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The Central and Peripheral Production of Pro-inflammatory Cytokine, IL-1β after Immune and Stress Stimulation in Rats

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ABSTRACT

Interleukin-1β (IL-1β), one of the pro-inflammatory cytokines, acts as an endogenous pyrogen and is an important mediator of behavioral and physiological responses to immune stimulation as well as exposure to stressors. The objective of the present study was to examine the pattern of central or peripheral IL-1β response to lipopolysaccharide (LPS) or exposure to the foot shock stress (FS) in rats. After treatment of LPS (100μg/kg) or exposure to the FS [ten times (0.8 mA) foot shocks for 5 sec each and 90 sec interval], body temperature and IL-1β levels in plasma, spleen and brain were measured. Both LPS and FS stimuli elicited increased body temperature but showed different patterns of peripheral IL-1β levels. LPS produced a widespread increase in IL-1β levels in the plasma, spleen and brain, whereas FS produced a significant increase in IL-1β levels only in the brain regions but not in plasma and spleen. The present study suggests that IL-1β is, centrally or peripherally in different patterns, regulated by immune stimulation or exposure to stressors and IL-1β plays an important role in mediating responses of sickness-like behaviors induced by immune stimuli or stressors.

Key words: interleukin-1β (IL-1β), brain, periphery, foot shock (FS), lipopolysaccharide (LPS), pro-inflammatory cytokines

INTRODUCTION

The immune system and the central nervous system (CNS) form a bi-directional communication network through cytokines which act as signaling molecules of the immune system (Maier, 2003). Especially, IL-1β, a pro-inflammatory cytokine, plays an important role in communicating between the immune system and the CNS. In recent years, there has been increasing recognition that IL-1β plays an important role in behavioral and physiological alterations produced by immune stimulation or exposure to stressors and similarities between the neurochemical, endocrine and behavioral consequences of immune challenge and stressors have been described frequently. IL-1β has been detected in the CNS after injury to the brain or peripheral immune activation. For example, IL-1β has been found to be present in the brain after peripheral administration of lipopolysaccharide (LPS, a component of the cell walls of gram-negative bacteria, potent activator of the immune system) (Laye et al., 1994; Buttini and Boddeke, 1995; Nguyen et al.,

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1998). Also, exposure to acute stress induces IL-1β protein in various brain regions including the hypothalamus and hippocampus in rats (Nguyen et al., 1998; O'Connor et al., 2003). However, the pattern of IL-1β production in the brain may be stressor specific. For instance, increased IL-1β has been observed in the hypothalamus after a variety of stressors such as tail shock (Nguyen et al., 2000) and immobilization (Shintani et al., 1995a) while other stressors such as maternal separation (Hennessy et al., 2004), predator exposure (Plata-Salaman et al., 2004), forced swim (Deak et al., 2003) and restraint (Deak et al., 2005) had no effect on central IL-1β expression (Blandino et al., 2005). Together, these findings provide converging lines of evidence that IL-1β is an important mediator of behavioral and physiological responses to exposure to stressors. Shintani et al. (1995b) has reported that central administration of IL-1β produces neurochemical, endocrine and behavioral changes that are similar to those produced by stressors. Direct administration of IL-1β, either peripherally or into the CNS, produces fever (Kluger, 1991) reduced food and water intake (Plata-Salaman et al., 1998), reduced social interaction (Bluthe et al., 1996) increased plasma ACTH and glucocorticoids (Weiss et al., 1991), and alterations in peripheral immune parameters (Brown et al., 1991). Correspondingly, intra-cerebroventricular IL-1ra, IL-1 receptor antagonist, has been reported to blunt or block several behavioral effects of peripheral immune stimulation (Kent et al., 1996) and reported to block the brain monoamines (NE, DA, and 5-HT) or ACTH responses to immobilization stress (Dantzer et al., 1993). Increased peripheral IL-1β has been observed in both blood and spleen after stressor exposure (Nguyen et al., 2000; O'Connor et al., 2003). It is, therefore, of great interest to explore the regulatory mechanisms of the production of IL-1β in the CNS and periphery. The purposes of the present experiments were to determine whether IL-1β could be altered of rat brain and periphery under immune stimulation of inflammatory immune system such as treatment of LPS and exposure of stressor such as foot shock, how is the patterns of response to each.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighting 310～360 g were obtained from Orient Corp (Gyeonggi-do, Korea). Animals were housed in groups of three with continuous access to food and water ad libitum. They were maintained on a 12 : 12 h light : dark cycle (lights on 08 : 00 h) regulated at 22°C room temperature. The experiments began at least 7 days after they had been acclimatized to their new environment. The experimental procedures were carried out according to the animal care guidelines of the NIH and the Catholic University of Korea Institutional Animal Care.

Experimental design

The experiment consisted of three groups (each groups consist of 8 animals): the non-treated group (non-treated animals), the LPS group (lipopolysaccharide; LPS, 100μg/kg., i.p after 2 hr) and the foot shock group [ten times (0.8 mA) foot shocks for 5 sec each and 90 sec interval].

Lipopolysaccharide (LPS) treatment

The LPS group were injected intraperitoneally (i.p.) with lipopolysaccharide (LPS: *Escherichia coli* serotype: 0111:B4; from Sigma-Aldrich, Pool, UK; 100μg/kg) dissolved in sterile, endotoxin-free 0.9% saline. Two hours after the LPS injection, rats were sacrificed.

Application of foot shock stress

To undertake foot shock experiments, a dark- colored chamber (28×23.5×20 cm), which had a floor grid for foot shock made of stainless steel rods of 3 mm in diameter with 10-mm intervals between the rods. All current was delivered to the grid floor by a shock generator. A shock generator with the ability to vary the current intensity was connected to the floor grid through a shock scrambler. The generator and scrambler were switched on and off by means of a time controller set for the predetermined time period used for foot shock. Rats were given an electrical foot shock through the floor grid by delivering electric currents (ten, 0.8-mA, foot shock for 5 sec each and 90 sec interval) and sacrificed immediately.

Measurement of core body temperature

Rats were deeply anesthetized with sodium pentobarbital (80 mg/kg, administered intraperitoneally) and core temperature was measured by thermometer (Letica pocket System, Spain) for three minutes.

Tissue collection and measurement of brain IL-1β

Brain regions (hypothalamus, hippocampus) and peripheral tissues (spleen) were quickly dissected on a cold plate and frozen immediately. All tissue was stored at 70°C until the time of assay. On the day of the assay, each tissue was thawed ice cold buffer (pH 7.2) containing 10 mM Tris-HCl (pH 7.4), 5 mM NaF, 1 mM Na_3VO_4, 1 mM EDTA, 1 mM EGTA extract and measure IL-1β. A commercially available ELISA (enzyme linked immunosorbent assay) designed to detect IL-1β was used to measure levels of IL-1β (assay designs, USA).

Blood sampling for corticosterone (CORT) and plasma IL-1β

Blood was collected through the ascending aorta and centrifuged for 15 min at 12,000 rpm (4°C). Then, separated plasma was frozen at 70°C until corticosterone assay. Plasma corticosterone level was determined using of Corticosterone Enzyme Immunoassay Kit (assay designs, USA) and plasma IL-1β level was determined using of Enzyme Immunometric Assay Kit (assay designs, USA).

Cytokine antibody array for measurement of spleen cytokines

The supernatants from spleen were analyzed with cytokine antibody arrays using Ray Bio Rat Cytokine Antibody Array V (Ray Biotech, Inc., Norcross, GA) according to the manufacturer's instructions. Briefly, cytokine array membranes were blocked in 2 ml of blocking buffer for 30 min and then incubated with 1 ml of samples at 4°C for overnight (about 16 h). Samples then were decanted from each container, and the membranes were washed three times with 2 ml of wash buffer I, followed by two washes with 2 ml of wash buffer II at room temperature with shaking. Membranes then were incubated in 1 : 250-diluted biotin-conjugated primary antibodies at room temperature for 2 h and washed as described above, before incubation in 1 : 1,000-diluted horseradish peroxidase-conjugated streptavidin for 1 h. Membranes were then washed thoroughly and exposed to a peroxidase substrate (detection buffers C and D; Ray Biotech, Inc., Norcross, GA) for 1 min in the dark before imaging. Membranes were exposed to X-ray film (Fuji medical X-ray film). Signal intensities were quantified and analyzed with Scion Imaging System. Biotin-conjugated immunoglobulin G served as a positive control at six spots, where it was used to identify membrane orientation and to normalize the results from different membranes that were being compared. For each spot, the net optical density level was determined by subtracting the background optical level from the total raw optical density level.

Statistical analysis

All data were analyzed by one-way ANOVA test using the statistical software SPSS (for windows OS). The data were expressed as the mean±standard error of the mean (SEM). For the comparison among the groups, Scheffe post hoc tests were performed and differences among the groups were considered statistically significant at $p<0.05$.

RESULTS

The effect of LPS and FS on core body temperature

Following treatment of LPS or exposure to the FS, body temperature was recorded for 3 minutes. Animals treated to the LPS or to the FS had significantly higher body temperature compared with the non-treated group ($F(2, 21)=23.757$, $p<0.001$). Both the LPS ($p<0.05$) and the FS group ($p<0.001$) had significantly higher body temperature compared with the non-treated group. Also, rats treated to the FS showed higher body temperature than the LPS group ($p<0.01$). The level of body temperature of rats in each group is shown in Fig. 1.

Plasma corticosterone

Plasma corticosterone levels were determined in each group of rats. Animals treated to the LPS or to the FS had significantly higher plasma corticosterone levels compared with the non-treated group ($F(2, 20)=26.414$, $p<0.001$). The FS stressor ma-

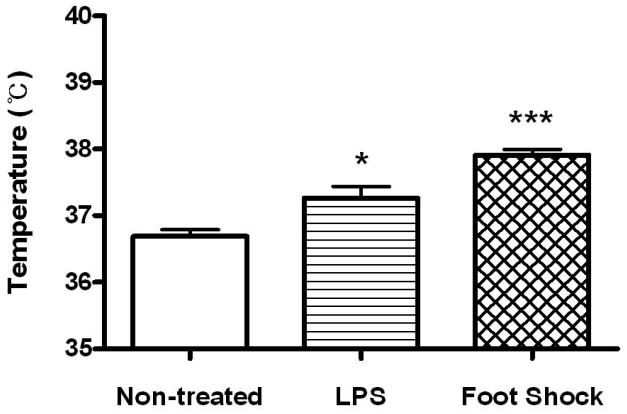


Fig. 1. Core body temperature in each group. Body temperature was measured for three minutes. Significance with Scheffe's test following a one-way ANOVA is indicated as *: $p<0.05$ and ***: $p<0.001$ compared to non-treated group. Vertical lines indicate S.E.M.

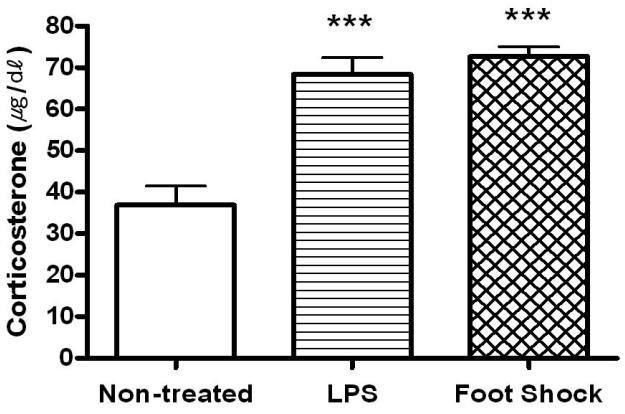
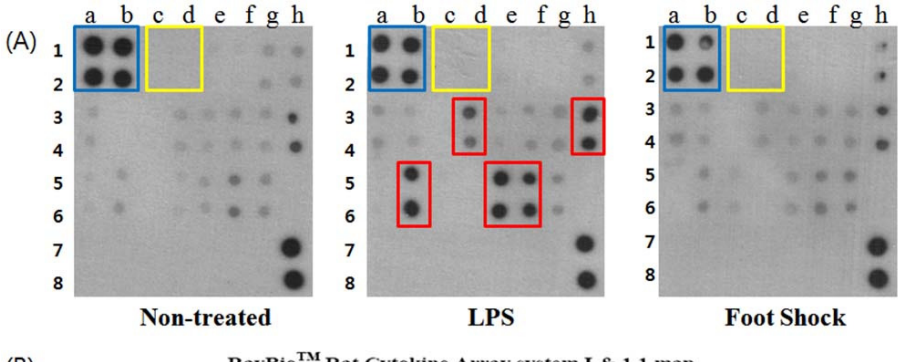


Fig. 2. Plasma corticosterone levels (μg/dl) in each group. Significance with Scheffe's test following a one-way ANOVA is indicated as ***: $p<0.001$ compared to the non-treated group. Vertical lines indicate S.E.M.



(B) RayBio™ Rat Cytokine Array system I & 1.1 map

	a	b	c	d	e	f	g	h
1	Pos	Pos	Neg	Neg	CINC-2	CINC-3	CNTF	Fractalkine
2	Pos	Pos	Neg	Neg	CINC-2	CINC-3	CNTF	Fractalkine
3	GM-CSF	IFN-γ	IL-1α	IL-1β	IL-4	IL-6	IL-10	LIX
4	GM-CSF	IFN-γ	IL-1α	IL-1β	IL-4	IL-6	IL-10	LIX
5	Leptin	MCP-1	MIP-3α	β-NGF	TIMP-1	TNF-α	VEGF	BLANK
6	Leptin	MCP-1	MIP-3α	β-NGF	TIMP-1	TNF-α	VEGF	BLANK
7	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	Pos
8	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	Pos

Fig. 3. Cytokines induced by LPS and Foot Shock in spleen detected by cytokine antibody array. (A) The cytokine array image shows the results obtained by exposure of membranes to X-ray film. (B) The layout shows the locations of each antibody in the array membrane. Blue box: positive control, Green box: negative control, Red box: p markedly increased cytokine in the LPS group.

nipulation significantly elevated the levels of plasma corticosterone relative to the non-treated group ($p<0.001$). The magnitude of the corticosterone response was similar to that of the LPS group ($p<0.001$). Therefore, there was no significant difference between the FS and the LPS groups (Fig. 2).

Cytokine expression in spleen

Spleen supernatants were subjected to cytokine antibody array analysis (Fig. 3). In the LPS group, IL-1β, monocyte chemoattractant protein 1 (MCP-1), TIMP-1 and TNF-α were markedly increased, compared with those of the non-treated and the FS groups (Fig. 3 and Fig. 4). IL-1β was significantly increased in the LPS group ($F(2, 3)=11.003$, $p<0.05$) compared with non-treated group ($p<0.05$). There was no significant difference between the FS and the LPS groups ($p=0.083$) (Fig. 4A). MCP-1

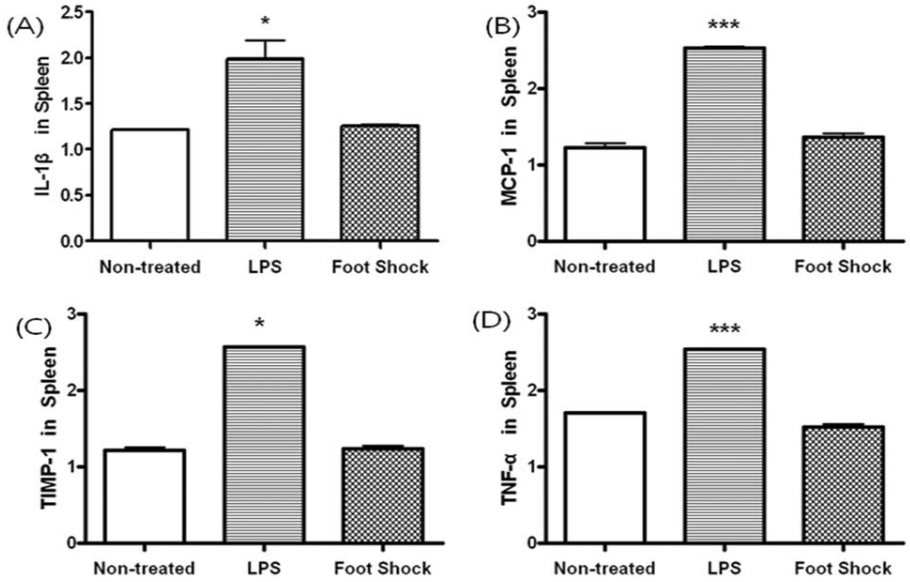


Fig. 4. Average optical intensities for each cytokine spot induced by LPS and foot shock in spleen. In Fig. 3A, cytokine spots showing significant difference in relative optical density compared to non-treated group were analyzed such as IL-1β, MCP-1, TIMP-1and TNF-α. Significance with Scheffe's test following a one-way ANOVA is indicated as *: $p<0.05$, ***: $p<0.001$ compared to the non-treated group. Vertical lines indicate S.E.M.

was significantly increased in the LPS group ($F(2,3)=198.867$, $p<0.001$) compared with the non-treated group ($p<0.001$) and FS group ($p<0.001$) (Fig. 4B). TIMP-1 was significantly increased in the LPS group ($F(2, 3)=11.003$, $p<0.05$) compared with the non-treated group ($p<0.05$) (Fig. 4C). TNF-α was significantly increased in the LPS group ($F(2, 3)=264.561$, $p<0.0001$) compared with the non-treated group ($p<0.001$) and FS group ($p<0.001$) (Fig. 4D).

Effects of LPS and FS on the plasma IL-1β levels

In the LPS group, IL-1β were markedly increased ($F(2, 15)=12.558$, $p<0.01$). As similar pattern with spleen IL-1β, FS again had no effect on plasma IL-1β ($p=0.731$), compared to the non- treated group. However, LPS produced a robust increase in plasma IL-1β ($p<0.01$) compared to the non-treated group and compared to the FS group ($p<0.05$) (Fig. 5).

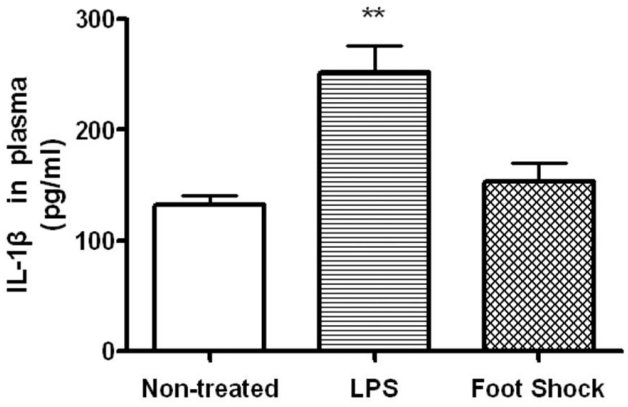


Fig. 5. IL-1β levels obtained from plasma sample of the each group. Significance with Scheffe's test following a one-way ANOVA is indicated as **: $p<0.01$ compared to the non-treated group. Vertical lines indicate S.E.M.

Effects of LPS and FS on the hypothalamic IL-1β levels

IL-1β brain levels in regions of hypothalamus (Fig. 6) were examined ($F(2, 15)=15.420$, $p<0.001$). As can be seen, IL-1β in the hypothalamus was significantly induced by the LPS injection ($p<0.01$) and FS treatment ($p<0.001$) compared with the non treated group for the hypothalamus. However, there was no significant difference between the LPS and the FS groups.

Effects of LPS and FS on the hippocampal IL-1β levels

IL-1β brain levels in regions of hippocampus were examined. ($F(2, 15)=20.130$, $p<0.001$) As can be

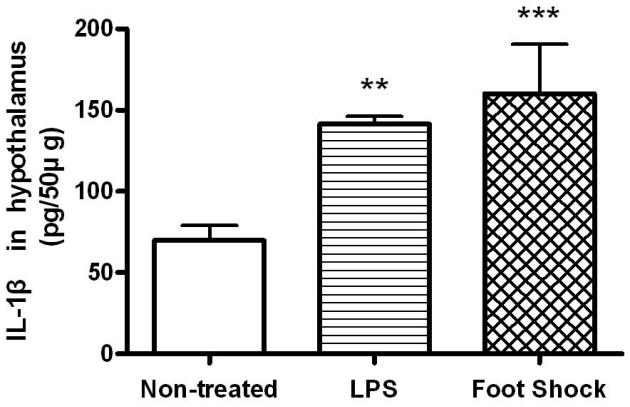


Fig. 6. IL-1β levels obtained from hypothalamus in rat brain of the each group. Scheffe's test following a one-way ANOVA is indicated as **: $p<0.01$, ***: $p<0.001$ compared to the non-treated group. Vertical lines indicate S.E.M.

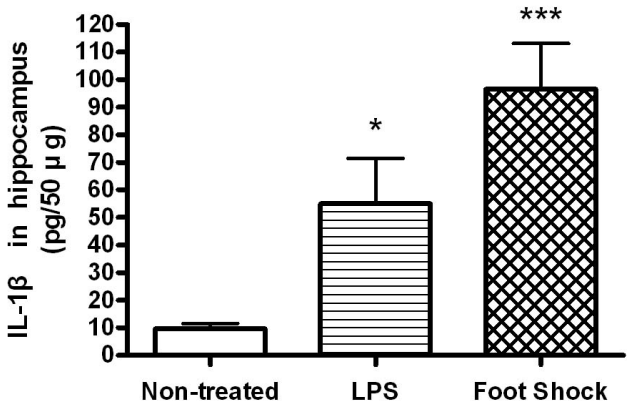


Fig. 7. IL-1β levels obtained from hippocampus in rat brain of the each group. Scheffe's test following a One-Way ANOVA is indicated as *: $p<0.05$, ***: $p<0.001$ compared to the non-treated group. Vertical lines indicate S.E.M.

seen (Fig. 7), IL-1β in the hypothalamus induced by the LPS injection ($p<0.05$) and FS treatment ($p<0.001$) compared with the non treated group for the hippocampus. However, there was no significant difference between the LPS and the FS groups.

DISCUSSION

The purposes of the present experiments were to determine whether IL-1β could be altered in rat brain and periphery under immune stimulation of inflammatory immune system such as treatment of LPS and exposure of stressor such as foot shock, how is the patterns of response to each. As the first step of the experiment, body temperature was measured since LPS and stress can influence physiological processes including hyperthermia (Singer et al., 1986; Briese et al., 1991; Poole et al., 1997). Our data showed that a significant elevation of body temperature was caused after exposure of the foot shock stress compared with the non-treated and the LPS groups. Also, body temperature was increased at two hours after the intraperitoneal injection of the LPS group compared with the non-treated group, like other experiment (Johnson et al., 2003). Deak et al. (1997) showed that peripheral administration of acute LPS produces a fever response by the production of IL-1β in the CNS. Correspondingly, central administration of IL-1β in the anterior hypothalamus produces fever (Fontana et al., 1994). Also, inescapable tailshock, a stressor which has been widely documented to increase central expression of IL-1β, produced a fever response that was sustained for at least 24～48 h following stressor termination. In present study, both the LPS and the foot shock group showed increased IL-1β proteins in hypothalamus region. Together, these findings provide support for the view that increased central IL-1β appears to be particularly potent stimulator of fever. Plasma corticosterone (CORT) level was also determined since this hormone is a major indicator of stress. After foot shock, plasma concentrations of CORT were significantly increased like following administration of LPS that is consistent with other's results (Yelvington et al., 1984 and 1987). It has been reported that CORT suppress IL-1β in peripherally and centrally. Indeed, Goujon et al. (1995) reported that adrenalectomy enhanced brain IL-1β mRNA increases produced by peripheral administration of LPS. An increase in plasma IL-1β was not detected in animals after foot shock but significantly increased in animals after administration of LPS. Correspondingly, foot shock failed to increase splenic IL-1β, but significantly increased in animals after administration of LPS. Although, an increase in plasma IL-1β was not detected in animals after foot shock, an effect of stress on brain IL-1β still existed. The brain regions examined in the present experiments were chosen based on previous observations implicating them in stress responses and peripheral immune challenge. The hypothalamus is

a critical site mediating responses to IL-1β such as HPA activation and fever induction (Propes and Johnson, 1997). The hippocampus is another stress-related region involved in memory function (Kim and Diamond, 2002). The effects of LPS treatment and foot shock on brain levels of IL-1β were therefore examined in hypothalamus and hippocampus. IL-1β brain levels in regions of hypothalamus and hippocampus were significantly produced by the LPS injection and foot shock treatment compared with the non treated group. However, there was no significant difference between the LPS and the foot shock groups. A number of stressors have indeed been reported to increase IL-1β mRNA and protein levels in brain, although these increases were specific to the regions of hypothalamus and hippocampus and did not occur in other brain regions (Maier, 2003). However, the pattern of IL-1β production in the brain may be stressor specific. For instance, increased IL-1β has been observed in the hypothalamus after a variety of stressors such as tail shock (Nguyen et al., 2000) and immobilization (Shintani et al., 1995b) while other stressors such as maternal separation (Hennessy et al., 2004), predator exposure (Plata-Salaman et al., 2004), forced swim (Deak et al., 2003) and restraint (Deak et al., 2005) had no effect on central IL-1β expression (Blandino et al., 2005). Additionally, present study indicates that foot shock also induce brain IL-1β levels.

In summary, LPS produced widespread increase of IL-1β in the brain, spleen and plasma, but foot shock produced significant increase in IL-1β only in brain but not in spleen and plasma.

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