to its action in other brain regions (cerebral cortex, midbrain, cerebellum and medulla oblongata) under the doses employed.

Effects of LPS and indomethacin on DA concentrations

As a general rule, mere control concentrations of DA (0.96–1.95 μg g^{-1}) were obviously higher than parallel NE concentrations (0.21–0.70 μg g^{-1}) in different brain regions. In LPS injected rats, a gradual increase of DA concentrations was noticed up to 12 hrs in cerebral cortex (2.1 μg g^{-1}), thalamus-hypothalamus (3.1 μg g^{-1}), midbrain (3.3 μg g^{-1}), cerebellum (1.8 μg g^{-1}), pons (4.0 μg g^{-1}) and medulla oblongata (3.21 μg g^{-1}) over parallel control values (1.2, 1.8, 1.40, 0.99, 2.0 and 1.7 μg g^{-1}, respectively). After 24 hrs, concentrations came back to near control levels (0.21 μg g^{-1}) only in cerebral cortex (1.28 μg g^{-1}); whereas in other regions, a significant rise ($p<0.01$) was still found.

In LPS-injected rats, pre-injection with indomethacin resulted in apparent attenuation of LPS-related rise in DA levels. Pretreatment with indomethacin significantly reduced DA concentrations, particularly after 24 hrs in all brain regions (cerebral cortex, thalamus-hypothalamus, midbrain, cerebellum, pons and medulla oblongata) (1.24, 1.76, 1.37, 1.36, 1.75 and 1.65 μg g^{-1}, respectively) compared to LPS alone treatments (1.56, 2.51, 2.62, 1.60, 3.90 and 3.02 μg g^{-1}, respectively). In almost all brain regions, DA concentrations came back very close to control values after 24 hrs of joint treatment of LPS and indomethacin. However in cerebellum, a substantial attenuation did not seem to have taken place up to the end of the experimental duration.

DISCUSSION

It is widely reported that the endotoxin LPS can elicit strong effects on the autonomic and immune-neuroendocrine network (Szelényi & Vizi 2007) and produce brain mediated effects including changes in body temperature (Romanovsky *et al.* 1996) and cerebral catecholamine stimulation (Wang *et al.* 2004; Flierl *et*

Fig. 3. Time- related changes (2–24 hrs) in DA concentrations (μg g^{-1} body wt) in 6 brain regions of albino Wistar rat, *Rattus norvegicus,* post-administration of the bacterial endotoxin "LPS" (250 μg g^{-1} body wt) and co-administration of LPS with indmethacin (20 mg Kg^{-1} body wt) (LPS-IND) versus control levels. Values are given as mean ± SEM.

al. 2009). In view of these considerations, the purpose of the present study was to analyze the protective role played by indomethacin against LPS-induced changes in body temperature and the levels of brain NE and DA.

Mitigating action of indomethacin against LPS-produced hyperthermia

Intra-peritoneal administration of 250 μg Kg^{-1} LPS in *R. norvegicus*, produced a significant initial hypothermia after 2 hrs, followed by hyperthermia at 8, 12 and 24 hrs. A similar initial decrease and a subsequent increase in body temperature was reported following injection of LPS doses lower and higher than the current dose in rat (Mohamed *et al.* 2004), in mice (Kozak *et al.* 1994) and in the ox *Bos taurus* (Kozak *et al.* 1985). Hypothermia could be an initial protective response towards LPS challenge aimed at delaying the induction of proinflammatory cytokines (Kimura *et al.* 2002). Hypothermia mostly reflects shifts of thermoeffector thresholds to a lower body temperature and/or heat dissipation mediated through changes in vascular resistance that cannot be compensated by an increase in metabolic heat production (Romanovsky *et al.* 1996).

The subsequent LPS-produced fever in *R. norvegicus* (8–24 hrs) was completely abolished by pretreatment with indomethacin (20 mg Kg^{-1} body wt). LPS-induced hyperthermia could be attributed to more than one mechanism. Rather than by a direct action, the febrile response to LPS classically suggests the interaction of pyrogenic cytokines (tumor necrosis factor-α "TNF-α", IL-1β and IL-6), secondarily produced in response to LPS challenge, with thermosensitive neurons in the brain causing the production of PGE2 and consequently of fever (Blatteis *et al.* 2000). However, the mechanism of prompt fever induction following intravenous administration of a pyrogenic dose of LPS in experimental animals was found controversial (Perlik *et al.* 2005). Fever appears within 10–15 min (Elmquist *et al.* 1996), whereas, the first cytokine to appear (TNF-α), is not detectable until 30 min after LPS treatment (Jansky *et al.* 1995). This temporal disconnect was reported to be less obvious after intraperitoneal administration of low to moderate LPS doses, when the latency of fever onset is ~60 min (Blatteis *et al.* 2000).

To account for the rapidity of the fever response to intravenous LPS, some authors showed that the peripheral pyrogenic signal could be transmitted to the preoptic-anterior hypothalamic area (presumptive locus of the febrigenic controller) via a neuronal rather than a humoral mechanism (Watkins *et al.* 1995; Ek *et al.* 1998). It was also shown that the onset of fever following intravenous LPS injection was correlated with the appearance of LPS in the liver's Kupffer cells (Li & Blatteis 2004), the body's principal clearinghouse of LPS (Ruiter *et al.* 1981) and source of pyrogenic cytokines (Dinarello *et al.* 1968).

From another standpoint, our study provided evidence of a role of PGs synthesis in the development of LPS-induced fever in *R. norvegicus*, because this response was abolished by the COX inhibitor indomethacin. In other mammals, intraperitoneal injection of indomethacin was shown to inhibit peripherally and centrally the two isoforms of the enzyme COX (the inducible COX-2 and the constitutive COX-1) (Vane 1971). Injection of another COX inhibitor (ketorolac) into the preoptic anterior hypothalamus POAH in rats markedly attenuated the fever produced by LPS, indicating that PG synthesis in this area is necessary for the production of fever (Scammell *et al.* 1998).

Taken together, we concluded that LPS-induced fever could be controlled by both humoral and neuronal mechanisms and that the most probable mechanism could be the direct interaction of pyrogenic cytokines with thermosensitive neurons in the brain causing the production of PGE2 in the preoptic anterior hypothalamus and consequently of fever (Blatteis *et al.* 2000). Yet another mechanism of direct neuronal action suggesting the implication of the vagus nerve and its hepatic branch (Simons *et al.* 1998; Perlik *et al.* 2005) in conveying the pyrogenic signal to the brain is worth considering.

Catecholamine distribution in brain regions under normal condition

NE and DA were unequally distributed in different brain regions of albino rat; the pons possessed unrivaled maximum levels of both NE and DA (0.72 and 2.05 μg g^{-1}, respectively), whereas the lowest values were found in cerebral cortex and cerebellum (0.20, 0.23 for NE and 1.21, 0.96 μg g^{-1} for DA, respectively). Earliest reports showed some opposing and some supporting data. In the cat brain, Vogt 1954 showed a contradictory result when he reported that NE has highest concentration in the hypothalamus and a consistent result when he found low levels of DA in the cerebellum and cerebral cortex. NE was, as well, found in highest concentration in the hypothalamus in the normal monkey (Segal *et al.* 1972), and its concentration in the hypothalamus was found to be approximately 3 times that of the dog and cat (Vogt 1954). The high levels of NE and DA in the pons could be attributable to the fact that noradrenergic neurons can be found mostly in seven regions of the pons and medulla and one region of the thalamus; yet, the most important noradrenergic system has neurons originating from locus coeruleus, a nucleus found in the dorsal pons (Glowinski & Iversen 1966). Additionally, earlier studies showed that pons and medulla oblongata are known to contain noradrenergic cell bodies and that the NE transporter has even a higher affinity for DA than NE (Taylor & Snyder 1970).

LPS stimulation of neurotransmitter biosynthesis in the brain

During the present experiments, LPS stimulated the release of both DA and NE in almost all brain regions examined in *R. norvegicus*. A time-dependent manner

attended by a regionally nonselective manner characterized the elevated catecholamine release in response to LPS administration. As a general rule, i.p. injection of LPS challenged catecholamine biosynthesis in six regions in the brain (cerebral cortex, thalamus-hypothalamus, midbrain, cerebellum, pons and medulla) with a prevalent peak at 12 hrs. Both Lavicky and Dunn (1995) and Dunn (1992) found similar release of NE and DA following LPS injection in rat and mouse, respectively. In a limited conformity with the current data, Dunn (1992) found peak responses for both DA and NE around 2 hrs following LPS administration in all mouse brain regions examined. This timely different manner from the current prevailing 12 hrs peak could be a species-specific variation between *Mus musculus* and the present model and could reflect the relative time required to deplete immune defences for each species.

The neurochemical changes attended by LPS-stimulated catecholamine release could have been mediated through induction of prostaglandin synthesis when the bacterial endotoxin acts directly on brain tissue by crossing areas of weak blood-brain barrier (Dunn 1992) or when cytokines are released after an endotoxin immune challenge acting as a Ca^{2+} ionophore and allowing calcium influx into the cell (Liang *et al.* 1985). This is normally followed by activation of phospholipase A_2 and the subsequent formation of prostaglandins that have stimulatory effects on catecholamine synthesis (Liang *et al.* 1985; Coceani *et al.* 1989). More specifically, it seems that LPS could stimulate IL-1 production from stimulated peripheral immune cells, which consecutively stimulate different levels of the hypothalamo-pituitary-adrenal (HPA) axis (Beishuizen & Thijs 2003) for catecholamine production. Catecholamine levels peaked 12 hrs post-injection were only reduced to near baseline levels in midbrain in case of NE and cerebral cortex in case of DA. It seems that this limited catecholamine mitigation over extended periods of endotoxemia (24 hrs) is due to the phenomenon of endotoxin tolerance. The toll-like receptor family was suggested as a major receptor for LPS and an element in endotoxin tolerance that can serve important counter inflammatory functions (Fan & Cook 2004).

Neuroprotective potential of indomethacin

In virtually all brain regions tested, catecholamine values in LPS treated *R. norvegicus* surpassed their respective controls. Yet coadministration of indomethacin with LPS provoked substantial mitigation versus catecholamine biosynthesis, predominately after 24 hrs. Time-based neurochemical changes showed that the mitigating effects of indomethacin for both NE and DA took place primarily after 24 hrs; namely, in 11 out of 12 brain regions after 2 hrs, 9/12 after 8 hrs, 11/12 after 12 hrs and 12/12 after 24 hrs. This timely dependent manner of indomethacin mitigation is attended by a regionally selective alleviation for NE and DA. The alleviation potential was most apparent in the pons after 24 hrs (1.6 folds decrease for NE and 2.17 folds for DA). However, thalamus-hypothalamus exhibited the least alleviating effects of indomethacin after 24 hrs among all brain regions tested (1.05 and 1.09, respectively). Thus while thalamus-hypothalamus exhibited the slowest response, the pons was the fastest brain region to respond to the attenuating action of indomethacin for both NE and DA. Complement to the above, medulla oblongata displayed two contradictory responses towards indomethacin administration showing a high alleviation response with respect to DA (1.83 folds) and a slight response for NE (1.08%). It seems that the inflammatory potential of LPS acted in a regionally nonselective manner as regards NE and DA, while indomethathin alleviating potential proceeded in a, more or less, regionally selective manner. An alleviating action towards LPS challenge was reported in rat brain for NE in pons and medulla using indomethacin (Mohamed *et al.* 2005), for DA in rat striatum using two other NSAIDs (tolmetin and sulindac) (Dairam *et al.* 2006) and for DA in mice brain using Diclofenac (another NSAID) (Hassanein *et al.* 2004).

Indomethacin acts primarily on cyclooxygenose-1 (COX 1) (Foegh *et al.* 1998), which is a rate limiting enzyme in PGE2 synthesis that inhibits both its release (Ferreira *et al.* 1971) and synthesis (Vane 1971). The enzyme cyclooxygenose (prostaglandin G/H synthase) catalyzes the stepwise conversion of arachidonic acid into two short-lived intermediates, prostaglandin G (PGG) and prostaglandin H (PGH) (Goppelt-Strube 1995). Pharmacological blockade of PGE2 synthesis was shown to attenuate many of the LPS-induced responses, such as activation of noradrenergic neurotransmission in hippocampus (Linthorst *et al.* 1996), and increased blood-brain barrier permeability (DeVries *et al.* 1996). It was earlier suggested that the activation of noradrenergic, dopaminergic and epinephrine-containing neurons in hypothalamus, as well as dopaminergic neurons in other regions is associated with the acute phase response to endotoxin in rat brain (Masana *et al.* 1990). Recently, indomethacin was shown to have a neuroprotective role and could modify dopaminergic neurotransmission in the brain (Antony *et al.* 2010). It seems that prostagalndins play a fundamental role in catecholamine responses in all rat brain regions examined, whether in LPS acute phase endotoxemia or in indomethacin mitigating potential. Although a timely dependent and regionally nonselective manners characterized the elevated catecholamine release in response to LPS administration, indomethacin mitigation of catecholamine biosynthesis was characterized by timely-dependent and regionally-selective manners. In conclusion, this study provided evidence of a role of prostaglandin synthesis in the development of LPS-induced fever and catecholamine biosynthesis given that both responses were abolished by the cyclooxygenase-inhibitor indomethacin. Additionally, indo-

methacin proved to possess potent therapy potential that protects cerebral dopaminergic and noradrenergic systems against LPS-induced acute phase reactions.

ACKNOWLEDGEMENTS

The Authors extend their appreciation to the "Deanship of Scientific Research", King Saud University, for funding this work through the research group Project No. RGP-VPP-069.

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