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Dopamine Dynamics in the Amygdala Influence Emotional Changes in the Early Stage of Systemic Inflammatory Response Syndrome in Rats

La Dinámica de la Dopamina en la Amígdala Influye en los Cambios Emocionales en la Etapa Temprana del Síndrome de Respuesta Inflamatoria Sistémica en Ratas

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SUMMARY: Systemic inflammatory response syndrome (SIRS) is a potentially fatal reaction to various forms of tissue damage and infections that cause damage to various organs. Furthermore, the brain is damaged earlier than other organs, resulting in diffuse brain dysfunction. The central clinical symptom of SIRS is delirium and emotional changes are involved in disease development. Although the amygdala is known to play a major role, the mechanisms underlying emotional changes in the early stages of SIRS have not been elucidated. Therefore, changes to dopamine levels in the amygdala were observed using an in vivo model of lipopolysaccharide (LPS)-induced SIRS to clarify the biochemical mechanisms activated in the early stages of SIRS. Extracellular dopamine was collected from the amygdala of free moving rats via microdialysis and then analyzed by high-performance liquid chromatography. In addition, emotional changes were assessed with the open field and sucrose preference tests. In the LPS group, dopamine release in the amygdala increased remarkably immediately after LPS administration, peaking at 120 min. Thereafter, dopamine release temporarily decreased, but then significantly increased again after 180 min. The present results suggest that diffuse brain dysfunction in the early stages of SIRS may involve altered dopamine levels in the amygdala.

KEY WORDS: Dopamine; Amygdala; Rat; Systemic inflammatory response syndrome; Sepsis-associated encephalopathy.

INTRODUCTION

Systemic inflammatory response syndrome (SIRS), which has been linked to sepsis-associated encephalopathy (SAE), is characterized by widespread damage to endothelial cells due to uncontrolled inflammation and multiorgan failure (Widmann & Heneka, 2014). Although a frequent sequel to sepsis, acute altered mental status is associated with a significantly increased risk of mortality. Brain dysfunction due to sepsis has been largely ignored as a cause of altered mental status in critically ill patients primarily because of the lack of precise, well-established clinical and biological markers to assess brain dysfunction associated with SAE (Iacobone *et al*., 2009). However, recent studies have reported that SAE is a relatively common cause of altered mental status in critically ill patients admitted to the intensive care (Iacobone *et al*., 2009).

The clinical spectrum of SAE ranges from mild inattentiveness or disorientation, agitation, and hypersomnolence to more severe disturbances of consciousness and coma (Chaudhry & Duggal, 2014). A recent study found that SAE can induce sustained brain lesions (Widmann & Heneka, 2014). Cerebral dysfunction in SAE reflects systemic metabolic, inflammatory, and hemodynamic disturbances associated with SIRS, rather than abnormal function of the central nervous system. The implications of sepsis and SIRS on cerebral function are

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profound and the body of knowledge regarding SAE is growing. Further research, however, is clearly necessary to improve recovery from SAE and reduce long-term consequences on cerebral function. Evidence suggests the coexistence of immune dysfunction with psychological changes in patients with various neuropsychiatric and non-neurological disorders. Immunotherapies are increasingly used clinically but often affect mood (Hodes *et al*., 2015).

Psychological stress and depression have been intimately linked to peripheral inflammation due to increased levels of circulating proinflammatory cytokines, including interleukin IL-6 and tumor necrosis factor TNF-α (Felger & Lotrich, 2013). Behavior is a major, highly preserved, and adaptive component of the stress response. The behavioral response to stress is variable, ranging from aggressiveness, anxiety, or hyperalertness to lethargy, and is mainly controlled by the amygdala and hippocampus, which are vulnerable to hemodynamic and metabolic (i.e., hypoxemia and hypoglycemia) insults. The administration of lipopolysaccharide (LPS) is a widely used approach to investigate the mechanisms underlying activation of systemic inflammation induced by psychopathological effects. Previous studies have showed that local or systematic administration of LPS both could damage dopaminergic neurons (Reinert *et al*., 2014). And Li *et al*. (2020) found that specific inhibitors of dopamine D1 or D2 receptors both partly reduced the protective effect of L-DA on the learning and memory of lipopolysaccharides (LPS) treated mice and L-DA administration could prevent and treat SAE via dopamine D1 and D2 receptors. Furthermore, behavioral responses are mainly controlled by the amygdala and hippocampus. These behavioral changes are induced at the molecular level by proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α. The basolateral amygdala (BLA) is a key brain region involved in mood and may mediate some of the behavioral modifications induced by inflammation. Therefore, modification of behavior can be adaptive and physiologic, i.e., behavioral features of the response to stress, or maladaptive and pathophysiological, i.e., consequences of SAE (Chaudhry & Duggal, 2014). The results of the present study provide the first evidence supporting a functional separation between the brain structures underlying cytokine-induced disease behavior and cytokine-induced behavior and provide important clues about the neuroanatomical brain circuitry that cytokines may influence. These behavioral changes are induced at the molecular level by proinflammatory cytokines (e.g., IL-1β, IL-6, and TNF-α). The BLA is a key region of the brain involved in mood and may mediate some of the behavioral effects associated with inflammation. However, the mechanisms underlying emotional changes in the early stages of systemic inflammation remain unclear.

Therefore, the aim of the present study was to explore functional separation among the brain structures that regulate behavioral modifications associated with cytokine-induced disease to elucidate the neuroanatomical brain circuitry influenced by cytokines.

MATERIAL AND METHOD

Study approval. This study was conducted in strict accordance with the recommendations of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The research protocol with animal experimentation was approved by the Scientific Ethics Committee of Aichi Medical University School of Medicine (Protocol Number: 2020-101). All surgery was performed under sodium pentobarbital anesthesia and every effort was made to minimize suffering.

Animals. Male Sprague Dawley rats (initial body weight [BW], 180–190 g; BW at time of microdialysis, 290–420 g) were purchased from CLEA Japan, Inc. (Tokyo, Japan) and housed in an animal care facility at a constant temperature of 26 ± 2°C under a 12-h light/dark cycle with *ad libitum* access to food and water. Data from a total of 44 rats were included in the analysis.

LPS stimulation. The rats were intraperitoneally injected with LPS from *Escherichia coli* O127:B8 (Sigma-Aldrich Corporation, St. Louis, MO, USA) dissolved in 1 mL of sterile saline solution at 10 or 20 mg/kg BW/day. The dose of LPS was selected to elicit an inflammatory-induced transient sickness, as described in a previous study (Lacroix & Rivest, 1997). All rats were euthanized?within 24 hours of LPS or saline administration.

Surgical implantation of cannulas. Prior to surgery, the rats were anesthetized with 0.3 mg/kg BW of medetomidine, 4.0 mg/kg BW of midazolam, and 5.0 mg/kg BW of butorphanol. After induction of anesthesia, a stereotaxic frame was used to insert a guide cannula into the left BLA complex at 2.4 mm posterior and 5.2 mm lateral to the bregma and at a depth of 7.2 mm from the surface of the bregma (Kawano *et al*., 2018). Ear bars with dulled tips were used to stabilize the head and avoid damage to the eardrums. After surgery, the anesthesia was reversed by subcutaneous administration of atipamezole hydrochloride at 0.75 mg/kg BW. Then, each rat was placed in an individual cage with opaque sides (height, 30 cm; width, 25 cm; depth, 15 cm) and allowed to recover for at least 1 day.

Microdialysis. The left amygdala reportedly plays a much larger role in emotional processing than the right amygdala (Gur *et al*., 2002). A previous study with methamphetamine-

sensitized and fear-conditioned rats reported hyperactivity of dopamine in the left amygdala (Oshibuchi *et al*., 2009). Therefore, dopamine levels in the left amygdala of conscious freely moving rats were measured by microdialysis one day after implantation of the microdialysis probes (AI-12-2; Eicom, Kyoto, Japan; membrane length, 2.0 mm; outer diameter, 0.5 mm; molecular weight cutoff, 20,000 Da). Ringer's solution (147 mM Na^+, 4 mM K^+, 2.3 mM $Ca2^+$, 155.6 mM Cl^-) was used as the perfusate for microdialysis and samples were collected at a flow rate of 2 μL/min. Following an acclimation period of 60 min, preinjection extracellular dopamine levels were measured for 60 min after the start of microdialysis.

LPS at 10 or 20 mg/kg BW or saline solution was administered at 60 min after the start of microdialysis. Time-based changes in extracellular dopamine levels were measured for 280 min following injection at 60 or 340 min after the start of microdialysis.

At the end of the experiment, the rats were given an overdose of chloral hydrate and brains were fixed with 4 % paraformaldehyde via intracardiac infusion. The brains were cut into sagittal sections (50 mm) and the dialysis probes were located with reference to an atlas (Paxinos & Watson, 2006). If the dialysis probe was misplaced, the rat was excluded from analysis.

Measurement of extracellular dopamine levels. Samples were collected every 20 min. Extracellular dopamine levels were measured in real time with a high-performance liquid chromatography-based neurochemical analyzer (HITEC-500: Eicom) equipped with an auto-injector (ESA-20; Eicom) and a CA-5ODS column (2.1 × 150 mm; Eicom). The mobile phase consisted of 134.49 g/L of NaH_2PO_4, 49.40 g/L of Na_2HPO_4, 1 % methanol, 800 mg/L of sodium 1-decanesulfonate, and 50 mg/L of disodium ethylenediaminetetraacetate. The voltammetric detector was equipped with a graphite working electrode set at +0.45 V relative to an Ag/AgCl reference electrode. Use of the auto injector enabled measurement of dopamine levels without sample decomposition or loss through oxidation.

Histological analysis. At the end of the study, the rats were administered an overdose of sodium pentobarbital (100 mg/kg) and transcardially perfused with physiological saline followed by 10 % buffered formalin. The brains were post-fixed in 10 % buffered formalin for 1 to 10 days and then cryoprotected by immersion in 25 % sucrose for 2 days. The location of the microdialysis probe in the amygdala was histologically determined by staining of 50 μm-thick serial coronal sections with cresyl violet. Only data obtained from rats with correctly implanted probes were included for analysis. Data were considered compromised by bleeding around the trace of the probe, probes extending beyond the range of the BLA, or probes inside the striatum. Data from rats with amygdala catheterization failure or significant hemorrhaging proximal to the membrane of the microdialysis probe were also excluded.

Western blot analysis. Total protein was isolated using a modified protocol adapted from a method previously described for analysis of serum cytokine levels (Noguchi *et al*., 2017). Western blot analysis of the amygdala was performed with antibodies against total tyrosine hydroxylase (TH), which appeared on the blot as a single band corresponding to a molecular mass of approximately 63 kDa. Total TH levels were calculated relative to Na+/K+-ATPase levels.

Behavioral tests. Behavioral tests, which included the open field (OF) and sucrose consumption (SC) tests, were used to evaluate the anxiety- and depression-like behaviors of rats. Each behavioral test was conducted by the same technician between 09:30 and 16:00 h. Prior to testing, the rats were acclimated to the experimental room for at least 1 h.

OF test. The OF test was employed to identify psychomotor disturbances characteristic of anxiety in an open environment (Huang *et al*., 2012). Briefly, each rat was placed in an observation cage (height, 50 cm; width, 50 cm; depth, 30 cm; Muromachi Kikai Co., Ltd., Tokyo, Japan) and spontaneous locomotor activity (i.e., distance traveled, time spent in locomotion, rearing counts, and time in rearing) was measured in a square arena using photo-beam detectors for monitoring horizontal and vertical activity. The percentage of path length in the center region was calculated using ImageJ software (*https://imagej.nih.gov/ij/*). The rats were allowed to freely explore the observation cage for 240 min while data was collected.

SC test. During SC test, rats were free to choose from granulated sucrose, food and tap water for 24 hours. Prior to starting the experiment, the rats in the individual home cages were exposed to granulated sucrose, food, and tap water for 48 hours. After acclimation to granulated sucrose for 48 hours, granulated sucrose intake was measured for 240 min.

Multiplex electrochemiluminescence assay. Measurements of IL-1β and TNFα were performed by EMD Millipore Corporation (St. Charles, MO, USA) using a multiplex electrochemiluminescence assay with custom multiplex plates for detection (Meso Scale Diagnostics LLC, Rockville, MD, USA) in accordance with the manufacturer's standard protocol. Signals were detected with a SECTOR® Imager 6000 (Meso Scale Diagnostics LLC) and interpreted with

DISCOVERY WORKBENCH® software (Meso Scale Diagnostics LLC). Serum cytokine levels were normalized to the total protein concentration of each sample. If the measurements were out of range, the cytokine or chemokine (>10,000 pg/ml) was diluted and the assay was repeated. For samples with no detection of cytokines, a value of zero was assigned.

Statistical analysis. Dopamine values are expressed as a percentage of the mean of three basal samples, where absolute units are given. Rats with unstable basal levels were excluded from analysis. For each rat, the data were rescaled to set the average concentration of the three stable basal samples to 100 %. Two-way analysis of variance (ANOVA) was used to identify potential correlations between dopamine levels and freezing behavior following LPS administration to the saline-treated groups. Using group as a factor, repeated measures ANOVA and one-way ANOVA were used to identify significant differences between groups. Bonferroni's test was used for multiple comparisons or simple main effect testing when the main effect or interaction was determined as significant. All statistical analyses were conducted using GraphPad Prism, version 7.0b for Mac OS X version (GraphPad Software Inc., San Diego, CA). A probability (p) value of <0.05 was considered statistically significant.

RESULTS

Data from 3 rats were excluded because of amygdala catheterization failure or the presence of severe hemorrhaging around the membrane of the microdialysis probe or along the insertion path. Accordingly, data from a total of 17 rats (3 groups of 4~6 each) were included for analysis (Fig. 1A). Data from a total of 44 rats were included in the analysis (Fig. 1B).

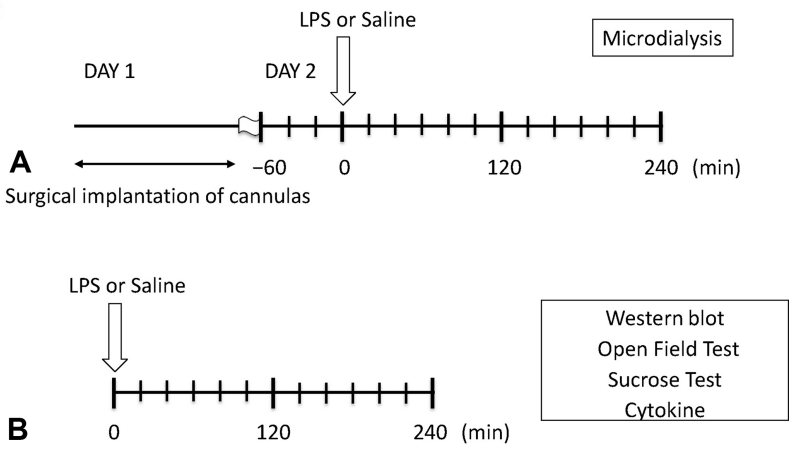

Fig 1. Summary of the experimental schedule.

Effects of LPS on extracellular dopamine release from the amygdala

The duration of the effects of LPS on extracellular dopamine levels in the amygdala relative to dopamine levels prior to treatment of naive rats is illustrated in Figure 1A. The techniques used for surgical implantation, microdialysis, and measurements of extracellular dopamine levels are described in the Materials and methods section. Correct placement of the microdialysis probe is illustrated in the diagrams presented in Figures 2A and 2B.

The line graphs in Figure 3A illustrate relative changes to extracellular dopamine levels over time following intraperitoneal injection of LPS (10 or 20 mg/kg BW) or saline (0.2 mL/kg BW in the same volume) beginning at 0 min. Extracellular dopamine levels had significantly increased following intraperitoneal injection of LPS at 20 mg/kg BW as compared to the control group ($p < 0.05$). This effect was maintained over 180 min and stabilized beginning at 40 min. In contrast, there was no significant difference in extracellular dopamine levels between the control group and the group treated with LPS at 10 mg/kg BW ($p < 0.05$). As illustrated in Figure 3B, total TH levels tended to decrease in the group treated with LPS at 20 mg/kg BW ($p < 0.05$). Post hoc analysis confirmed that LPS caused a significant decrease in total TH levels relative to the saline controls at 240 min ($p < 0.05$).

Brain dysfunction in the early stages of acute inflammation leads to anxiety-induced and antidepressant behavioral responses. Initially, two anxiety disorder tests, OF and SC, were tested after LPS (20 mg/kg) administration (Fig. 1b). The OF test was employed to investigate locomotor activity (Fig. 4a). The results of the OF test showed that locomotor activity decreased in the LPS-treated group over time as compared to the control group. The amount of time spent in the center of the field was strongly correlated with the level of anxiety, a characteristic referred to as “risk-taking behavior”. Risk-taking behavior was decreased in the LPS group and locomotor activity was significantly reduced immediately after LPS administration and completely suppressed after 120 min. To further assess these differences, the SC test was conducted, which revealed similar anxiogenic-like responses and significantly reduced intake of sucrose ($p < 0.05$). Images of the facial expressions of the rats at 120 min after saline injection (Fig. 4c) and 120 min after LPS injection are shown in Figure 4d.

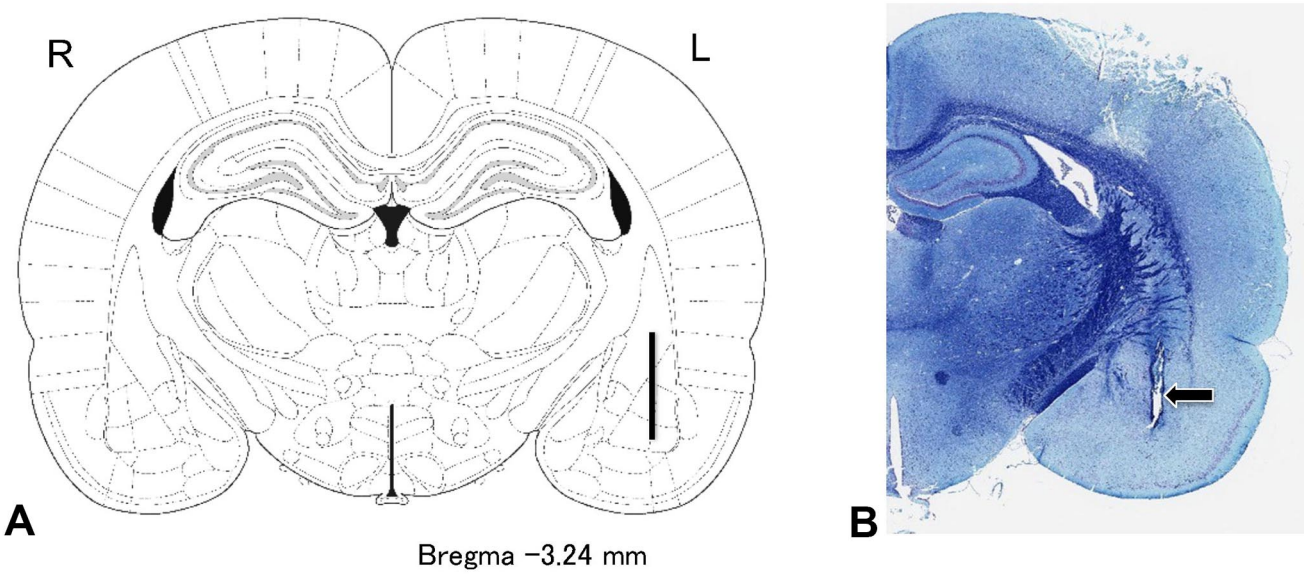

Fig 2. (A) Schematic of dialysis probe placement in the basolateral complex of the amygdala. The open bar represents the location and orientation of the dialysis membrane. Indicated is the anterior-posterior coordinate in mm relative to the bregma. R, right hemisphere; L, left hemisphere. (B) Micrograph of the slice preparation. A brain slice preparation stained with cresyl violet, showing a typical probe placement. The arrow indicates the area of the probe in the basolateral complex of the amygdala, which comprises the lateral and basolateral nuclei. The dotted lines indicate the major subdivisions of the amygdala. CeA, central nucleus of the amygdala; BLA, basolateral complex of the amygdala.

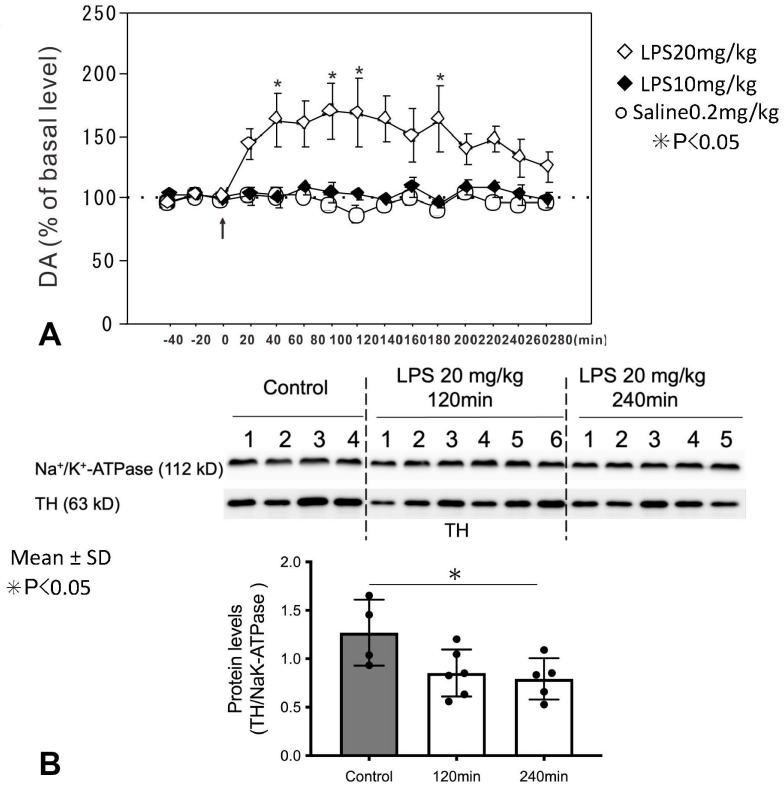

Fig 3. (A) Time course of the LPS effects on basal dopamine levels. The vertical axes represent changes in dopamine levels. The horizontal axis represents the time (min) from injection. (B) Measurement of total-TH levels in the amygdala (n = 5 per group).

Serum cytokine levels during the early stages of acute inflammation. Serum cytokine levels were measured at baseline (pre-LPS treatment) and at 120 and 240 min following a single injection of LPS at 20 mg/kg BW (n=10). Expression patterns of cytokines measured after a single injection are shown in Figure 5. There were notable differences in serum levels of IL-6, IL-1β, and TNF-α with significant increases in TNF-α at 2 h after LPS injection and IL-6 and IL-1β at 4 h.

IL-6. Serum IL-6 concentrations were measured to assess the extent of the inflammatory response to LPS. In the LPS-treated groups (relative to the control group), serum IL-6 concentrations had increased by 14398.60-fold at 120 min after injection of LPS and by 21574.34-fold at 240 min (Fig. 5A).

IL-1β. Serum IL-1β concentrations were measured to assess the extent of the inflammatory response to LPS. In the LPS-treated groups (relative to the control group), serum IL-1β concentrations had increased by 303.44-fold at 120 min after injection of LPS and 873.51-fold at 240 min (Fig. 5B).

TNF-α. Serum TNF-α concentrations were measured to assess the extent of the inflammatory response to LPS. Serum TNF-a concentrations increased as compared to the control group at 240 min after LPS injection (Fig. 5C).

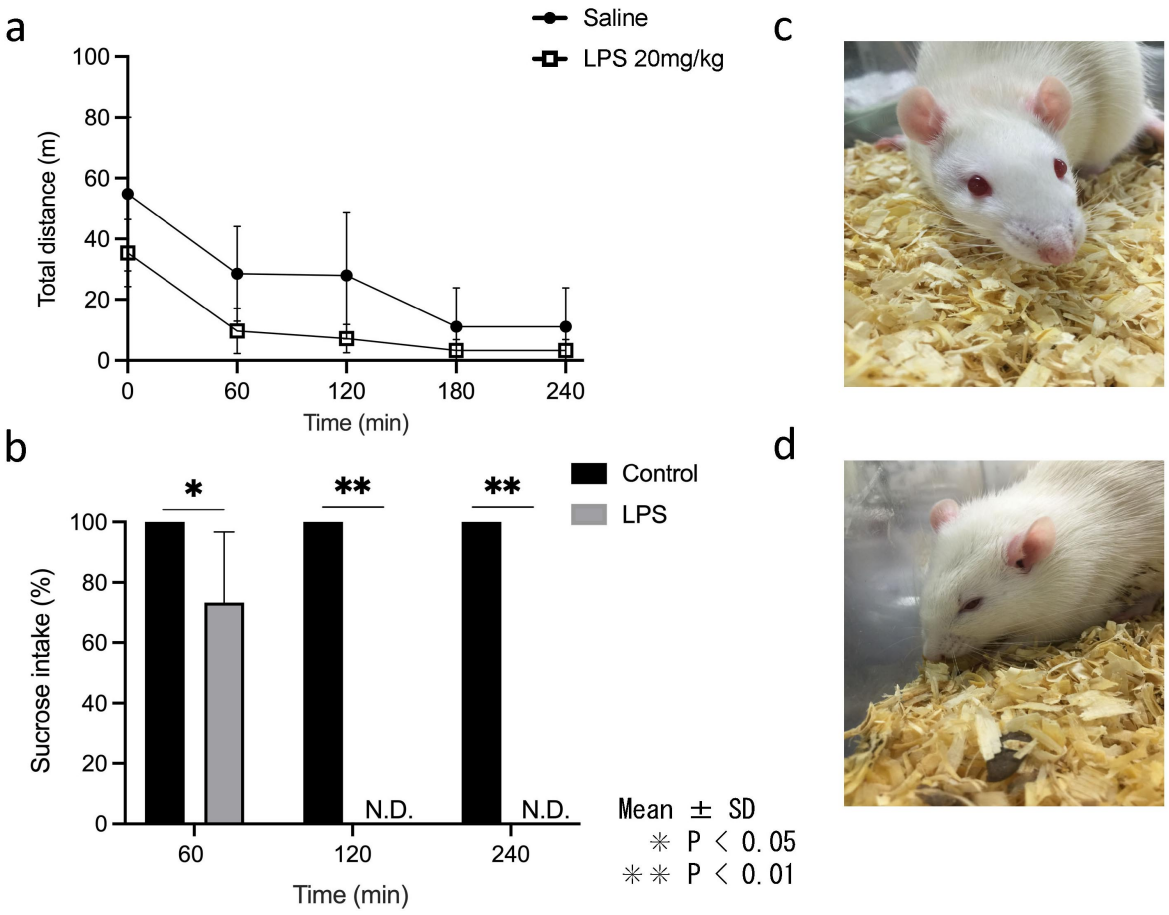

Fig 4. Anxiety-like behavioral indexes of the (a) OF and (b) SC tests. p<0.01 and p< 0.05 was taken as the overall level of significance. Facial expressions in LPS- and Saline- treated rats. Examples of facial expressions in the LPS-treated rat are shown in (c) saline-diagonal view and (d) LPS-diagonal view.

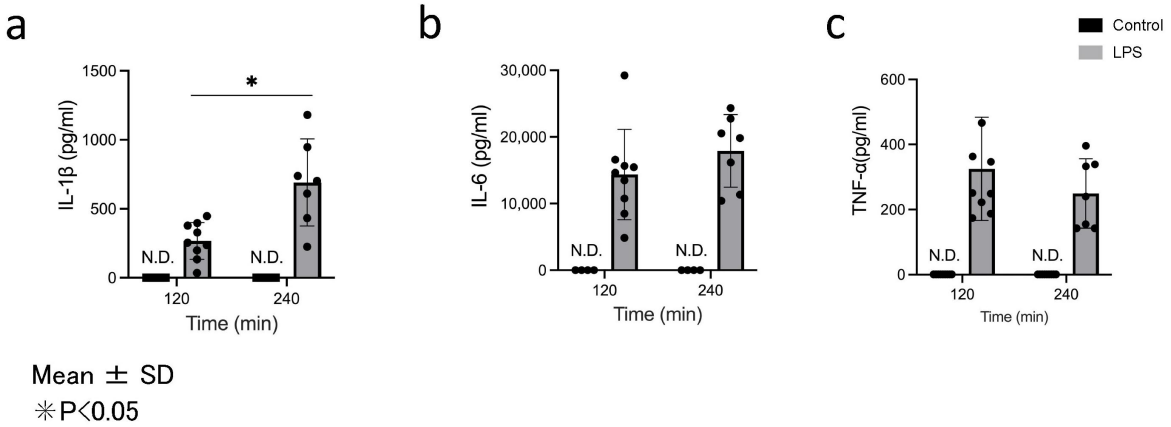

Fig 5. Time course of three cytokines in serum at 0, 120 and 240 min after LPS injection. p < 0.05 was taken as the overall level of significance.

DISCUSSION

This study is the first to provide direct neurochemical evidence that dopamine release is increased in the amygdala in the early stage of SIRS in a rat model, while locomotor activity is decreased. These findings suggest that SAE in the early stages of SIRS may involve dopamine dynamics in the amygdala.

Systemic inflammation caused an early increase in dopamine levels in the amygdala

Sickness behavior is now recognized as part of a motivational system to promote recovery from infection (Iacobone *et al*., 2009). Several mechanisms have been implicated in the pathogenesis of SAE, including disruption of the blood–brain barrier, neuronal apoptosis, endothelial activation, hyperinflammation due to the release of inflammatory cytokines, oxidative stress, and impaired neurotransmission due to altered neurotransmitter levels (Heming *et al*., 2017). Previous studies have suggested that cognitive impairment may be caused by febrile infection during or after fever (Heming *et al*., 2017). About 40 % of SAE patients experience long-term and irreversible sequela, including memory impairment, depression, anxiety, and cognitive disturbances (Widmann & Heneka, 2014). This clinical syndrome is described as sickness behavior induced by increased production of proinflammatory cytokines after infection that affect brain function (Chaudhry & Duggal, 2014). SAE is being increasingly recognized as a cause of cognitive dysfunction in critically ill patients. The amygdala mediates behavioral changes via the stress network and responses to behavioral features of illness (anorexia, anxiety, and avoidance). The amygdala is crucial for the formation, retrieval, and expression of emotion memories during fear conditioning. Dopaminergic transmission underlies multiple effects in fear memory processing. Furthermore, dopamine release in the amygdala is involved in the extinction of fear conditioning (Kawano *et al*., 2018). Thus, multiple mechanisms may underlie the effects of dopamine on processing of fear memory. A recent genetic manipulation study revealed that sub-threshold dopamine release in the amygdala leads to long-term depression that limits less salient experiences from forming persistent memories (Kwon *et al*., 2015). In a mouse model treated with LPS, the amygdala microcircuitry was shown to define anorexic behavior (Douglass *et al*., 2017).

Currently, SAE is considered the most common cause of encephalopathy in medical and surgical intensive care units, with more than half of septic patients developing encephalopathy (Chaudhry & Duggal, 2014). However, relatively few studies have addressed the causes of SAE. Previous studies have suggested that dopamine release in the amygdala is involved in memory processing, suggesting that dopamine may be involved in the early stages of SAE.

The nucleus accumbens and amygdala are considered a series of pathways associated with the stress response. Extracellular dopamine is increased by stress load at multiple sites in the brain, including the nucleus accumbens and amygdala, which has been associated with the later stage of fear memory processing, such as reconsolidation (Iwata *et al*., 2016). In the present study, administration of LPS at 20, but not 10, mg/kb BW led to an immediately increase in dopamine levels and this effect was maintained over a period of 180 min and stabilized beginning at 40 min (Fig. 3A). Furthermore, administration of LPS at 20 mg/kg BW led to a significant decrease in total TH levels at 240 min (Fig. 3B). TH is a monooxygenase that catalyzes the first rate-limiting step in the biosynthesis of catecholamines, including dopamine, noradrenaline (NA), and adrenaline (Nagatsu & Nagatsu, 2016). The results of this study suggest that total TH is depleted from the amygdala at 240 min after LPS administration at 20 mg/kg BW, which is consistent with dopamine levels in the amygdala measured over time, which peaked at 60 min, was maintained until around 180 min, and then gradually decreased.

Administration of LPS affected freezing behavior

The behavioral symptoms of sickness do not occur immediately after the onset of the immune response, but rather develop over time. Following peripheral administration of LPS, many sickness-related behaviors are induced within 2–6 h and then gradually wane (Dantzer *et al*., 2008). There was no immediate difference in locomotor activity (i.e., total distance travelled) in the OF test between saline- and LPS-treated rats, but these behaviors were significantly affected after 120 min, when electrical activity and NA levels in the amygdala of rats treated with LPS were markedly increased as compared to those injected with saline, demonstrating that the various behavioral changes of the LPS-challenged rats were timed to the increases in both amygdaloidal neuronal activity and NA release. The SC test provides a measure of the ‘‘hedonic’’ state or the ability to experience pleasure (Jacques *et al*., 2019). Impairment of this state (i.e., anhedonia), as evidenced by decreased sensitivity to reward, is a fundamental feature of clinical depression (Huang *et al*., 2012).

At present, rodent grimace scales have renewed interest in measuring the affective component of pain and have been promoted as a means to overcome the shortfalls of nociceptive threshold testing. Five facial features (action

units) considered indicators of "pain" by the mouse grimace scale were identified in rats treated with LPS at 20 mg/kg BW (Fig. 4D) (Langford *et al*., 2010).

An increase in the frequency of anxiety-like behavior could be a direct consequence of increased amygdaloidal activity since the amygdala is known to play a critical role in the generation of fear and anxiety (Engler *et al*., 2011). However, patterns of anxiety-like behavior cannot clearly be dissociated from the observed reduction in locomotor activity and explorative behavior, which more likely result from suppressed activity of other brain regions, such as the hippocampus, ventral tegmental area, and dorsal striatum. Nevertheless, the amygdala might be involved in modulation of these behaviors as well, since bilateral injection of NA into the amygdala has been shown to dose-dependently suppress locomotor activity and explorative behavior in rats. Pro-inflammatory cytokines play an essential role in the development of sickness behavior, as IL-1b and TNF-a exhibit anxiogenic potential (Dantzer *et al*., 2008). Amygdaloidal IL-1b, IL-6, and TNF-a mRNA expression levels were significantly higher in the LPS-treated rats than the saline-treated group at 120 min after injection and had further increased at 240 min, indicating that these cytokines are de novo synthesized in the amygdaloid complex in response to peripheral immune activation. This is consistent with previous reports showing that engagement of immune-to-brain communication ultimately leads to the production of proinflammatory cytokines in the central nervous system (Engler *et al*., 2011). The results of the present study showed that serum levels of IL-1b increased before serum TNF-a, similar to the results of a previous study (Iwata *et al*., 2016). However, the mechanism underlying triggering of amygdaloidal cytokine expression remains unclear. Several studies have suggested that NA might be involved in the induction of cytokine expression in the brain. Hence, the increased expression of proinflammatory cytokines in the amygdala of LPS-treated rats might be directly linked to increased NA levels. The vast majority of studies of immune-to-brain communication have been conducted with the use of rodents, but initial evidence indicates that systemic inflammation may also affect human amygdala function. For example, depressive-like symptoms and anxiety can be transiently elicited in healthy human subjects by peripheral administration of cytokine-inducing agents, such as LPS and typhoid vaccine (Wright *et al*., 2005). Furthermore, functional magnetic resonance imaging studies have shown that vaccination with typhoid vaccine leads to changes in amygdaloidal neuronal activity during emotion-related and cognitive tasks (Harrison *et al*., 2009). These findings suggest that in humans, similar to rodents, the amygdala is involved in the integration of behavioral and immune responses, although further studies are needed since the data are not conclusive. Such studies could also provide important insights into a potential link between the early stages of SIRS and the clinical manifestations of SAE.

The current study aimed to elucidate changes in dopamine levels in the amygdala that also occur in animal models of LPS-induced inflammation at even lower concentrations than previously reported (Engler *et al*., 2011). Taken together, the results of this study offer novel insights into different features of the amygdaloidal response to experimental immune activation in rats and provides further evidence that the amygdala plays an important role in the integration of immune-derived signals to coordinate behavioral and autonomic responses. This study focused on the early phase after experimental stimulation of the immune response to determine the length of time required from the propagation of immune-related information from the periphery to the amygdala.

CONCLUSION

Systemic inflammatory response syndrome (SIRS) is characterized by widespread damage to endothelial cells due to uncontrolled inflammation and multiorgan failure, and it can lead to death in some patients.

The main clinical symptom of SIRS is delirium, and emotional changes are involved in the development of this disease. Although the amygdala is known to play a key role in the pathogenesis of SIRS, the mechanisms underlying the development of emotional changes in the early stages of SIRS are unknown. Therefore, changes in the dopamine levels in the amygdala were observed using an in vivo model of lipopolysaccharide (LPS)-induced SIRS to elucidate the biochemical mechanisms that are activated in the early stages of SIRS.

The results of this study offer novel insights into the different features of the amygdaloidal response to the experimental immune activation in rats and provide further evidence that the amygdala plays an important role in the integration of immune-derived signals to coordinate behavioral and autonomic responses.

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RESUMEN: El síndrome de respuesta inflamatoria sistémica (SRIS) es una reacción potencialmente fatal a diversas formas de daño tisular e infecciones que causan injuria a varios órganos. Además, el cerebro se daña antes que otros órganos, lo que provoca una disfunción cerebral difusa. El síntoma clínico central del SIRS es el delirio y los cambios emocionales están involucrados en el desarrollo de la enfermedad. Aunque se sabe que la amígdala desempeña un papel importante, no se han dilucidado los mecanismos que subyacen a los cambios emocionales en las primeras etapas del SRIS. Por lo tanto, en el estudio se provocaron cambios en los niveles de dopamina en la amígdala utilizando un modelo *in vivo* de SRIS inducido por lipopolisacáridos (LPS) para dilucidar los mecanismos bioquímicos activados en las primeras etapas del SRIS. La dopamina extracelular se recogió de la amígdala de ratas en movimiento libre mediante microdiálisis y luego se analizó mediante cromatografía líquida de alta resolución. Además, se evaluaron los cambios emocionales con las pruebas de campo abierto y de preferencia de sacarosa. En el grupo de LPS, la liberación de dopamina en la amígdala aumentó de manera notable inmediatamente después de la administración de LPS, alcanzando un máximo a los 120 minutos. A partir de entonces, la liberación de dopamina disminuyó temporalmente, pero luego volvió a aumentar significativamente después de 180 min. Los resultadosactuales sugieren que la disfunción cerebral difusa en las primeras etapas del SIRS puede implicar niveles alterados de dopamina en la amígdala.

PALABRAS CLAVE: Dopamina; Amígdala; Rata; Síndrome de respuesta inflamatoria sistémica; Encefalopatía asociada a sepsis.

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