THE Bacterial endotoxin lipopolysaccharide enhances seizure susceptibility in mice: involvement of proinflammatory factors: nitric oxide and prostaglandins

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Abstract—Central nervous system (CNS) inflammation in cases such as head trauma, infection and stroke has been associated with the occurrence of epileptic seizures. Microglia, the principal immune cells in the brain, readily become activated in response to injury, infection or inflammation. The bacterial endotoxin lipopolysaccharide (LPS) induces the activation of microglia and the production of proinflammatory factors including nitric oxide (NO) and prostaglandins (PGs). We examined the effect of LPS on seizure susceptibility of mice, by using the sensitive test, threshold of clonic seizures induced by i.v. infusion of pentylenetetrazole. LPS decreased the seizure threshold in a dose- and time-dependent manner. Pretreatment of mice with the NO synthase inhibitor, Nω-nitro-arginine methyl ester or cyclooxygenase inhibitor, piroxicam or the opioid receptor antagonist, (−)-naloxone completely reversed the proconvulsant effect of LPS.

These results indicate that NO, PGs and endogenous opioid peptides seem to be involved in LPS-induced decrease in seizure threshold. © 2003 IBRO. Published by Elsevier Ltd.

Key words: epilepsy, microglia, naloxone, neuroinflammation, pentylenetetrazole.

Epilepsy, which is characterized by recurrent seizures, is the second most common neurologic disorder after stroke (Porter and Meldrum, 2001). The causes of seizures are numerous and include the full range of neurologic diseases, from infection to neoplasm and head injury (Porter and Meldrum, 2001).

Consequent to infection, post-traumatic brain injuries and ischemia, an inflammatory state emerges in the central nervous system (CNS) (Willmore, 1990; Leib et al., 1998; Matyszak, 1998; Minghetti and Levi, 1998). Inflammatory responses in the brain are thought to be mainly associated with activation of microglia, the resident immune cells of the brain (Kreutzberg, 1996; Minghetti and Levi, 1998; Streit et al., 1998). Activated microglia produce a variety of proinflammatory and cytotoxic factors, including nitric oxide (NO), reactive oxygen species, arachidonic acid metabolites and also pro- and anti-inflammatory cytokines (Lee et al., 1993; Leib et al., 1998; Minghetti and Levi, 1998; Schwartz et al., 1998; Liu et al., 2000a,b; Matoth et al., 2000). Although the convulsant and/or anticonvulsant effects of most of these mediators has been extensively described by in vitro and/or in vivo seizure models, contribution of the combination of these proinflammatory factors to the seizure occurrence is poorly studied.

The bacterial endotoxin lipopolysaccharide (LPS) is a general stimulator of microglia to produce the pro-inflammatory and/or cytotoxic factors (Hetier et al., 1990; Lee et al., 1993; Liu et al., 2000a,b; Matoth et al., 2000; Turrin et al., 2001; Schuligoi et al., 2003). Recently, peripheral or central administration of LPS to animals has been used as a model to produce neuroinflammation (Hauss-Wegrzyniak et al., 2000; Liu et al., 2000a,b,c; Turrin et al., 2001).

In the present study, the acute effect of LPS on susceptibility of mice to seizures induced by pentylenetetrazole (PTZ) was examined. The most popular and widely-used animal seizure model is the traditional PTZ test. PTZ is an inhibitor of γ-amino butyric acid (GABA)ergic neurotransmission, and i.v. infusion of PTZ is a sensitive model to determine the seizure threshold (Loscher and Schmidt, 1988).

NO and prostaglandins (PGs) are among the inflammatory mediators implicated in the pathogenesis of seizures and epilepsy (Bhaduri et al., 1995; Del-Bel et al., 1997; Medeiros et al., 1997; Alexander et al., 1998; Paoletti et al., 1998; Jelenkovic et al., 2002). We used a selective inhibitor of NO synthase, Nω-nitro-arginine methyl ester (L-NAME) and an inhibitor of cyclooxygenase, piroxicam, to determine the possible involvement of NO and PGs in seizure threshold changes induced by LPS.

Naloxone, an antagonist of the opioid receptors, is reported to inhibit microglial activation and possesses neuroprotective effect against LPS-induced central inflammatory response (Liu et al., 2000a,b,c). The effect of naloxone on LPS-induced changes in seizure susceptibility was evaluated in this study, as well.

EXPERIMENTAL PROCEDURES

Animals

Adult male NMRI mice, 28–35 days old, weighing 20–28 g, from Institute Pasteur of Iran (Tehran, Iran; n=600) were used throughout this study. The animals were housed in standard Plexiglas...
cages with free access to food (standard laboratory rodent’s chow) and water. The animal house temperature was maintained at 23±3.0 °C with a 12-h light/dark cycle (light on from 6:00 AM). All animal experiments were carried out in accordance with the European Communities Council Directive in such a way to minimize the number of animals and their suffering. Each animal was tested once.

Materials
PTZ, LPS (Escherichia coli serotype 026:B6), (−)-naloxone hydrochloride, piroxicam and L-NAME were purchased from Sigma (Poole, UK). All drugs were dissolved in sterile physiological saline except piroxicam, which was dissolved in sesame oil. Solutions were prepared freshly on the day of use and administered i.p. PTZ was administered i.v. All drugs were administered in volumes of 0.1 ml/10 g of mice body weight.

LPS injection and recording of body temperature
Mice rectal temperature was recorded by a thermistor probe connected to a digital thermometer (testo 915-1, Lenzkirch, Germany). The probe was lubricated, inserted approximately 1 cm into the rectum and kept there for 5 s and after recording the body temperature the probe was removed. This procedure was repeated every 0.5 h several times to eliminate the stress of probe insertion and the related effects on body temperature. The mice body temperature was recorded from 8:00 AM to 4:00 PM and the animals were restrained gently, only during the recordings. After adaptation of the animals, four groups of 10 mice were selected. One group received no injection and the body temperature was recorded at 8:00 AM. The other three groups were treated i.p. with the sterile physiological saline (10 ml/kg, as control) or LPS (0.1 and 1 mg/kg) at 7:30 AM and after 0.5 h (at 8:00 AM, which is equal to the recording time in mice with no injection) the body temperature was recorded. The body temperature of all four groups of mice was measured 0.5 h later and then every 1 h up to 8 h and eventually at 24 h.

Threshold of clonic seizure induced by PTZ
PTZ (10 mg/ml) was infused into the tail vein of freely moving mice at a constant rate (0.3 ml/min) using a 30 gauge dental needle which was connected by polyethylene tubing to a Hamilton microsyringe (Loscher and Schmidt, 1988). The onset of a general clonus was used as the endpoint. The general clonus was characterized by clonus of all four limbs with transient loss of righting reflex (Loscher and Schmidt, 1988). The volume of PTZ solution required to attain the endpoint was recorded. The dose of PTZ (mg/kg of mice weight) inducing general clonus was calculated and considered as an index for clonic seizure threshold.

Time course and dose effect of LPS on seizure threshold
At the dose of 1 mg/kg, LPS produced considerable hypothermia (began at 0.5 h, peaked at 1 h and returned to the control level at 4 h after administration) and hyperthermia (peaked at 8 h and dropped to the control level at 24 h) in mice. Based on these results, the effect of LPS (1 mg/kg) on the clonic seizure threshold was determined at 0.5, 1, 4, 8, 12 and 24 h after i.p. administration to separate groups of mice (n=10). In order to find whether the effect of LPS on seizure threshold is dose-dependent, six groups of 10 mice received i.p. LPS (0.001, 0.01, 0.1, 1 or 10 mg/kg) or sterile physiological saline (10 ml/kg) and after 1 h (the time that LPS produced maximum decrease in seizure threshold), PTZ-induced clonic seizure threshold was determined.

Effect of L-NAME on seizure threshold in LPS-treated mice

Experiment 1. Four groups of 10 mice each, were pretreated i.p. with the doses of 1, 5 or 10 mg/kg of L-NAME or 10 ml/kg of sterile physiological saline and after 1.5 h, PTZ-induced clonic seizure threshold was determined. Four other groups of mice were pretreated i.p. with either L-NAME (1, 5 or 10 mg/kg) or sterile physiological saline (10 ml/kg, control) and after 0.5 h received LPS (1 mg/kg) and the seizure threshold was determined 1 h thereafter.

Experiment 2. The procedure was just like experiment 1 but the PTZ seizure threshold was determined at the times of 8.5 h after L-NAME injection alone or 8 h after injection of LPS to L-NAME-treated mice.

Effect of piroxicam on seizure threshold in LPS-treated mice
The procedure was the same as experiments 1 and 2, except that piroxicam at 1, 5 and 10 mg/kg was used instead of L-NAME. The control group received sesame oil 10 ml/kg, i.p.
Effect of naloxone on seizure threshold in LPS-treated mice

The procedure was the same as experiments 1 and 2, except that naloxone at 0.1, 1 and 2 mg/kg was used instead of L-NAME.

The doses of L-NAME, piroxicam and naloxone were the doses that are reported to inhibit NO synthase, cyclooxygenase or block opioid receptors, respectively (Paoletti et al., 1998; Jelenkovic et al., 2002; Liu et al., 2000c; Santos and Rao, 1998). LPS produced maximum decrease in seizure threshold at 1 h and 8 h after administration. Therefore, these time points were selected to evaluate the effect of L-NAME, piroxicam and naloxone on LPS-induced changes in seizure threshold.

Statistical analysis

Data are presented as means ± S.E.M. One-way analysis of variance followed by Tukey’s post hoc test were used to analyze the data (SPSS for Windows; SPSS Inc., Chicago, IL, USA). * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the corresponding control value.

RESULTS

Effect of LPS on body temperature

During the 8-h recording, there was a gradual decrease in body temperature of the mice receiving no injection or saline (control group). There was no difference in body temperature of both groups in different recording times. Therefore, only the results of saline group are shown in Fig. 1. Moreover, Fig. 1 demonstrates that LPS (1 mg/kg, i.p.) produces significant hypothermia at 1 h after administration where the mice body temperature is 36.6 ± 0.2 °C compared with saline group (37.4 ± 0.1 °C) at this time. This is followed by gradual increase in the body temperature and at 8 h post-administration the body temperature in LPS (1 mg/kg) group is higher (by 0.9 °C) than saline group at this time point (37.5 ± 0.1 °C versus 36.6 ± 0.1 °C).
Effect of LPS on clonic seizure threshold

LPS decreased the clonic PTZ-induced seizure threshold in a dose-dependent (Fig. 2A) and time-dependent (Fig. 2B) manner. The maximum decrease in seizure threshold was induced by the dose of 1 mg/kg of LPS and at 1 h after injection. The decrease in seizure threshold was a sustained effect, which began 0.5 h after LPS injection, continued up to 12 h and reached to the control level at 24-h post-injection period.

Effect of L-NAME, piroxicam and naloxone on seizure threshold in LPS-treated mice

L-NAME, piroxicam and naloxone at the doses used, had no effect on clonic seizure threshold. However, they completely abolished the decreasing effect of LPS on seizure threshold at both 1 h and 8 h post-LPS administration periods (Figs. 3, 4 and 5).

DISCUSSION

Seizures and epilepsy are common sequels to acute brain insults such as stroke, traumatic brain injury and infection (Temkin et al., 2001; Herman, 2002) where there is a certain CNS inflammatory processes (Willmore, 1990; Thompson et al., 1993; Li et al., 1997; Leib et al., 1998). Microglia are the principal immune cells in the brain and they become activated in response to injury, infection or inflammation (Kreutzberg, 1996; Streit et al., 1998). The bacterial endotoxin LPS is a general inflammatory stimu-
lus, which activates microglia to release the inflammatory mediators (Hetier et al., 1990; Lee et al., 1993; Liu et al., 2000a,b; Matoth et al., 2000; Turrin et al., 2001; Schuligoi et al., 2003).

In this study, LPS produced a biphasic effect on body temperature, with an initial hypothermia, followed by a febrile phase. This is in agreement with previous reports that the inflammatory state induced by LPS is characterized by a biphasic changes in body temperature (Paul et al., 1999; Yirmiya et al., 2001).

Our results show that acute peripheral administration of LPS decreases the clonic seizure threshold in mice. This effect is time- and dose-dependent. In LPS-treated mice the seizure threshold shows a minimum at the 1 and 8 h post-injection periods (just the times that LPS produces the maximum hypothermia and hyperthermia, respectively). Then the threshold gradually increases and eventually reaches to the control level at 24 h after administration of LPS. However, Yuhas et al. (1995, 2002) reported that LPS (1 mg/kg, i.p.) did not affect sensitivity to PTZ. Two explanations can be given for this discrepancy: 1) the type of LPS used in these studies is different; while they used LPS obtained from *Shigella flexneri*, we used LPS of *E. coli*. 2) We evaluated the effect of LPS in PTZ i.v.-infusion model for clonic seizures. However, Yuhas et al. (1995, 2002) used the injection of a fixed dose of PTZ (50 mg/kg,

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**Fig. 4.** Effect of piroxicam on LPS-induced decrease in threshold of clonic seizures at 1 h (A) or 8 h (B) post-LPS administration periods. Histograms represent mean ± S.E.M. for 10 mice. *** P<0.001 compared with control group. * P<0.05 and ** P<0.001 compared with LPS (1 mg/kg) group.
It is possible that anticonvulsant (or convulsant) effect of an agent is missed because of the strong seizure stimulus of fixed doses of a chemoconvulsant such as the i.p. PTZ (Loscher and Schmidt, 1988); this can be avoided by use of the sensitive i.v. PTZ threshold test where the proconvulsant effects of a drug can be detected as well (Loscher and Schmidt, 1988).

LPS stimulates microglial activity and production of proinflammatory factors including tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), superoxide free radicals, PGs and NO (Hetier et al., 1990; Lee et al., 1993; Liu et al., 2000a,b; Matoth et al., 2000; Turrin et al., 2001; Schuligoi et al., 2003).

NO is a known chemical messenger involved in the pathogenesis of seizures and epilepsy with excitatory (Del-Bel et al., 1997; Alexander et al., 1998; Jelenkovic et al., 2002) or anticonvulsant role (Jayakumar et al., 1999; de Vasconcelos et al., 2000; Marangoz and Bagirici, 2001). In the present study, pretreatment of mice with the NO synthase inhibitor, l-NAME significantly inhibited the modulation of PTZ seizure threshold by LPS at both 1 and 8 h after administration. It seems that NO is involved in LPS-induced in-

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![Figure 5](image-url)

**Figure 5.** Effect of naloxone on LPS-induced decrease in threshold of clonic seizures at 1 h (A) or 8 h (B) post-LPS administration periods. Histograms represent mean ± S.E.M. for 10 mice. *** $P < 0.001$ compared with control group. $^a P < 0.05$, $^b P < 0.01$ and $^c P < 0.001$ compared with LPS (1 mg/kg) group.
crease in susceptibility of mice to clonic seizures induced by PTZ. In agreement with this suggestion, it has been found that LPS induces NO production by rat glial cell cultures 40-fold above the control level (Mattoth et al., 2000) and that the activation of NO production can decrease the PTZ-induced seizure threshold (Osonoe et al., 1994; Nidhi et al., 1999).

Apart from NO, activated glial cells produce PGs; for example PGE2 production has increased nine-fold above the control level following 48-h incubation with LPS (Mattoth et al., 2000). The present study shows the inhibitory effect of the cyclooxygenase inhibitor piroxicam on LPS-induced decrease in clonic seizure threshold. There are studies suggesting that PGs have a proconvulsant activity. The PGE analog, misoprostol has lowered the threshold for convulsions induced by PTZ (Madeiros et al., 1997).

Moreover, prevention of PGE2 elevation in the rat brain abolishes the expression of seizures (Paoletti et al., 1998). Thus, it can be suggested that PGs be also involved in the increase in seizure susceptibility of mice treated with LPS.

We found that the opiate receptor antagonist, (-)-naloxone completely inhibited the LPS-induced decrease in seizure threshold. Previous research demonstrated that LPS activates endogenous opioid system. Endogenous opioid levels are higher in plasma, cerebrospinal fluid and hypothalamus of LPS-treated animals (Carr et al., 1982) and i.p. injection of LPS increases endogenous morphine level in rat brain (Gourmon et al., 2000). Endogenous opioids appear to mediate at least some of the effects of LPS (Yirmiya et al., 1994); naloxone attenuates the physiological effects of LPS and bimodal body temperature response to LPS (Holaday, 1984; Blatteis et al., 1991). The febrile effect of LPS is mediated via μ-opioid receptors (Benamar et al., 2000). On the other hand, contradictory roles for endogenous opioids in the seizure have been suggested; while some studies support the anticonvulsant effect of endogenous opioids (Koide et al., 1993; Yehuda and Mostofsky, 1993; Simonato and Romualdi, 1997), the others suggest a proconvulsant effect (Di Giannuario et al., 1994; Schroeder et al., 1998; Di Giannuario et al., 2001) depending on the seizure model, animal species, site of action and type of opioid receptor involved (Frenk, 1983; Ramabadran and Bansimth, 1990). One of the endogenous opioids, which is claimed to be mainly involved in LPS effects, is β-endorphin (Carr et al., 1982; Benamar et al., 2000). In some studies β-endorphin has increased the vulnerability to seizure (Yehuda and Mostofsky, 1993) and induced electroencephalographic nonconvulsive limbic seizures, which were antagonized by naloxone (Di Giannuario et al., 1994, 2001). Therefore, opioid system may be involved in the LPS-induced decrease in clonic seizure threshold. However, Liu et al. (2000b,c) recently found that both (-)-naloxone and its inactive enantiomer as an antagonist for the classical opioid receptors, (+)-naloxone, inhibited, with equal potency, the LPS-induced activation of microglia and generation of NO and TNF-α, in mixed neuron-glial cocultures. They concluded that this inhibitory effect may not be related directly to binding of naloxone to the classical opioid receptors but rather to the negative regulation of the glial cells inflammatory response (Liu et al., 2000b,c). The same mechanism may be proposed for the inhibitory effect of naloxone on LPS-induced decrease in seizure threshold. Nevertheless, comparison of the effects of the (-)- and (+)-isomers of naloxone on LPS-induced increase in seizure susceptibility is needed for precise conclusion to be drawn.

There is evidence that inflammatory cytokines e.g. IL-1β are involved in seizures and epilepsy. A significant upregulation in mRNA of IL-1β is observed in some brain regions such as amygdala and hippocampus 2 h following the last generalized kindled seizures (Plata-Salaman et al., 2000). Limbic seizures induced by kainate, bicuculline or electrical stimulation, enhance IL1-β, IL-6 and TNF-α mRNA expression in hippocampus (Vezzani et al., 2002). Moreover, intrahippocampal injection of IL-1β has proconvulsant effect in these seizure models (Vezzani et al., 2002). Therefore, role of IL-1β in proconvulsant effect of LPS, observed in our study, should be borne in mind.

In conclusion, the present study reveals that LPS decreases clonic seizure threshold in mice. The proinflammatory mediators NO and PGs, and also endogenous opioid systems seem to mediate the effect of LPS.

REFERENCES


