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LIPOPOLYSACCHARIDE ATTENUATES PHRENIC LONG-TERM FACILITATION FOLLOWING ACUTE INTERMITTENT HYPOXIA

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Abstract

Lipopolysaccharide (LPS) induces inflammatory responses, including microglial activation in the central nervous system. Since LPS impairs certain forms of hippocampal and spinal neuroplasticity, we hypothesized that LPS would impair phrenic long-term facilitation (pLTF) following acute intermittent hypoxia (AIH) in outbred Sprague-Dawley (SD) and inbred Lewis (L) rats. Approximately three hours following a single LPS injection (i.p.), the phrenic response during hypoxic episodes is reduced in both rat strains versus vehicle treated, control rats (SD: $84 \pm 7\%$ vs. $128 \pm 14\%$ baseline for control, $p < 0.05$; L: $62 \pm 10\%$ vs. $90 \pm 9\%$ baseline for control, $p < 0.05$). At 60 min post-AIH, pLTF is also diminished by LPS in both strains: (SD: $22 \pm 5\%$ vs. $73.5 \pm 14\%$ baseline for control, $p < 0.05$; L: $18 \pm 15\%$ vs. $56 \pm 8\%$ baseline for control, $p < 0.05$). LPS alone does not affect phrenic burst frequency in either rat strain, suggesting that acute LPS injection has minimal effect on brainstem respiratory rhythm generation. Thus, systemic LPS injections and (presumptive) inflammation impair pLTF, a form of spinal neuroplasticity in respiratory motor control. These results suggest that ongoing infection or inflammation must be carefully considered in studies of respiratory plasticity, or during attempts to harness spinal plasticity as a therapeutic tool in the treatment of respiratory insufficiency, such as spinal cord injury.

Keywords

Respiratory plasticity; Lipopolysaccharide; Intermittent Hypoxia; Phrenic long term facilitation; Inflammation

1. INTRODUCTION

Plasticity in the neural network controlling breathing results from physiological changes and/or prior experiences (Mitchell and Johnson, 2003). Respiratory plasticity may be essential to maintain appropriate ventilatory responses in the face of changing life circumstances. Inflammation alters plasticity in other neural systems, including the hippocampus (Hennigan et al., 2007; DiFilippo et al., 2008) and spinal cord (Young et al., 2007; Bessis et al., 2007). Here we demonstrate that systemic LPS and (presumptive) inflammation impair spinal plasticity in respiratory motor control.

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One experimental treatment frequently used to initiate inflammation is lipopolysaccharide (LPS). LPS directly and indirectly activates microglia, the resident immune cells of the central nervous system. LPS-induced immune responses result from the LPS-binding protein linked to toll-like receptor 4, toll-like receptor 2 and MD-2 (Lu et al., 2008; Palsson-McDermott and O'Neill, 2004). LPS activated microglia increase expression of pro-inflammatory molecules and decrease their expression of growth/trophic factors (Guan and Fang, 2006). Inflammation impairs hippocampal synaptic plasticity via multiple inflammatory molecules, including cytokines and Interleukins (Di Filippo et al., 2008). Conversely, systemic inflammation can induce synaptic plasticity, including plasticity in the spinal dorsal horn associated with chronic pain (Trang et al., 2009; Keller et al., 2007).

Acute intermittent hypoxia (AIH) induces a form of spinal, serotonin-dependent respiratory plasticity known as phrenic long-term facilitation (pLTF). pLTF is a progressive increase of phrenic motor output that can last for hours following the final hypoxic episode (Fuller et al., 2000; Mitchell et al., 2001; Feldman et al., 2003; Macfarlane et al., 2008; Dale-Nagle et al., 2010). pLTF requires serotonin-dependent synthesis of new BDNF in cervical spinal regions encompassing the phrenic motor nucleus (Baker-Herman and Mitchell, 2002; Baker-Herman et al., 2004). Here, we hypothesized that a single LPS injection would attenuate AIH-induced pLTF and suppress respiratory chemoreflexes (hypoxia and hypercapnia) in two frequently studied rat strains (Lewis and Sprague-Dawley rats).

2. METHODS

Experiments were performed on 3 to 4-month-old male Sprague–Dawley and Lewis rats (Harlan Laboratories, Indianapolis, IN, USA). All experiments were approved by the Animal Care and Use Committee at the School of Veterinary Medicine, University of Wisconsin–Madison and conformed to policies laid out by the National Institutes of Health in the *Guide for the Care and Use of Laboratory Animals*.

2.1 Electrophysiological experiments

As described previously (Bach and Mitchell, 1996; Baker-Herman and Mitchell, 2002; MacFarlane and Mitchell, 2008), anesthesia was induced using isoflurane, the rats were tracheotomized and pump ventilated (Rodent Ventilator, model 683; Harvard Apparatus, South Natick, MA, USA). LPS (3 mg/kg, Sigma-Aldrich, St. Louis, MO, USA, L4130) or vehicle (Saline 0.9%) were injected intraperitoneally at this time. Briefly, rats were maintained on isoflurane (3.5%, in 50% O₂) and then slowly converted to urethane anesthesia (1.8 g/kg, i.v.; Sigma-Aldrich). The rats were then paralyzed with pancuronium bromide (i.v., 3 mg/kg). The depth of anesthesia was assessed periodically by testing blood pressure and phrenic nerve responses to toe pinch with a hemostat. One hour after beginning surgery, an i.v. infusion was initiated, consisting of sodium bicarbonate (0.84%) and Hetastarch solution (3%) diluted in lactated Ringer's solution; these intravenous fluids helped maintain blood volume and acid–base balance. Rats were bilaterally vagotomized and a catheter was inserted into the right femoral artery. Arterial blood samples were drawn periodically and analyzed for arterial P_{O₂} and P_{CO₂}, pH and base excess using a blood gas analyzer (ABL 800, Radiometer, Copenhagen, Denmark). Rectal temperature was monitored throughout the procedure.

The left phrenic nerve was dissected using a dorsal approach, cut distally, desheathed, and placed on bipolar silver recording electrodes (submerged in mineral oil). Rats were allowed at least 1 hour for stabilization prior to beginning an experimental protocol. Nerve activity was amplified (gain, 10,000; A-M Systems, Everett, WA, USA), bandpass-filtered (100 Hz to 10 kHz), and integrated (CWE 821 filter; Paynter, Ardmore, PA, USA; time constant, 50 ms). The signal was digitized, recorded, and analyzed using a WINDAQ data acquisition

system (DATAQ Instruments, Akron, OH, USA). To establish baseline nerve activity, rats were ventilated with hyperoxia ($F_{IO_2} \sim 0.50$; arterial partial pressure of oxygen (Pa_{O_2}) ~ 280 mm Hg), with CO_2 added to the inspired gas. After the stabilization period, the apneic threshold was determined by progressively lowering the inspired CO_2 . Steady state baseline nerve activity was established with arterial partial pressure of carbon dioxide (Pa_{CO_2}) set 2–3 mmHg above the CO_2 recruitment threshold (Bach and Mitchell, 1996). End-tidal CO_2 was monitored throughout the experiment using a flow-through capnograph (Novamatrix, Wallingford, CT, USA). Arterial blood gases and blood pressure were monitored and corrected as necessary to ensure that post-hypoxia values were maintained near baseline levels. Pa_{CO_2} was adjusted by manipulating inspired CO_2 ; negative base excess values lower than -3 mEq/L were corrected with i.v. sodium bicarbonate, and progressive reductions in blood pressure were offset with i.v. injections of a lactated Ringer's/Hetastach solution. Data were included in the analysis only if they complied with the following criteria: (1) Pa_{O_2} during hypoxia was between 35 and 45 mm Hg; (2) Pa_{O_2} during the hyperoxic baseline and recovery periods was >180 mmHg (except for the first 15 min blood sample with the drug treated group); and (3) Pa_{CO_2} remained within 1.5 mmHg of baseline throughout the post-hypoxia protocol. Phrenic nerve activity was recorded continuously. Blood samples (50 μ l in a heparinized plastic catheter) were drawn before and during the first hypoxic episode, and at 15, 30, and 60 min post-AIH to ensure that blood gases met the criteria outlined above. Measurements of phrenic nerve burst amplitude and frequency were evaluated for 1 min immediately prior to each blood sample. At the end of the experiment, rats were humanely killed by an overdose of urethane.

2.2 Experimental groups

Rats were divided into time control (TC, $n=20$) and acute intermittent hypoxia (AIH, $n=37$) groups. TC rats were maintained in hyperoxia ($F_{IO_2} \sim 0.5$) throughout an experiment; TC rats were pre-treated with either vehicle ($n=5$ for each strain) or LPS ($n=5$ for each strain). AIH rats (3, 5-min episodes of isocapnic hypoxia, $F_{IO_2} \sim 0.11$; separated by 5 min hyperoxic intervals; see Fig. 1), were pre-treated (i.p., 2.5 hours prior the first hypoxic episode) with either vehicle ($n=10$ for Lewis, $n=8$ for Sprague-Dawley) or LPS ($n=7$ for Lewis, $n=12$ for Sprague-Dawley). LPS (3 mg/kg, i.p., Sigma) was freshly made, mixed and sonicated in saline (0.9%) prior injection.

2.3 Data analysis

Peak amplitude and frequency (bursts per minute) of phrenic nerve activity were averaged in 1-min bins at each recorded data point (baseline, end of the first hypoxic exposure, and 15, 30 and 60 min post-AIH). At all time points, changes in nerve burst amplitude are normalized as a percentage change from baseline, whereas burst frequency is expressed as an absolute change from baseline. Phrenic nerve responses were compared between rats receiving vehicle (saline) versus LPS injections, and TC versus AIH treatment. Statistical comparisons were made for time and drug treatment effects using two-way, repeated measures ANOVA. Bonferroni's *post hoc* test was used to identify statistically significant individual comparisons. Differences were considered significant if $p < 0.05$. All values are expressed as mean $\pm$ SEM.

3. RESULTS

3.1 Differences between Lewis and Sprague Dawley rats

In anaesthetized animals, baseline Pa_{O_2} and Pa_{CO_2} were lower in Sprague Dawley versus Lewis rats (248 ± 16 mmHg vs. 304 ± 8 mmHg, $p < 0.05$; 45.4 ± 1 mmHg vs. 51.7 ± 0.8 mmHg, respectively, $p < 0.05$; Table 1). On the other hand, mean arterial pressure was higher in Sprague Dawley versus Lewis rats (129 ± 4 mmHg vs. 68 ± 4 mmHg, respectively; $p < 0.05$).

These differences are maintained during hypoxic episodes (38 ± 3 mmHg vs. 49 ± 3 mmHg for PaO_2 ; 45.7 ± 1.2 mmHg vs. 51.6 ± 1.2 mmHg for PaCO_2 ; 105 ± 9 mmHg vs. 40 ± 3 mmHg for mean arterial pressure; Table 1). During baseline conditions, phrenic nerve burst amplitude is higher in Sprague Dawley versus Lewis rats (0.17 ± 0.03 μV vs. 0.09 ± 0.01 μV , $p < 0.05$; Figure 2A). The hypoxic phrenic response was also higher in Sprague Dawley rats (128 ± 14 % vs. 90 ± 8 % baseline, $p < 0.05$; Figure 2B). Phrenic burst frequency increases during hypoxia in Sprague Dawley rats (57 ± 3 bursts/min during hypoxia vs. 45 ± 1 burst/min during baseline in vehicle-treated rats, $p < 0.05$; Figure 2C). However, no hypoxic frequency increase was observed in Lewis rats (45 ± 2 burst/min vs. 45 ± 1 burst/min for vehicle-treated rats, $p < 0.05$; Figure 2C). Significant strain differences were not observed in the magnitude of pLTF (Sprague Dawley: 74 ± 14 % baseline; Lewis: 56 ± 9 % baseline, Figure 3C).

3.2 LPS effects

In anesthetized animals, acute LPS exposure (3 hrs) does not have any effect on measured physiological parameters (Table 1) or raw baseline nerve activity in either rat strain (Sprague Dawley: $0.23 \pm 0.03 \mu\text{V}$ vs. $0.17 \pm 0.03 \mu\text{V}$ for vehicle treated rat; Lewis: $0.13 \pm 0.03 \text{V}$ vs. $0.01 \pm 0.03 \text{V}$ for vehicle treated rat; Figure 1; 2A), whereas the response to a mild hypoxia (inspired $\text{O}_2 \sim 10\%$) is impaired in both rat strains ($83.5 \pm 6.7\%$ vs. $128 \pm 14\%$ baseline for Sprague Dawley vehicle treated; $62 \pm 10\%$ vs. $90 \pm 8.7\%$ baseline for Lewis vehicle treated; figure 2B). The burst frequency increase during hypoxia is not significantly altered by LPS in Sprague Dawley rats (43.2 ± 0.8 burst/min during baseline vs. 53.9 ± 0.7 burst/min following LPS; $p < 0.05$), but is enhanced in Lewis rats which received LPS versus vehicle treated rats (50.8 ± 2 burst/min vs. 45.3 ± 2 burst/min respectively; $p < 0.05$; Figure 2C). LPS does not have any effect on frequency over time (Figure 3A), but reduces pLTF in Sprague-Dawley rats ($22 \pm 5\%$ baseline at 60 min post-AIH, $p < 0.05$; vs. $73.5 \pm 14\%$ baseline at 60 min post-AIH for vehicle, $p < 0.05$ vs. LPS treated). LPS eliminates pLTF in Lewis rats (LPS: $18.4 \pm 15\%$ baseline at 60 min post-AIH, $p > 0.05$ vs. baseline; vehicle: $56 \pm 8\%$ baseline; Figure 3B,C). After LPS, no differences were observed between AIH and time control rats ($16.2 \pm 10\%$ baseline vs. $22 \pm 5\%$ baseline for Sprague-Dawley vs. $18.4 \pm 15\%$ baseline for Lewis rats; Figure 3C).

4. DISCUSSION

This is the first demonstration that acute (3 hr) systemic injections of LPS impairs respiratory motor plasticity, specifically AIH-induced pLTF.

4.1 Rat strain differences

We found small but significant physiological differences between out-bred Sprague Dawley and inbred Lewis rats. For example, PaO_2 and PaCO_2 are lower in Sprague Dawley vs. Lewis rats, even with the same inspired gas conditions. Further, mean arterial pressure is higher in Sprague Dawley rats. Differences in baseline PaCO_2 could reflect differences in the CO_2 apneic and recruitment thresholds, and are consistent with previously published work (Golder et al., 2005). The lack of hypoxic frequency response in Lewis rats has been reported previously (Golder et al., 2005). Despite this deficiency, both strains express pLTF in phrenic burst amplitude following AIH; the magnitudes of pLTF in both rat strains are comparable to previous reports (Fuller et al., 2000; Fuller et al., 2001; Golder and Mitchell 2005; Baker and Mitchell, 2000; MacFarlane and Mitchell, 2008; Baker-Herman and Mitchell, 2008).

4.2 LPS, inflammation and respiratory plasticity

Several studies have shown that a single injection of LPS induces CNS neuroinflammation, even with peripheral injections (Schnydrig et al., 2007; Guo and Schluesener, 2006). Single

LPS injections increase GFAP and ED1 (a marker for activated macrophages/microglia) immuno-staining in the CNS, which correlate to astrocytic and microglial activation, respectively (Guo and Schluesener, 2006). Acute peripheral LPS injections also increase the number of activated glial cells into the rat spinal cord (Guo and Schluesener, 2006). Microglia, once activated, release inflammatory mediators that can interact with their cellular environment, including nearby neurons. For example, IL1 β and TNF- α production increase in the hippocampus and spinal cord, with peak expression 6h post injection (Tanaka et al., 2006; Shen et al., 2008). LPS also induces TNF- α expression in spinal neurons (Shen et al., 2008), and decreases brain tissue BDNF mRNA levels (Schnydrig et al., 2007). In these same rat strains, it has been found that microglia freshly isolated from brainstem/spinal cord exhibit significant induction of inflammatory mediators, with mRNA levels for inducible nitric oxide synthase and cyclooxygenase 2 increasing markedly at 3 hours post-LPS injection (Watters and Crader, personal communication).

Glial cells, which implement the LPS-induced CNS immune response, interact with and modulate neuronal function (Bessis et al., 2006), including synaptic transmission, neuronal excitability and several forms of synaptic plasticity (Di Filippo et al., 2008). Inflammatory mediators induced by LPS can impair or induce synaptic plasticity (Di Filippo et al., 2008; Keller et al., 2007; Trang et al., 2009). Further, LPS can disrupt integrated behaviors, such as spinal learning in rats with spinal injury (Young et al., 2007) or learning and memory (Tanaka et al., 2006). LPS increases TNF- α expression in endothelial cells surrounding the carotid body, diminishing the ventilatory chemoreflex response to hypoxia (Fernandez et al., 2008). We found similar effects on the rat hypoxic phrenic response (Figure: 2B), although the site of LPS effects cannot be determined. However, changes in carotid body sensitivity may account for diminished hypoxic phrenic responses observed here.

LPS-induced neuroinflammation disrupts AIH-induced pLTF versus inducing plasticity, as occurs in the spinal dorsal horn (Trang et al., 2009). LPS may: 1) modulate the normal molecular cascade leading to pLTF, or 2) add a novel form of plasticity that offsets pLTF. Modulation of the cellular mechanisms giving rise to pLTF must be indirect (via glia or other inflammatory cells) since CNS neurons do not express the LPS receptor, the toll like receptor 4 (TLR4; Lehnardt et al., 2003) and since LPS does not cross the brain blood barrier (Singht and Jiang, 2004). There is little evidence that LPS affects 5-HT release during hypoxia (Karmouty Quintana et al., 2005). On the other hand, LPS reduces brain BDNF and TrkB mRNA expression (Tanaka et al., 2006; Schnydrig et al., 2007; Guan and Fang, 2006). Since new BDNF synthesis is both necessary and sufficient for pLTF (Baker-Herman et al., 2004), diminished BDNF function could play a role in abolishing pLTF. Reactive oxygen species are also necessary for pLTF (MacFarlane et al., 2008), largely reducing serine/threonine phosphatases that constrain pLTF expression (Wilkerson et al., 2008; MacFarlane and Mitchell, 2008). Additional reactive oxygen species formation in activated microglia may disrupt the normal ROS/phosphatase balance (Qin et al., 2005), thereby impairing pLTF. The mechanism whereby LPS impairs pLTF is not yet known and requires further investigation.

5. Conclusions

Here we show that LPS impairs pLTF, a spinal BDNF-dependent form of plasticity in respiratory motor control. Although the mechanism of impairment is not yet known, these results raise the critical point that ongoing infection (and subsequent inflammation) and/or endogenous inflammation (during for example traumatic or degenerative neural disorders) must be carefully considered in studies of respiratory plasticity, or during attempts to harness respiratory plasticity as a therapeutic approach for pathologies such as spinal cord injury (Mitchell, 2007).

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Figure 1.
Representative integrated neurogram from the phrenic nerve activity of an anaesthetized rat before, during and after AIH (Hypoxia 1 to 3) with 3 mg/kg of LPS i.p. (bottom trace) or vehicle treated rats (top trace). Also indicated are magnified bins (5 s duration) showing the time points for data analysis and blood sampling throughout the protocol of pLTF. White dashed line indicates the baseline. Note after the last hypoxia (Hx3) the gradual increase in phrenic nerve burst amplitude above baseline amplitude (i.e. LTF, top trace) compared to LPS treated rats. Hx = Hypoxia.

Figure 2. Phrenic nerve burst amplitude, frequency during baseline and first hypoxia from Sprague-Dawley and Lewis rats treated with 3 mg/kg LPS i.p. or vehicle

A. Phrenic nerve baseline burst amplitude for Sprague Dawley and Lewis rats with LPS or Vehicle. Note that the treatment has no effect on the baseline amplitude in both rat strains. There is a difference in amplitude between the rat strains. B. Phrenic nerve burst amplitude during the first hypoxic episode. Note that the LPS reduces in both rat strains the hypoxic response, and Lewis rat have a lower hypoxic response compared to Sprague Dawley rats. C. Phrenic nerve burst frequency during normoxia and the first hypoxia for LPS treated or Vehicle injected Sprague Dawley and Lewis rats. Note for the Lewis rats the absence of increase in burst frequency during hypoxia. LPS does not have any effect on the ventilatory response to hypoxia on both rat strains. a; $p < 0.05$ vs. strain-matched baseline; b; $p < 0.05$ vs. strain-matched vehicle; *, $p < 0.05$ vs. Sprague treatment-matched.

Figure 3. Phrenic long term facilitation from Sprague-Dawley (SD) and Lewis rats treated with 3 mg/kg LPS i.p. or vehicle

A. Phrenic burst frequency during, baseline, 15 min, 30 min and 60 min post-hypoxia. Note the absence of statistical differences between all the treated/strain groups. B. Phrenic long-term facilitation time course in different treated groups. Note that LPS treated rats have a reduced pLTF expression at 60 min post-hypoxia compared to the vehicle treated rats. C. 60 min post-hypoxia pLTF for time control (which did not receive any hypoxia), Sprague Dawley and Lewis rats with/without LPS. Note that LPS induces a reduction of the pLTF magnitude at 60 min in Sprague Dawley, and Lewis rat with LPS do not express pLTF (compared to a time control and baseline). a; $p < 0.05$ vs. strain-matched baseline; b; $p < 0.05$ vs. strain-matched LPS; *, $p < 0.05$ vs. time control.

Table 1

Physiological variables for anesthetized Sprague Dawley (SD) and Lewis rats during electrophysiological experiments.

Group	Body Temperature	Pao ₂	Paco ₂	pH	MAP
Baseline					
<i>Vehicle</i>	37.1 ± 0.2	248.3 ± 15.9	45.4 ± 1	7.352 ± 0.004	129.1 ± 3.5
SD <i>LPS</i>	37.4 ± 0.1	262 ± 10.2	45 ± 0.6	7.357 ± 0.008	126.6 ± 2.8
<i>Vehicle</i>	37.4 ± 0.2	303.7 ± 8.4 ^c	51.7 ± 0.8 ^c	7.33 ± 0.01	67.8 ± 3.9 ^c
LEWIS <i>LPS</i>	38 ± 0.2 ^c	277.3 ± 11.8	50.4 ± 1.7 ^c	7.331 ± 0.01	78 ± 5.7
Hypoxia					
<i>Vehicle</i>	37.1 ± 0.2	38 ± 2.6 ^a	45.7 ± 1.2	7.338 ± 0.005	104.9 ± 9.4 ^a
SD <i>LPS</i>	37.4 ± 0.1	35.5 ± 1.23 ^a	43.4 ± 0.9	7.347 ± 0.008	98.3 ± 5.5 ^a
<i>Vehicle</i>	37.3 ± 0.3	48.6 ± 2.8 ^{a,c}	51.6 ± 1.2 ^c	7.339 ± 0.009	40.3 ± 2.8 ^{a,c}
LEWIS <i>LPS</i>	37.9 ± 0.2 ^c	42.4 ± 1.6 ^{a,c}	50.9 ± 1.2 ^c	7.347 ± 0.01	58.8 ± 6.4 ^{a,b,c}
15 min					
<i>Vehicle</i>	37.2 ± 0.2	185.1 ± 16.2 ^a	45.8 ± 0.8	7.334 ± 0.009	129.1 ± 2.5
SD <i>LPS</i>	37.6 ± 0.1	203.3 ± 10.9 ^a	44.8 ± 0.9	7.344 ± 0.01	126.3 ± 2.9
<i>Vehicle</i>	37.7 ± 0.3	230.4 ± 21 ^a	51.4 ± 1.5 ^c	7.318 ± 0.01	63.5 ± 5.3 ^c
LEWIS <i>LPS</i>	38 ± 0.2 ^c	238 ± 21.8	51.5 ± 1.4 ^c	7.342 ± 0.01	56.9 ± 4.3 ^{a,c}
30 min					
<i>Vehicle</i>	37.3 ± 0.2	225 ± 14.4	45.9 ± 1	7.346 ± 0.006	121.8 ± 2.8
SD <i>LPS</i>	37.6 ± 0.1	240 ± 11.1	45.5 ± 0.5	7.353 ± 0.008	113.7 ± 3.5 ^a
<i>Vehicle</i>	37.8 ± 0.2	287.1 ± 7.5 ^c	53.3 ± 1 ^c	7.327 ± 0.01	57.3 ± 3.7 ^c
LEWIS <i>LPS</i>	38.1 ± 0.2 ^c	261 ± 12.3	51.2 ± 2.1 ^c	7.352 ± 0.01	59.4 ± 6.9 ^{a,c}
60 min					
<i>Vehicle</i>	37.4 ± 0.2	234.6 ± 11.3	45.5 ± 1.2	7.352 ± 0.008	109.2 ± 3.3 ^a
SD <i>LPS</i>	37.6 ± 0.1	249.5 ± 7.5	44.7 ± 0.5	7.368 ± 0.005	103.7 ± 3.4 ^a
<i>Vehicle</i>	37.8 ± 0.3	287 ± 8.7 ^c	52.2 ± 1 ^c	7.336 ± 0.01	54.1 ± 2.6 ^{a,c}
LEWIS <i>LPS</i>	38.1 ± 0.2 ^c	263.3 ± 10.3	50.8 ± 1.7 ^c	7.361 ± 0.01	49 ± 12.3 ^{a,c}

Values are: body temperature, degrees Celsius; PaO₂, PaCO₂ and MAP, mmHg.

^a p<0.05 versus strain- and treatment-matched vehicle;

^b p<0.05 versus strain-matched baseline;

^c p<0.05 versus treatment-matched Sprague-Dawley rat.