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The effects of repeated endotoxin exposure on rat brain metabolites as measured by ex vivo ¹HMRS

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Abstract

Abnormalities in brain chemistry induced by acute or chronic treatment with LPS were studied in the rat model. Ex vivo brain metabolites were measured using proton magnetic resonance spectroscopy, whereas serum corticosterone levels were determined using radioimmunoassay. We observed increased lactate levels in all measured brain regions and decreased choline in the hypothalamus after chronic LPS treatment. Acute LPS treatment led to an elevation of corticosterone, whereas chronic LPS treatment led to attenuation of the HPA response. These findings suggest that chronic inflammation induced by LPS could lead to cell loss/dysfunction, and hence, desensitization of the HPA axis, particularly in the hypothalamus.

Keywords

Lipopolysaccharide; ¹HMRS; Lactate; Choline; Hypothalamus; Corticosterone

1. Introduction

During a bacterial infection, an endotoxin, such as lipopolysaccharide (LPS), can trigger an immune response in the host. Prior exposure to sub-lethal doses of LPS allows an animal to survive a subsequent lethal dose of the same endotoxin (Harbuz et al., 2002; West and Heagy, 2002). This phenomenon is called endotoxin tolerance and, while it has long been thought that endotoxin tolerance may be a defense mechanism, some researchers now believe that it may actually be a dysfunction of the host's immune system (Curley, 1996; Ayala et al., 2000; Varma et al., 2001; Song et al., 2002).

The hypothalamic–pituitary–adrenal (HPA) axis is activated in response to an immune challenge (Elenkov et al., 1992; Hadid et al., 1999; Beishuizen and Thijs, 2003), and is strongly activated by LPS (Beishuizen and Thijs, 2003). Corticosterone, a glucocorticoid stress hormone with anti-inflammatory effects, is released by the adrenal gland in response to activation of the HPA axis (Elenkov et al., 1992; Giovambattista et al., 1997; Hadid et al.,

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1999). Repeated exposure to an escalating dosage of LPS can result in desensitization of the HPA axis (Chautard et al., 1999; Beishuizen and Thijs, 2003), which could inhibit the anti-inflammatory processes and result in a prolonged inflammatory response.

Systemic inflammation has been linked to the pathogenesis of several neurodegenerative disorders (Harms et al., 1997; Liu et al., 2002; Barcia et al., 2004), and patient's suffering from such disorders exhibits alterations in several brain metabolites, including choline and lactate (Harms et al., 1997; Govindaraju et al., 2000). An alteration in choline indicates membrane turnover (Tong et al., 2004), and changes in lactate may reflect an oxygen deficiency in a particular tissue (LaManna et al., 1996; Govindaraju et al., 2000).

Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) are useful tools to measure changes in the brain. While MRI gives a better understanding of the morphological changes in the brain, MRS allows quantification of specific metabolites, such as choline and lactate, in the brain (Gasparovic et al., 1999; Schuhmann et al., 2003; Bo et al., 2004; Geurts et al., 2004; Jones and Waldman, 2004).

Using ex vivo proton MRS (¹H MRS), we tested the hypothesis that both acute and chronic exposure to LPS will increase lactate levels, whereas chronic LPS treatment will decrease choline in the hypothalamus (indicating cell loss). We also hypothesize that corticosterone will be elevated following acute LPS exposure and activation of the HPA axis, but attenuated after chronic LPS treatment, indicating desensitization of the HPA axis.

2. Materials and methods

2.1. Animals

Sprague–Dawley male rats (225–250 g) were obtained from Harlan Inc., Indianapolis, IN. Animals were pathogen free as confirmed by serology, bacteriology, and parasitological analyses. They were housed 2 or 3 per transparent plastic cage with chopped corn bedding in a temperature/humidity controlled environment and a 12-h light:dark cycle. They were fed a standard rat diet and water ad libitum, and were allowed a period of 5 to 7 days to adapt to the environment before any treatment was given. This animal study was conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at Seton Hall University.

2.2. LPS treatment and tissue collection

Forty rats were divided into 4 groups: Group 1, Saline/Saline (S+S); Group 2, Saline/LPS (S+L); Group 3, LPS/Saline (L+S); and Group 4, LPS/LPS (L+L). Groups 3 and 4 were treated chronically with escalating dosages of LPS administered intraperitoneally (i.p.) for a period of 10 days as follows: LPS was given twice a day, in the morning (08:00) and afternoon (17:00), starting with 250 µg/kg on Days 1 and 2, 500 µg/kg on Days 3 and 4, 1 mg/kg on Days 5 and 6, 2 mg/kg on Day 7, 4 mg/kg on Day 8, 8 mg/kg on Day 9, and finally, 16 mg/kg on Day 10. Groups 1 and 2 received injections with an identical volume of saline at the same times for 10 days. On Day 11, the animals in Groups 1 and 3 were given a single injection of saline, Group 4 animals received a single injection of 32 mg/kg LPS, and the animals in Group 2 were given a single acute dose of LPS (32 mg/kg). All the animals

were decapitated 2 h later, one animal from each group in sequence. Trunk blood was collected to prepare serum for corticosterone measurement. Brain tissue was collected and dissected over ice immediately following decapitation. All dissections were performed in the same order and timing throughout. The brain was placed ventral side up on a glass dish over an ice bed. A coronal slice was made at the level of the optic chiasm, and the hypothalamus was removed. A second coronal cut was made in the rostral half of the brain, and the olfactory bulbs and frontopolar cortex were removed. The striatum was separated from the frontal cortex. Cortical tissue was removed, and the thalamus and remaining striatum were dissected out and frozen (the rostral and caudal striatal halves were combined). As regions were removed, they were placed in liquid nitrogen, then placed in a -80°C freezer.

2.3. Acid extraction

The frozen brain regions were weighed and homogenized in five volumes of 0.04 M HClO_4 (according to their wet weight), and centrifuged at 15,000 rcf ($\times g$) at 4°C for 10 min. The supernatant was collected, and the pellets were homogenized and centrifuged again as described above. The supernatants of the two homogenizations were combined, and 3-(trimethylsilyl) propionic [1,d4] acid sodium salt (TSP) was added to a final concentration of 2.5 mM.

2.4. ^1H MRS

The brain extract (0.4 ml) was transferred to a 5 mm, series 400, 7 NMR tube (Aldrich, WI), and analyzed in a Bruker 400 MHz NMR (9T) with a 5 mm QNP probe at room temperature. The acquisition parameters were: 30° pulse, $6\text{ }\mu\text{s}$ 4100 Hz spectral width with 128 averages, and 4 s repetition time. The following metabolites were identified by ^1H chemical shift positions: *N*-acetyl-aspartate (NAA), glutamate (Glu), γ -aminobutyric acid (GABA), total choline (Cho), total creatine (Cre), lactate (LA), alanine (Ala), and myo-inositol (MI). Following Fourier transformation, phasing, and baseline correction, areas under each peak were integrated to determine the concentration of each metabolite, relative to the value of the TSP standard, taking into account the difference in tissue weight of the sample and the hydrogen atoms responsible for each peak in the spectrum.

2.5. Radioimmunoassay (RIA) for serum corticosterone levels

Serum levels of corticosterone were measured using a commercially available radioimmunoassay (RIA) kit and following the protocol provided by ICN Pharmaceuticals, Inc. (Orangeburg, NY) without modifications. In short, [^{125}I]-tracer and anti-corticosterone (in that order) were added to diluted rat serum. After vortexing the solution, centrifuging it at $1000\times g$ for 15 min, and decanting the supernatant, the precipitate was counted on a Wallace Wizard I470 Automatic Gamma Counter.

2.6. Statistical analyses

Statistical analyses of the metabolite concentrations were performed using a two-way analysis of variance (ANOVA). The metabolite of interest (e.g., lactate) was used as the dependent value, whereas the treatments (i.e., repeated, single LPS treatment) were used as independent factors. A post hoc Student's *t*-test was performed when significant effects or

differences were observed. Correlations were performed using listwise deletions and Fisher's r to z (to obtain p values), and only for those metabolites and regions that showed significant effects by ANOVA. All statistical analyses were performed with StatView for Windows 5.0.1 (SAS Institute Inc.). A p value of <0.05 was considered statistically significant.

3. Results

3.1. Lactate levels

^1HMR S allowed for an accurate analysis of many metabolites in specific sections of the brain. Fig. 1 shows a representative ^1HMR S spectrum of the hypothalamus extract. While the levels of most metabolites did not significantly change in any of the groups of animals following any LPS administration regimen (Table 1), ANOVA analysis indicated that there was a significant increase in lactate in the hypothalamus ($p=0.002$), striatum ($p<0.0001$), thalamus ($p<0.0001$), and frontal cortex ($p<0.0001$). The lactate levels observed in the hypothalamus (Fig. 2A) after the chronic treatment with LPS (L+S, L+L) were 16–18% greater than in the animals treated with either an acute dose of LPS (S+L) or saline (S+S). A significant increase in lactate (29%) was also seen in the striatum (Fig. 2B) and thalamus (Fig. 2D) in the animals receiving a single acute injection of high dose LPS (S+L) compared to the saline control group (S+S). An even greater increase in lactate (up to 29%) was observed in the striatum (Fig. 2B), thalamus (Fig. 2D), and frontal cortex (Fig. 2C) when the animals were chronically treated with LPS (L+L) compared to the animals acutely treated with LPS (S+L).

3.2. Choline levels

Choline concentrations were significantly decreased (12–13%; $p=0.008$) in the hypothalamus (Fig. 3A) of the rats chronically treated with LPS (L+S and L+L) compared to either the acutely treated animals (S+L) or the saline treated animals (S+S). However, there was no significant difference in the choline levels in the striatum (Fig. 3B), frontal cortex (Fig. 3C), or thalamus (Fig. 3D) in any of the groups of animals.

3.3. Corticosterone levels

Serum corticosterone levels were also measured to confirm the activation of the HPA axis by LPS since glucocorticoids play a crucial role during inflammation (Nadeau and Rivest, 2003). As shown in Fig. 4, there was a significant increase in serum corticosterone in the rats acutely treated with LPS (S+L) compared to the saline control (S+S). However, when the rats were chronically treated with LPS (L+S, L+L), corticosterone levels, while still increased compared to the S+S control, were significantly decreased compared to the animals given a single acute injection of LPS (S+L). There was also a significant increase in corticosterone levels in the chronic LPS treated animals receiving a high dose of LPS on Day 11 (L+L) compared to those given saline on Day 11 (L+S). Corticosterone levels were found to correlate with hypothalamic choline levels only in those animals that received the final LPS dose [S+L and L+L] ($r=0.5$; $p=0.01$). For those animals that were not endotoxin tolerant [S+S and S+L], corticosterone levels showed a positive correlation with lactate levels ($r=0.3$; $p=0.01$); however, no significant correlation was found between

corticosterone levels and lactate in the rats receiving chronic LPS [L+S and L+L] ($r=0.2$; $p=0.08$). For animals that received the final saline injection [S+S and L+S], there was also a weak positive correlation between lactate levels and corticosterone ($r=0.2$; $p=0.04$), whereas those animals that received the final LPS injection [S+L and L+L] had a negative correlation between lactate and corticosterone ($r=-0.3$; $p=0.01$).

4. Discussion

In this study, we used ex vivo ^1H MRS to evaluate the effects of chronic versus acute exposure to the bacterial endotoxin, LPS, on the brain metabolites of rats. Chronic exposure to LPS desensitizes both the HPA axis and the immune response of the body (Chautard et al., 1999; Beishuizen and Thijs, 2003). However, the differential effects of acute versus chronic LPS exposure on brain metabolites have not been determined.

We first examined several brain metabolites. *N*-acetyl aspartate (NAA) is a neuronal marker used to determine neuronal integrity or loss (Urenjak et al., 1992; Ebisu et al., 1994). Myo-inositol is thought to be involved in the maintenance and regulation of glial cell volume (Brand et al., 1993; Nagatomo et al., 1995; Rumpel et al., 2003). Creatine is involved in high energy oxidative metabolism (Miller, 1991); whereas GABA and glutamate are used to evaluate changes in neuronal and glial cell function (Govindaraju et al., 2000). Alanine, an essential amino acid, has been shown to be increased following ischemia as well as in some tumors (Smart et al., 1994; Brulatout et al., 1996). An increase in choline indicates membrane breakdown from injury or increased turnover; whereas a decrease in choline may suggest cell loss or dysfunction leading to decreased choline turnover (Castillo and Kwock, 1998; Fulham et al., 1992). Lactate is an end product of anaerobic cellular metabolism, and stationary increases in lactate are seen in pathologies that involve anaerobic conditions. More transient increases in lactate occur during neuronal activation, which may be associated with lactate's role as a neuronal energetic substrate (Giove et al., 2003).

In our study, only lactate and choline were altered as predicted following exposure to LPS. Lactate levels increased in all areas of the brain examined following chronic LPS exposure. An increase in lactate suggests increased anaerobic metabolism. However, an increase in lactate has also been associated with activation of macrophage or glial cells (Bal-Price and Brown, 2001) as well as the production of nitric oxide and the resultant inhibition of cellular respiration and cell death (Xiang et al., 2004). Recent studies demonstrated that lactate may also serve as an oxidative substrate for neurons (Bouzier-Sore et al., 2003a,b). Lactate levels in the brain can change for many reasons, including feeding in rats (Goucham and Nicolaidis, 1999) and motor movement in humans (Kuwabara et al., 1995). Produced mainly by glycolysis in astrocytes, lactate is believed to be transported to the neurons by means of a mechanism called the astrocyte-neuron lactate shuttle hypothesis (ANLSH) [Bouzier-Sore et al., 2003a; Serres et al., 2003]. While our study does not provide evidence to support this theory, an alteration in cell-cell interaction and cellular metabolism or astrocyte activation are other possible explanations for the accumulation of lactate seen in our study.

In animals given an acute LPS treatment, both lactate and corticosterone levels increased, suggesting that these two substances were serving as parallel indicators of inflammation and

potential cellular stress. However, with chronic LPS exposure, the lactate response grew stronger, while the corticosterone response weakened, suggesting that the strained metabolic state in the brain hindered the function of the HPA axis and, thus, attenuated the corticosterone response.

Considering that some brain metabolites, including lactate, may change after death, every effort was made to minimize possible postmortem effects during the collection and freezing of the brain tissue (as described in Materials and methods). We are confident that the alterations in lactate levels that we observed were not due to postmortem effects for two reasons. First, those brain regions dissected last (longest postmortem interval) did not have the highest lactate levels. Second, other metabolites that are very sensitive to postmortem intervals (i.e., alanine and GABA) were not increased as was for lactate.

Several studies have shown that LPS can disrupt the blood–brain barrier and facilitate the recruitment and accumulation of immune cells such as macrophages to the site of injury (Banks et al., 1999; Xaio et al., 2001; Singh and Jiang, 2004). The increase in lactate shown in the present study may, thus, reflect such an accumulation of macrophages in these brain regions since macrophages have been shown to be a source of elevated lactate (Petroff et al., 1992). In addition, the greater increase in lactate following chronic LPS exposure as compared to acute LPS treatment would be consistent with greater macrophage recruitment to a site of chronic inflammation.

Chronic LPS treatment resulted in a significant decrease in choline only in the hypothalamus, suggesting decreased cell membrane turnover that could be due to cell loss or dysfunction within the hypothalamus. It appears that the hypothalamus may be particularly vulnerable to the toxic effects of prolonged exposure to bacterial endotoxins or persistent inflammation. The hypothalamus is also the initiation site of the HPA axis. The increase in corticosterone levels demonstrated that the HPA axis was strongly activated by both acute and chronic exposure to LPS. However, the corticosterone response was attenuated following chronic LPS exposure. The decrease in choline in the hypothalamus and the subsequent attenuation of the corticosterone response could occur due to repeated chronic activation of the HPA axis which resulted in cellular injury or negative feedback on the HPA axis. This finding is particularly important because of the crucial role that the HPA axis plays during an inflammatory response, and supports our hypothesis that LPS-induced persistent inflammation can desensitize the HPA axis.

We expected that metabolites, other than lactate and choline, would be altered by one or both of our LPS treatment regimens. In particular, a decrease in NAA is considered as one of the primary indicators of neuronal damage or cell loss. However, studies have shown that early disease states or brain insult may initially exhibit a change in metabolites involved in inflammatory or compensatory responses; then, over time, if neuronal damage occurs, lowered NAA levels are observed (Chang et al., 1999). In the current study, even the chronic LPS treatment was relatively short-lived (only 10–11 d), which may not be long enough to detect the visible changes in NAA levels indicative of neuronal damage. Future studies that address this timing issue will be important for determining if there is a ‘‘window of

opportunity'' for successful treatment of those individuals with chronic inflammatory diseases or infections in order to avoid permanent neuronal damage.

Our results support the hypothesis that acute and chronic exposure to LPS differentially alters specific metabolites in the rat brain, and that chronic LPS exposure has a significant and possibly harmful effect on brain chemistry, resulting in desensitization of the HPA axis. This study suggests that the phenomenon known as endotoxin tolerance may be associated with alterations in brain metabolites and dysregulation of the HPA axis. This study also demonstrates that ¹HMRS is a unique technique that is useful for studying the effects of systemic infection and inflammation on brain chemistry. Certainly, further work is needed to elucidate the molecular and cellular mechanisms that underlie the detrimental effects that occur in the brain during prolonged bacterial infection and persistent inflammation.

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Fig. 1.
A representative ^1H MRS spectrum of the hypothalamus extract. The peaks for choline and lactate are indicated (arrows). TSP is the internal standard.

Fig. 2.

The effect of LPS on lactate concentration in the brain measured by ex vivo ^1H MRS. Mean (mM $\pm$ S.E.M.) lactate concentration in the hypothalamus (A), striatum (B), frontal cortex (C), and thalamus (D). S+S (control): Twice daily saline injections for 10 d followed by a single injection of saline on Day 11; S+L (Acute): Twice daily saline injections for 10 d followed by a single acute injection of 32 mg/kg LPS on Day 11; L+S (Chronic 10 d): Twice daily injections for 10 d with escalating dosages of LPS (250 $\mu\text{g}/\text{kg}$ to 16 mg/kg) followed by a single injection of saline on Day 11; L+L (Chronic 11 d): Twice daily injections for 10 d with escalating dosages of LPS (250 $\mu\text{g}/\text{kg}$ to 16 mg/kg) followed by a single injection of 32 mg/kg LPS on Day 11. $n=10$ per group. Post-hoc t -test: * $p<0.05$, ** $p<0.01$, *** $p<0.0001$ compared to S+S; # $p<0.05$, ## $p<0.01$ compared to S+L.

Fig. 3.

The effect of LPS on choline concentration in the brain measured by ex vivo ^1H MRS. Mean (mM $\pm$ S.E.M.) choline concentration in the hypothalamus (A), striatum (B), frontal cortex (C), and thalamus (D). S+S (control): Twice daily saline injections for 10 d followed by a single injection of saline on Day 11; S+L (Acute): Twice daily saline injections for 10 d followed by a single acute injection of 32 mg/kg LPS on Day 11; L+S (Chronic 10 d): Twice daily injections for 10 d with escalating dosages of LPS (250 $\mu\text{g/kg}$ to 16 mg/kg) followed by a single injection of saline on Day 11; L+L (Chronic 11 d): Twice daily injections for 10 d with escalating dosages of LPS (250 $\mu\text{g/kg}$ to 16 mg/kg) followed by a single injection of 32 mg/kg LPS on Day 11. Post-hoc t -test: * $p < 0.05$ compared to S+S, # $p < 0.05$ compared to S+L.

Fig. 4.

The effect of LPS treatment on serum corticosterone level measured by RIA. Mean (ng/ml $\pm$ S.E.M.) corticosterone concentrations in the serum. S+S (control): Twice daily saline injections for 10 d followed by a single injection of saline on Day 11; S+L (Acute): Twice daily saline injections for 10 d followed by a single acute injection of 32 mg/kg LPS on Day 11; L+S (Chronic 10d): Twice daily injections for 10 d with escalating dosages of LPS (250 μ g/kg to 16 mg/kg) followed by a single injection of saline on Day 11; L+L (Chronic 11 d): Twice daily injections for 10 d with escalating dosages of LPS (250 μ g/kg to 16 mg/kg) followed by a single injection of 32 mg/kg LPS on Day 11. Post-hoc *t*-test: ****p* < 0.0001 compared to S+S; ###*p* < 0.0001 compared to S+L; \$\$\$*p* < 0.0001 compared to L+S.

Table 1

Brain metabolite concentrations (mM $\pm$ S.E.M.) in (A) hypothalamus, (B) stratum, (C) frontal cortex, and (D) thalamus measured by ^1H MRS

Brain metabolite ^a	Chronic saline		Chronic LPS	
	Acute saline	Acute LPS	Acute saline	Acute LPS
<i>A. Hypothalamus</i>				
Lactate ^b	12.9 $\pm$ 0.4	13.7 $\pm$ 0.7	14.9 $\pm$ 0.4	15.2 $\pm$ 0.5
Choline ^c	1.8 $\pm$ 0.1	1.8 $\pm$ 0.1	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1
NAA	7.5 $\pm$ 0.3	7.6 $\pm$ 0.4	7.2 $\pm$ 0.3	7.4 $\pm$ 0.2
Glutamate	9.1 $\pm$ 0.3	8.5 $\pm$ 0.5	8.4 $\pm$ 0.4	8.9 $\pm$ 0.3
GABA	5.2 $\pm$ 0.3	5.4 $\pm$ 0.3	5.2 $\pm$ 0.2	5.2 $\pm$ 0.2
Alanine	0.8 $\pm$ 0.06	0.6 $\pm$ 0.07	0.6 $\pm$ 0.06	0.7 $\pm$ 0.07
Creatine	7.9 $\pm$ 0.3	8.4 $\pm$ 0.4	7.9 $\pm$ 0.4	8.1 $\pm$ 0.2
Myo-inositol	10.2 $\pm$ 0.6	10.5 $\pm$ 0.5	10.4 $\pm$ 0.6	10.2 $\pm$ 0.4
<i>B. Striatum</i>				
Lactate ^b	9.8 $\pm$ 0.2	10.8 $\pm$ 0.3	11.4 $\pm$ 0.2	12.6 $\pm$ 0.6
Choline ^c	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1
NAA	6.6 $\pm$ 0.1	6.8 $\pm$ 0.2	6.6 $\pm$ 0.2	6.7 $\pm$ 0.2
Glutamate	11.2 $\pm$ 0.3	11.1 $\pm$ 0.4	11.5 $\pm$ 0.5	11.3 $\pm$ 0.5
GABA	3.6 $\pm$ 0.3	4.4 $\pm$ 0.3	3.7 $\pm$ 0.3	3.9 $\pm$ 0.4
Alanine	0.9 $\pm$ 0.05	1.1 $\pm$ 0.06	1.0 $\pm$ 0.04	1.1 $\pm$ 0.06
Creatine	8.4 $\pm$ 0.2	8.9 $\pm$ 0.3	8.3 $\pm$ 0.2	8.3 $\pm$ 0.3
Myo-inositol	7.5 $\pm$ 0.5	7.3 $\pm$ 0.4	7.4 $\pm$ 0.5	7.6 $\pm$ 0.4
<i>C. Frontal cortex</i>				
Lactate ^b	11.0 $\pm$ 0.4	11.8 $\pm$ 0.4	13.1 $\pm$ 0.3	13.3 $\pm$ 0.3
Choline ^c	1.8 $\pm$ 0.1	1.9 $\pm$ 0.2	2.2 $\pm$ 0.2	2.1 $\pm$ 0.2
NAA	8.0 $\pm$ 0.3	7.7 $\pm$ 0.3	7.8 $\pm$ 0.2	7.6 $\pm$ 0.2
Glutamate	11.4 $\pm$ 0.4	11.0 $\pm$ 0.3	11.0 $\pm$ 0.2	11.1 $\pm$ 0.4
GABA	2.5 $\pm$ 0.1	2.8 $\pm$ 0.2	2.7 $\pm$ 0.3	2.2 $\pm$ 0.2
Alanine	1.1 $\pm$ 0.05	1.1 $\pm$ 0.08	1.1 $\pm$ 0.08	1.1 $\pm$ 0.06
Creatine	7.7 $\pm$ 0.4	7.7 $\pm$ 0.3	7.2 $\pm$ 0.2	7.2 $\pm$ 0.2
Myo-inositol	7.5 $\pm$ 0.4	8.1 $\pm$ 0.6	8.7 $\pm$ 0.4	8.8 $\pm$ 0.6
<i>D. Thalamus</i>				
Lactate ^b	11.2 $\pm$ 0.4	12.6 $\pm$ 0.3	13.9 $\pm$ 0.3	14.4 $\pm$ 0.5
Choline ^c	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	1.4 $\pm$ 0.2	1.2 $\pm$ 0.1
NAA	6.9 $\pm$ 0.2	7.1 $\pm$ 0.2	6.9 $\pm$ 0.2	6.9 $\pm$ 0.1
Glutamate	8.5 $\pm$ 0.4	7.7 $\pm$ 0.4	8.3 $\pm$ 0.4	7.9 $\pm$ 0.3
GABA	3.4 $\pm$ 0.2	4.0 $\pm$ 0.3	3.6 $\pm$ 0.2	3.5 $\pm$ 0.2
Alanine	0.8 $\pm$ 0.08	0.7 $\pm$ 0.06	0.8 $\pm$ 0.06	0.7 $\pm$ 0.04
Creatine	6.9 $\pm$ 0.3	7.4 $\pm$ 0.3	7.0 $\pm$ 0.2	6.9 $\pm$ 0.4

Brain metabolite ^a	Chronic saline		Chronic LPS	
	Acute saline	Acute LPS	Acute saline	Acute LPS
Myo-inositol	9.2±0.6	8.9±0.4	8.9±0.4	9.0±0.3

^aBrain metabolite values are adjusted for changes in wet tissue weight and the number of hydrogen atoms represented in the selected ¹HNMR peak.

^bANOVA analysis indicated that there was a significant increase in lactate in the hypothalamus, striatum, thalamus, and frontal cortex.

^cCholine concentrations were significantly decreased (12 – 13%; $p=0.008$) in the hypothalamus.