Lipopolysaccharide Induces Atrial Arrhythmogenesis via Down-Regulation of L-Type Ca\textsuperscript{2+} Channel Genes in Rats

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SUMMARY

Septic shock has been reported as an independent risk factor for atrial fibrillation (AF), however, the mechanism remains unknown. We investigated whether lipopolysaccharide (LPS) could alter cardiac ion channel gene expression, thereby leading to atrial arrhythmogenesis.

LPS (2.5 mg/kg) was injected intraperitoneally into 10 week old Sprague-Dawley rats (n = 5). Hemodynamic data were obtained and the atrial appendages were removed after LPS injection (0, 3, 6, 12, and 24 hours) for an RNase protection assay for α1C, β2, α1G, and SCN5A. An electrophysiological study in isolated perfused hearts was performed before and 12 hours after the LPS injection. Heart rate and body temperature were significantly increased (P < 0.05) and mean blood pressure was slightly decreased (P < 0.1) at 12 hours after LPS injection. The mRNA levels of the L-type calcium channel gene (β2 and α1C) were significantly decreased at 6 and 12 hours after LPS injection. Atrial ERP became significantly shortened and the number of repetitive atrial responses induced by an extrastimulus were significantly increased after LPS injection.

LPS induced the down-regulation of L-type calcium channel gene expression and ERP shortening, which might be a mechanism underlying sepsis-induced AF. (Int Heart J 2009; 50: 353-363)

Key words: Atrial fibrillation, Septic shock, L-type calcium channel, Lipopolysaccharide, Electrical remodeling

It has been reported that septic shock is one of the independent risk factors for atrial fibrillation (AF) in the surgical intensive care unit.\textsuperscript{1-3} In a multivariate analysis of the risk factors for AF, the odds ratio of shock was high at greater than 6, and most cases of shock were septic shock.\textsuperscript{1} In addition, 50% of the
AF patients fulfilled the criteria for sepsis in the surgical intensive care unit. Moreover, it has been suggested that inflammation per se could induce or provoke AF. Although a causal relationship between septic shock and AF has been suspected, little is known about the precise mechanism of septic shock induced AF.

Lipopolysaccharide (LPS) is known to play a major role in the pathogenesis of septic shock and have various adverse physiological effects due to the biochemical regulation of systemic organs such as the kidneys, liver, intestinal tract, and brain. LPS also affects the ventricles via physiological and biochemical regulation which leads to LV dysfunction.

On the other hand, various predisposing factors including the thyroid, ethanol, and glucocorticoids have been reported to potentially lead to AF occurrence due to cardiac ion channel remodeling. Therefore, LPS might cause some biochemical regulation effects in rat atria via cardiac ion channel gene expression leading to AF occurrence.

To test the hypothesis that LPS might underlie the causal relationships, we investigated whether LPS could alter the temporal profile of cardiac ion channel gene expression related to the occurrence of AF in rat atria, thereby leading to atrial arrhythmogenesis.

**Methods**

**Preparation of experimental animals:** Sprague-Dawley female rats aged 10 weeks were used in the present study (n = 5). LPS from Escherichia coli (O111 B4; Sigma, St. Louis, MO) at a dose of 2.5 mg/kg was injected intraperitoneally into the rats.

**Measurement of the hemodynamic parameters:** To examine whether LPS had an effect on hemodynamic parameters, heart rate, blood pressure, and body temperature were measured at predetermined times after the LPS injection (0, 6, 12, 24 hours). The systolic, mean, and diastolic blood pressures were determined at 3 different times during each period using a tail-cuff method with a manometer (BP-98A, Softron, Tokyo). Heart rate was also determined using a manometer. To obtain stable hemodynamic data, a thermal pocket was used. Body temperature was measured from the rectum with a thermometer at 3 different times during each period.

**Electrophysiological study:** To investigate the arrhythmogenic substrates of the atrial tachyarrhythmias during LPS injection, an electrophysiological study (EPS) in the whole hearts was performed before and 12 hours after LPS injection.
The hearts were rapidly excised and retrogradely perfused with oxygenated Tyrode’s solution containing (mmol/L) NaCl 136.5, KCl 5.4, HEPES 5.5, Na$_2$HPO$_4$ 0.33, glucose 5.5, CaCl$_2$ 1.8, and MgCl$_2$ 0.53 (pH 7.4 adjusted with NaOH). After maintaining a 30 minute recovery period during sinus rhythm, single extrastimulus pacing at a basic cycle length of 200 msec was delivered from the left atrial appendage using a conventional electrode. Stimulation was performed with 1 ms rectangular pulses using a digital programmable stimulator (SEN 7203, Nihon Kohden) and a constant current source (SS401J, Nihon Kohden). The maximum number of repetitive atrial responses (RAR) and effective refractory period (ERP) of the atrium were determined. The intra-atrial conduction time was measured between the pacing artifact and the right atrial electrogram recorded during left atrial appendage pacing at a cycle length of 200 msec.

**RNA preparation and RNase protection assay:** To examine whether LPS could alter cardiac ion channel gene expression, the mRNA levels associated with inward currents including L-type calcium channel ($\alpha_1C$ and $\beta_2$), T-type calcium channel ($\alpha_1G$), and Na channel (SCN5A) mRNAs were measured by an RNase protection assay with a RPA III kit (Ambion), as previously reported.\(^{17,18}\)

Briefly, DNA templates of $\alpha_1C$, $\beta_2$, $\alpha_1G$, and SCN5A were prepared by a reverse transcript polymerase chain reaction (RT-PCR) (Access RT-PCR system, Promega) from total RNA isolated from the rat atria. Amplified cDNA fragments were subcloned into PCR II vectors (Invitrogen) and confirmed by sequencing. The primers specific for each channel are shown in Table I. These plasmids were used to synthesize the antisense digoxigenin-labeled RNA probes. A cardiac cyclophilin signal was used as the internal control. RNA Century Markers (Ambion) were used as the molecular marker for protected fragments. At a predetermined time after LPS injection (0, 3, 6, 12, and 24 hours), total RNA was extracted from the left and right atrial appendages by the AGPC method. Five to 10 $\mu$g of RNA was used for the hybridization, RNase digestion, and recovery of the protected RNAs, and transferred to a nylon membrane after being run on a denaturing gel. The membranes were incubated with anti-digoxigenin antibody (Roche) conjugated to alkaline phosphatase, and then the protected fragments

### Table I. PCR Primers Used for the Amplification of the Cardiac Ion Channel Genes

<table>
<thead>
<tr>
<th>Channel</th>
<th>Sense</th>
<th>Antisense</th>
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<tbody>
<tr>
<td>$\alpha_1C$ (287 nt)</td>
<td>5’-GATGCAAGAGCCTATGGGCTAGG</td>
<td>5’-GCATGCTCATGTGTTGGGGTTT</td>
</tr>
<tr>
<td>$\alpha_1G$ (245 nt)</td>
<td>5’-TGGAGGAGAAGTTCAGAGG</td>
<td>5’-ATCCCCATGCCAGTTGAGG</td>
</tr>
<tr>
<td>SCN5A (200 nt)</td>
<td>5’-ATCCCGTTCCTCTAGTGAG</td>
<td>5’-CATGGATGTCGGGCTAGG</td>
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were detected using CDPstar (Roche). The chemiluminescent signals were quantified using a lumino-image analyzer (ATTO Light Capture AE-6960).

**Statistical analysis:** To examine the time-course of the mRNA levels of the cardiac gene channels, the value at the control was arbitrarily set to 1 U for quantitative comparisons. The mean values of the mRNA level and hemodynamic data at different times after the injection were compared by ANOVA, while the Bonferroni modified *t* test was used for multiple comparisons. The EPS data were analyzed using Student’s unpaired *t*-test. A *P* < 0.05 was considered significant.

**Results**

**Hemodynamic changes induced by LPS:** The results of the hemodynamic changes after LPS administration are shown in Figure 1. Heart rate was significantly increased at 6 hours after the LPS injection (control 350 ± 36 versus 453 ± 30 bpm, *P* < 0.05, *n* = 5). Body temperature was significantly increased at 12 hours after LPS injection (control 36.8 ± 0.3 versus 38.9 ± 0.8 Celsius, *P* < 0.05, *n* = 5). Mean blood pressure gradually decreased and reached a trough level at 12 hours after the LPS injection.

*Figure 1.* Hemodynamic response after LPS administration. The vertical axis represents the actual value of the heart rate (HR), systolic blood pressure (SBP), mean blood pressure (mBP), diastolic blood pressure (DBP), and body temperature (BT) values. The horizontal axis shows the time after the LPS injection. *P* < 0.05 versus control.
after LPS injection (control 101 ± 11 versus 95 ± 10 mmHg, \( P < 0.1 \)).

**Atrial arrhythmogenesis induced by LPS:** The difference in sinus cycle length of Langendorff perfused hearts during EPS was not statistically significant between the control and LPS rats (control 242 ± 50 versus 231 ± 23 msec; \( P = 0.65, n = 5 \)). The results of the EPS are shown in Table II. The ERP of the atrium was significantly shortened at 12 hours after LPS injection (control 44.2 ± 5.3 versus 34.9 ± 4.5 msec; \( P < 0.05, n = 5 \)). In addition, LPS significantly increased the maximum number of repetitive atrial responses induced by a single extrastimulus, whereas fewer responses were inducible in control rats (control 2.0 ± 2.4 versus 12.8 ± 14.0 beats; \( P < 0.05, n = 5 \)) (Figure 2). The intra-atrial conduction time was prolonged at 12 hours after LPS injection (control 19.3 ± 1.7 versus 21.1 ± 2.0 msec; \( P = 0.17, n = 5 \)), however, the prolongation of the conduction did not reach statistical significance. During the induced atrial tachyarrhythmias, atrial electrograms revealed rapid and irregular intervals, consistent with the charac-

**Table II.** Results of the Electrophysiological Studies

<table>
<thead>
<tr>
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<th>ERP (msec)</th>
<th>Intra-atrial CT (msec)</th>
<th>Maximum number of RAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (( n = 5 ))</td>
<td>44.2 ± 5.3</td>
<td>19.3 ± 1.7</td>
<td>2.0 ± 2.4</td>
</tr>
<tr>
<td>LPS 12 hour (( n = 5 )</td>
<td>34.9 ± 4.5*</td>
<td>21.1 ± 2.0</td>
<td>12.8 ± 14.0*</td>
</tr>
</tbody>
</table>

*indicates the value was statistically significant \( (P < 0.05) \).

ERP indicates effective refractory period; intra-atrial CT, intra-atrial conduction time; and RAR, repetitive atrial responses.

**Figure 2.** The mRNA levels of α1C, β2, α1G, and SCN5A after LPS injection (0, 3, 6, 12, 24 hours) in RNase Protection Assay. Cardiac cyclophilin signal was used as internal control. The mRNAs were measured by RNase protection assay with a RPA III kit (Ambion).
Cardiac ion channel remodeling induced by LPS: The results of the RNase Protection Assay are shown in Figures 3 and 4. The mRNA level of $\beta_2$ was slightly increased at 3 hours ($1.56 \pm 0.55$ fold versus control; $P < 0.1$, $n = 5$), following which it began to decrease at 6 hours ($0.69 \pm 0.13$ fold versus control; $P < 0.05$, $n = 5$), and then reached a trough level at 12 hours ($0.43 \pm 0.27$ fold versus control; $P < 0.05$, $n = 5$) after the LPS injection. Thereafter it began to return to the baseline level. The mRNA level of $\alpha_{1C}$ was also significantly decreased at 6 hours ($0.60 \pm 0.35$ fold versus control; $P < 0.05$, $n = 5$) and 12 hours ($0.39 \pm 0.33$ fold versus control; $P < 0.05$) after the LPS injection. The trough level of $\alpha_{1C}$ was obtained at 12 hours, following which it began to return to the baseline level. In addition, the mRNA level of $\alpha_{1G}$ was significantly decreased at 6 hours ($0.41 \pm 0.23$ fold versus control; $P < 0.05$), and 12 hours ($0.25 \pm 0.17$ fold versus control; $P < 0.05$) after LPS injection. On the other hand, the mRNA level of SCN5A exhibited no significant differences among the groups after LPS injection. Therefore, from the investigation of the 4 genes, 3 could be down-regulated by the LPS with a trough level at 12 hours after injection.

Figure 3. Effect of LPS on mRNA levels of $\alpha_{1C}$, $\beta_2$, $\alpha_{1G}$, and SCN5A. The vertical axis represents the percent change compared to the control. *indicates a statistically significant value ($P < 0.05$). The mRNA levels of $\beta_2$, $\alpha_{1C}$, and $\alpha_{1G}$ gradually decreased and reached a trough level at 12 hours after LPS injection ($P < 0.05$), while SCN5A exhibited no significant differences among the groups after LPS injection.
The major findings of the present study were that (1) LPS changed the hemodynamic parameters: heart rate and body temperature increased and mean blood pressure decreased, (2) LPS induced ERP shortening leading to an increased RAR during the EPS using Langendorff perfused hearts, and (3) LPS caused a transient decrease with a trough at 12 hours in the mRNA levels of the α1C, β2, and α1G.

**Septic shock and AF:** A recent review showed that AF was found to occur in 10% to 65% of patients in the surgical intensive care unit, usually on the second or third postoperative day. The present study demonstrated that, with the hemodynamic changes caused by LPS injection, heart rate and body temperature were significantly increased and mean blood pressure gradually decreased after the LPS injection. These results indicated that LPS administration in rats induced similar hemodynamic changes as those observed in septic patients. An earlier study showed that the maximum serum level of endotoxins in Wistar rats was observed at 6 hours after the intraperitoneal injection of an endotoxin, which was consistent with the timing of the hemodynamic changes observed with the LPS injection. In the present study, heart rate and body temperature were sig-
nificantly increased at 6 and 12 hours after the LPS injection, respectively, while mean blood pressure was decreased at 12 hours after the injection.

A state of septic shock could favor AF occurrences due to the various physiological affects such as those from the autonomic nervous system, neurohumoral factors, and metabolic electrolyte imbalances due to renal failure. However, EPS was performed on a Langendorff apparatus retrogradely perfused with oxygenated Tyrode’s solution, so the results of the EPS were not directly influenced by the LPS induced autonomic nervous activity changes and neurohumoral factors. Therefore, the LPS induced biochemical modification of the rat atria, at least in part, contributed to the development of the atrial arrhythmogenesis. In fact, the difference in sinus cycle length during EPS was not statistically significant between control rats and LPS rats. Sinus cycle length was increased in vivo in rats injected with LPS. Therefore, in addition to biochemical modification of the calcium channel in rat atria, LPS might augment the ERP shortening leading to atrial arrhythmogenesis via these physiological changes in our in vivo model.

**Potential mechanism of the LPS induced ERP shortening:** LPS, a cell membrane component shed by gram-negative bacteria, is vital for the development of sepsis, and causes various physiological responses. Several studies have demonstrated that there is an endotoxemia-induced reduction in the L-type calcium current in ventricular myocytes in animal models. These studies showed that the endotoxin-induced cardiac contractile LV dysfunction was associated with a decrease in the functional L-type calcium channels. In representative traces of action potentials from ventricular myocytes before and 4 hours after an intraperitoneal injection of LPS, the action potential duration of the LPS myocytes was significantly shorter than that of the control myocytes. Another report revealed the stimulatory effect of Bay K 8644, known to be a dihydropyridine agonist selective for L-type calcium channels, on the calcium current in rat ventricular myocytes injected with endotoxin. These reports demonstrated that Bay K 8644 completely abolished the reduction of the calcium current induced by the LPS, indicating the reduced calcium current in endotoxin injected ventricular myocytes was carried by the L-type calcium channels. In the present study, the mRNA levels of α1C and β2 were already down-regulated 6 hours after the LPS injection and preceded the development of atrial arrhythmogenesis. Therefore, the ERP shortening in the rat atria induced by LPS might also be related to the down-regulation of the L-type calcium channels as well as the ventricular myocytes.

**LPS and cardiac ion channel gene expression of the L-type Ca channel:** In the present study, LPS immediately down-regulated the mRNA level of the L-type calcium channels. The majority of the toll-like receptor 4 (TLR4), the best-characterized ligand of LPS, was expressed at the level of the monocytes which
release cytokines such as interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and γ-interferon, and lipid mediator (eg, platelet activating factor), reactive oxygen, and nitrogen intermediates (eg, NO). An earlier study demonstrated that transforming growth factor (TGF-β) decreased the cardiac L-type calcium current in the myocytes of neonatal Wistar rats, suggesting that the cytokines released from the monocytes may also play an important role in the regulation of cardiac ion channel genes. On the other hand, it was reported that TLR4 was also expressed by the cardiomyocytes. Therefore, the TLR4 pathway within the cardiomyocytes might regulate the cardiac ion channel gene expression including that of β2 and α1C in addition to the indirect response to the cardiomyocytes.

**LPS and cardiac ion channel gene expression of T-type Ca channels:** Although previous studies and this study focused on the down-regulation of the L-type calcium channels associated with LPS injection, we found that α1G, a T-type calcium channel corresponding gene, was also down-regulated by LPS. An earlier report showed that the T-type calcium channel Cav3.1 in human atrial tissue with valvular heart disease was down-regulated in patients with AF as compared to those without AF. LPS may also affect the T-type calcium current, and is related to atrial arrhythmogenesis, however, little is known about the relationship between the T-type calcium current and atrial arrhythmogenesis. The contribution of LPS induced α1G down-regulation to atrial arrhythmogenesis is still uncertain.

**Limitations:** There are several limitations in the present study. First, we did not perform Western blot analysis, therefore, we could not confirm the down-regulation of calcium channels induced by LPS at the protein level. Second, we demonstrated LPS-induced atrial ERP shortening in the EPS, however, we did not evaluate the ionic currents in the cardiomyocytes in order to confirm the reduction in the calcium currents by whole-cell patch-clamp studies. Further studies that include Western blot analysis and a whole-cell patch-clamp study will be required to clarify the mechanism of septic shock-induced AF occurrence.

**Conclusion**

LPS immediately induced the down-regulation of L-type calcium channel gene expression at the pretranslational level, which led to ERP shortening that could be arrhythmogenic. These results implied that LPS-induced biochemical modification of ion channel genes might be one of the mechanisms underlying sepsis-induced paroxysmal AF.
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