Chronic Inhibition of Nitric Oxide Synthase Augments the ACTH response to Exercise

Ryan Jankord, Richard M. McAllister, Venkataseshu K. Ganjam, and M. Harold Laughlin

Department of Biomedical Sciences, College of Veterinary Medicine, University of Missouri, Columbia, Missouri 65211, USA.

Running head: Nitric Oxide and the HPA response to exercise

Corresponding author: Ryan Jankord, PhD
Department of Psychiatry
University of Cincinnati
Genome Research Institute, E216
2170 East Galbraith Road
Cincinnati, OH 45237
Phone: 513-558-3029
Fax: 513-558-9104
Email: ryan.jankord@uc.edu
ABSTRACT

Exercise can activate the hypothalamo-pituitary-adrenocortical (HPA) axis and regular exercise training can impact how the HPA axis responds to stress. The mechanism by which acute exercise induces HPA activity is unclear. Therefore, the purpose of this study was to test the hypothesis that nitric oxide modulates the neuroendocrine component of the HPA axis during exercise. Female Yucatan miniature swine were treated with L-NAME to test the effect of chronic nitric oxide synthase (NOS) inhibition on the ACTH response to exercise. In addition, we test the effect of NOS inhibition on blood flow to tissues of the HPA axis and report the effects of handling and treadmill exercise on the plasma concentrations of ACTH and cortisol. Chronic NOS inhibition decreased plasma NOx levels by 44%, increased mean arterial blood pressure by 46% and increased expression of neuronal NOS in carotid arteries. Vascular conductance was decreased in the frontal cortex, the hypothalamus and the adrenal gland. Chronic NOS inhibition exaggerated the ACTH response to exercise. In contrast, chronic NOS inhibition decreased the ACTH response to restraint, suggesting that the role of NO in modulating HPA activity is stressor dependent. These results demonstrate that NOS activity modulates the response of the neuroendocrine component of the HPA axis during exercise stress.

Key Words: HPA axis, neuroendocrine, restraint, pigs, L-NAME, stress
INTRODUCTION

During the stress response, release of ACTH secretagogues from the hypothalamus initiates the activation of the hypothalamo-pituitary-adrenocortical (HPA) axis. Exercise training can influence HPA function at the level of the hypothalamus and the pituitary, thereby facilitating adaptation to the exercise stimulus (24). Interestingly, adaptation to exercise training affects the HPA axis response to non-exercise stressors (7). To provide further insight into the mechanisms by which regular exercise training can affect HPA function the manner by which acute exercise bouts stimulate neuroendocrine activity need to be established. Therefore, this study was designed to test the role of nitric oxide in the activation of the neuroendocrine component of the HPA axis during exercise.

The primary site of control for the HPA axis resides in a set of neurons within the hypothalamic paraventricular nucleus (PVN) (14). The neurons within the PVN produce corticotropin-releasing hormone (CRH) and vasopressin (VP) and are capable of integrating extrinsic and intrinsic information so that a glucocorticoid response appropriate for the stressor encountered is achieved (14). Nitric oxide (NO) has been shown to affect the activation of these PVN neurons (27, 28, 33) and, at least in rodents, both stimulatory (19, 31) and inhibitory (9, 11, 29) roles for NO in the HPA stress response have been described.

In brain, NO may be derived from any one of three different nitric oxide synthase (NOS) isoforms: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS). In addition to its role in modulating neural activity (33, 34), NO is involved in the control of cerebral vascular tone (13, 15, 21, 23, 39). In fact, NO is the major contributor to cerebral blood
flow differences in sleep-wake states (39). Within the hypothalamus, NO can modulate neuroendocrine function and has been suggested to link neuronal-glial-vascular interactions (34).

The purpose of this study was to test the hypothesis that chronic NOS inhibition augments the ACTH response to exercise in adult Yucatan swine. Although NO has previously been suggested to couple neural activity and cerebral blood flow (13, 16), studies examining the role of NO in modulating neuroendocrine activity have not assessed the effects of NO on brain vascular tone. Therefore, this study incorporates blood flow measures and hormonal output data to test the effects of NOS inhibition on the ACTH response to exercise. For interpretation of results, basal HPA activity in female Yucatan swine was established and the effects of NOS inhibition on the HPA response to another stressor, i.e., restraint, determined.
METHODS

Animals.

Female Yucatan miniature swine (Sinclair Research Farm, Columbia, MO) were used for this study. All animals were sexually mature, between 22-42 kg in weight and between 7-13 months of age. Pigs were housed in a 12:12 h light/dark cycle and maintained in accordance with standards set forth by the University of Missouri Institutional Animal Care and Use Committee. Animals were singly housed in our swine facility and fed Purina minipig chow once daily. Animals were monitored for signs of heat and were not in estrous on test dates. Two groups of animals were used for this study: 1) a group of pigs used to describe HPA axis activity in female Yucatan miniature swine; and 2) a group of pigs used to test the effects of chronic NOS inhibition on HPA activity.

We selected adult Yucatan miniature swine to test the hypothesis that chronic NOS inhibition augments the ACTH response to exercise because there are a number of important similarities between the endocrine, metabolic and cardiovascular systems of pigs and humans and because these same systems respond similarly to exercise in pigs and humans. Similar physiologic characteristics include: 1) The maximal oxygen uptake/kg body weight of pigs is similar to that of humans (1, 2, 30); 2) Pigs are a good model in which to study lipoprotein metabolism (6) and whole body metabolism (1, 2, 30); 3) Pigs redistribute cardiac output, favoring blood flow to active skeletal muscle during exercise in a manner similar to that of humans (1, 2, 30); 4) Miniature pigs exhibit cardiorespiratory adaptations to exercise training that are similar to those reported for humans (1, 2, 30, 35-38). Because of the difference in surface area to volume ratio of
small rodents versus large mammals, the relative effects of exercise on metabolism and the cardiorespiratory system is much greater in large mammals like pigs and humans. Finally, the larger body size of pigs allows for sampling of blood during exercise and of tissues in the brain for measurement of regional blood flows. For these reasons we concluded that pigs are a unique and valuable model of these experiments.

**HPA axis activity in female yucatan swine.**

To assess hormonal rhythms and test the effects of blood collection and treadmill activity on ACTH and cortisol levels, animals underwent surgery to have catheters implanted in the jugular vein and connected to a vascular access port on their back. For surgery, animals received xylazine (2 mg/kg) and ketamine (10 mg/kg) administered IM and then isofluorane-in-O2. Animals were allowed to recover at least 5 days prior to inclusion in any study.

**Hormonal rhythms.**

To assess the circadian rhythm of ACTH and cortisol in female pigs, blood samples were collected with automated blood sampling equipment (Accusampler, DiLab). Pigs were individually placed in the sampling pen (1.2 x 1.8 m) for at least 4 d prior to initiation of blood collection. Pigs were connected and sampling commenced 2 h post handling. The blood collection device was programmed to collect 1 ml of blood every hour followed by an infusion of 1 ml of saline. Collected samples were placed in tubes with EDTA and kept at 4° C during the 24 h collection. Importantly, connection of pigs to sampling equipment did not restrict the pig's movement within the pen.
Handling and treadmill exercise.

To assess the hormone response to handling, blood samples were manually collected via vascular access ports while the animal was in its pen and after moving the animal out of its pen. When pigs were moved for blood collection they were placed on a scale that prevented their movement. For treadmill activity, animals were acclimated to treadmill activity over a two week period (4 d/wk, 5-10 min/d) where treadmill speed began at a slow walk and was slowly increased to a jogging pace (8 km/h). After acclimation, animals completed one exercise session per week, for three weeks, with blood samples being collected prior to and after each exercise session (10 min of treadmill jogging at 8 km/h). In comparison to the maximal exercise test described later in this manuscript, no electrical shock was used during treadmill activity in pigs used to assess the hormone response to treadmill jogging. Blood collection to determine the effects of handling and treadmill activity occurred in the afternoon between 1300 and 1700 h.

Effects of L-NAME on blood flow and HPA axis activity.

To determine the effects of L-NAME on tissue blood flow and the hormone response to stress animals had two catheters surgically placed, one in the left atrium and one in the aorta via the internal thoracic artery. Animals in this study represent a subset of pigs from a larger study examining the effects of L-NAME on exercise responses (22). Pigs recovered from surgery for 8-10 days prior to the exercise test. Restraint blood samples were collected 4 days after surgery. In the treatment group, L-NAME was added to the drinking water (0.01%) for ≥ 4 weeks prior to inclusion in studies making L-NAME available to drink whenever they were in their cage.
Exercise and restraint.

Restraint and exercise were used to determine the effect of L-NAME on the HPA response. Both stressors were conducted in the morning between 0800 and 1000 h. For the restraint stressor, pigs were removed from their pen, picked up by their hind limbs and placed flat on their back. Several investigators were used to restrain animals by holding down the pig’s front legs, rear legs and head while blood samples were obtained via jugular venipuncture. Blood samples were collected in chilled EDTA vacutainers within 5 min of placing the animal on its back. The time of collection occurred between 2-5 min and there was not a correlation between time and ACTH or cortisol, nor was there a group difference in mean restraint time.

The exercise stressor involved incremental exercise to exhaustion. Exhaustion was defined as an inability to locomote at the prevailing speed and grade. Every 5 min throughout the protocol the treadmill speed was changed. Animals began walking at 3.2 km/h and this walking stage was repeated between 5 min stages of 4.8, 8.0, 11.3 km/h. If the pig was able to complete the 11.3 km/h stage a 5% treadmill grade was then added to the speed of 11.3 km/h. Mild electrical shock was used sparingly to encourage running for the maximal exercise test. Between running stages blood samples were withdrawn from an arterial catheter and placed in chilled EDTA vacutainers.

Blood flow determination in conscious pigs.

The determination of blood flow was accomplished with the use of radiolabeled microspheres of 15 µm diameter (Perkin Elmer) (1, 5), using methods previously described (22). Approximately 2.2 million microspheres were infused and only experimental animals with at least 400 microspheres collected in the reference blood
sample were used for analysis (3). To obtain mean arterial pressure the externalized aortic catheter was connected to a blood pressure analyzer (MicroMed). After completion of the exercise protocol, animals were sedated and anesthetized. Following euthanasia, the frontal cortex, hypothalamus, pituitary and adrenal were dissected and weighed and radioactivity was determined with a gamma counter (LKB Wallac 1282).

Blood flow was normalized to tissue mass and vascular conductance was calculated by dividing blood flow by mean arterial pressure. Blood flow to right and left kidney was measured to confirm good microsphere-blood mixing and animals with bilateral difference >15% were excluded from analysis. Anterior and posterior pituitary samples were pooled in order to assure a minimum of 200 microspheres per sample (17). Adrenal data is the combined mean values from left and right adrenals as there was no difference in blood flow between the two sides.

**Blood assays.**

All blood samples were collected in chilled EDTA containers, centrifuged and plasma was stored at -80° C until analysis. Both ACTH and cortisol were measured by chemiluminescent assays (Immulite, DPC), as previously described (18). Blood lactate was measured spectrophotometrically by the enzymatic conversion of lactate and NAD to pyruvate and NADH by bovine heart LDH (glycine, 330 mM; hydrazine, 270 mM; NAD 1.3 mM; bovine heart LDH, 200 U/ml; Sigma) (25). NO metabolite (NOx) concentration was determined using a chemiluminescent analyzer (Sievers NOA 280i, Boulder, CO). Presented mean NOx data is from the larger ongoing study since only a small number of animals in this subset had systemic NOx concentration determined (22).
mRNA analysis of NOS isoforms.

The right common carotid artery was dissected, quickly cleaned of fat and connective tissue using a dissection microscope, and slit longitudinally. Endothelium was scraped from the luminal surface of the vessel into 1.0 ml of Trizol reagent (MCR, Cincinnati, OH). Total RNA was isolated according to the vendor’s recommended protocol. The phenol chloroform extraction of RNA included a separation centrifugation step of 12,000 g at 4ºC and the observation of an undisturbed interphase was used to confirm separation of the RNA from DNA.

First-strand (i.e., cDNA) synthesis was performed on 1.0 μg isolated RNA using reverse transcriptase (200 U/μg) and oligo (dT) primers (Invitrogen, Carlsbad, CA). cDNA was amplified via polymerase chain reaction (PCR). Primers for DNA polymerase to amplify eNOS cDNA were 5’-GCC TGA ACA GCA CAG GAG TT-3’ (forward) and 5’-GCT CTT CTA GCC GTG TGT CC-3’ (reverse), designed according to GenBank accession number GI: 30794678. Primers to amplify nNOS cDNA were 5’-CAA GGG TCA AGA ACT GGG AGA CTG-3’ (forward) and 5’-GTG CTG AGA AGG AAG CAT GAC G-3’ (reverse), developed from published sequence data (32). Primers to amplify iNOS cDNA were 5’-CGT TAT GCC ACC AAC AAT GG-3’ (forward) and 5’-GAG CTG AGC GTT CCA GAC C-3’ (reverse) adapted from published data (12), GenBank accession number GI: 1777979. GAPDH cDNA was amplified with the primers 5’-TCA AGA AGG TGG TGA AGC AG-3’ (forward) and 5’-TGT CGT ACG AGG AAA TGA GC-3’ (reverse), designed according to GenBank accession number GI: 2407183. The eNOS, iNOS and nNOS primers were intron spanning and yielded products of 100-200 base pairs, a size requirement for mRNA quantification with SYBR Green real-time PCR.
technology. Melt curves performed after PCR amplification confirmed the amplification of a single, one size product.

Real-time PCR cycles (40X; Cepheid SmartCycler, Sunnyvale CA) consisted of 5 s at 95°C (denaturing), 60 s at 58°C (annealing), and 60 s at 72°C (extension). Annealing and extension times were reduced to 20 s and 30 s, respectively, for nNOS and iNOS. The reaction mixture included Brilliant SYBR Green QPCR Master Mix (dNTPs, DNA polymerase, SYBR Green; Stratagene, La Jolla, CA), MgCl₂ (2.5 mM), primers (100 nM), and 5.0 μl cDNA (diluted 5-fold in nuclease-free water). Primer concentrations were increased to 200 nM for nNOS and 300 nM (iNOS). Samples were analyzed in duplicate.

PCR standard curves were generated for the primer set of each NOS isoform, as well as for GAPDH (housekeeping gene). The amplicon of each message of interest was purified, cloned into a plasmid (TOPO TA pCR2.1; Invitrogen, Carlsbad, CA), and propagated in bacteria. Purified constructs were linearized with NcoI and quantified by spectrophotometry. The PCR cycle thresholds for serial dilutions of the constructs were used to generate standard curves for PCR amplification of 5.0 μl of cDNA from each mRNA and recorded as number of molecules per reaction. This value was normalized to the corresponding value for GAPDH.

Statistical analyses.

Data are presented as mean ± SE and analysis was completed using SigmaStat 3.1 software. One-way repeated measures ANOVA with Holm-Sidak pairwise multiple comparison method was used to determine the effect of time on plasma ACTH and cortisol. A 2-way repeated measures ANOVA was used to compare the effects of
treadmill speed and L-NAME on the ACTH and cortisol response to exercise. A $t$-test was run for other group comparisons.
RESULTS

ACTH and cortisol rhythms.
The circadian rhythm for ACTH and cortisol in Yucatan miniature swine is presented in Figure 1. During this 24 h period blood sample collection was automated so that there would be no animal handling performed during the experiment. There was a statistically significant main effect of time on ACTH and cortisol. Animals were fed only once per day at approximately 1000 h. The increase in morning ACTH and cortisol began prior to the start of the light phase.

Effects of animal handling and treadmill activity.
Compared to automated sampling, manual sampling of blood in the pen significantly increased ACTH but did not affect cortisol while removal of the animal from its pen significantly increased both hormones (Fig. 2). There was no difference in ACTH or cortisol between placing animals on the scale or on the treadmill. There was a significant increase in ACTH and cortisol during treadmill exercise.

Chronic NOS inhibition.
Table 1 presents the effects of chronic NOS inhibition on plasma NOx, mean arterial pressure and tissue blood flow. Treatment with L-NAME decreased NOx levels by 44%, increased mean arterial blood pressure by 46% and decreased blood flow to the frontal cortex and the hypothalamus. Blood flow also decreased in the pituitary and adrenal but these differences were not statistically significant. Because chronic NOS inhibition increased arterial pressure we calculated the effects of treatment on tissue vascular conductance and the results are presented in Figure 3. In response to L-NAME treatment, there was a statistically significant decrease in vascular conductance in the
frontal cortex, the hypothalamus and the adrenal gland. Since L-NAME was administered through the drinking water, water intake for all animals was measured the final week of treatment. There was no difference in water consumption between L-NAME treated pigs (2874 +/- 248 ml/d) and the control pigs (3008 +/- 969 ml/d).

To evaluate the effects of chronic NOS inhibition on vascular NOS expression we measured expression of message for eNOS, iNOS and nNOS in carotid artery endothelium. As shown in Figure 4, real time PCR analysis reveals that there is substantial expression of eNOS and nNOS while there is very little iNOS message in carotid endothelium. Also, chronic L-NAME treatment increased expression of nNOS mRNA in carotid artery endothelial cells, but did not significantly alter eNOS or iNOS message (Fig 4).

**Exercise and Restraint.**

Treadmill walking increased ACTH and cortisol, as shown in Figure 2. In a separate group of pigs we determined whether there was a dose response relationship between treadmill running speed and changes in plasma ACTH and cortisol (Fig 5). There was a significant main effect of exercise on cortisol, blood pressure and blood lactate. Statistical analysis of the data set indicates a main effect of L-NAME \( (P < 0.05) \) and a main effect of treadmill speed \( (P < 0.001) \) on the ACTH response to exercise. The interaction between L-NAME and treadmill speed on the ACTH response to exercise did not reach statistical significance \( (P = 0.081) \).

The cortisol response to exercise was not affected by L-NAME treatment (Fig 5). Chronic NOS inhibition did not alter the percentage change in blood pressure produced by treadmill exercise nor did it affect the blood lactate response to exercise. Mean Pre
exercise blood pressure data is presented in Table 1. Chronic NOS inhibition with L-NAME produced a statistically significant reduction in the ACTH response to restraint ($P = 0.01$, Fig 6), but did not affect the cortisol response to restraint.
**DISCUSSION**

The key finding of our study is that chronic NOS inhibition increased the ACTH response to exercise, indicating that nitric oxide modulates the neuroendocrine component of the HPA axis during exercise. The decrease in hypothalamic blood flow in response to chronic L-NAME treatment raises the possibility that manipulations affecting NO bioavailability may affect the neuroendocrine component of the HPA axis through changes in local blood flow.

The establishment of the basal circadian rhythm for ACTH and cortisol in female Yucatan miniature swine demonstrates the benefit of automated sampling and allows for an appreciation of the effects of handling, exercise and restraint on ACTH and cortisol. If the pig is cooperative one can enter the pen and collect blood samples with no change in cortisol and a small increase in ACTH. Frequent handling, however, would not be ideal for blood sample collection over a 24 h period. Moving the animal out of the pen induces a stress response and our results indicate that placing pigs on a treadmill induced no greater hormonal change than being placed on the scale. An increase in ACTH and cortisol in response to treadmill walking was still apparent even after a few weeks of acclimation to treadmill walking (data not shown). The large increase in ACTH in response to sub-maximal treadmill activity is greater in female pigs and did not occur in male pigs (18).

Chronic inhibition of NOS with L-NAME decreased systemic NOx levels and increased blood pressure. The increase in blood pressure would be expected to produce a change in baroreceptor activity (26), but given that L-NAME treatment lasted several weeks there was enough time for resetting of the baroreceptors to occur (20).
The decrease in vascular conductance in the frontal cortex and hypothalamus suggests that NO production helps maintain basal cerebral blood flow, consistent with previous reports (15, 21, 23, 39). Chronic NOS inhibition also appeared to result in compensatory increases in expression of nNOS in carotid artery endothelial cells (Fig 4). This adaptation may have resulted from chronically decreased NOx levels in the blood.

Our results indicate that chronic NOS inhibition augmented the ACTH response to exercise. Further, our results indicate that this effect may be specific to exercise stress since chronic NOS inhibition decreased the ACTH response to restraint. The observation that NOS inhibition resulted in increased ACTH response to exercise but decreased response to restraint suggests that NO release from NOS can serve as an inhibitor or activator of the neuroendocrine component of the HPA axis in swine. Previous work in rodents has ascribed both stimulatory (19, 31) or inhibitory (9, 11, 29) roles to NO in affecting HPA activity. A stimulatory role for NO is supported by the observation that microinfusion of NO into the PVN increases the release of ACTH (31).

The inhibitory effect of NO during prolonged stressor exposure is consistent with the hypothesis that NO in the hypothalamus plays an inhibitory role during states of increased neuronal activity (34). That is, the time difference in stressor duration between restraint (<5 min) and exercise (45 min) may partly explain the differential effects of L-NAME on ACTH response to exercise versus restraint. Interestingly, s.c. injection of L-NAME in rats initially decreased the ACTH response to electroshocks in rats during the first 5-20 min but increased the ACTH response after 30-60 min compared to controls (28), a similar time pattern to what we observed with pigs during treadmill exercise.
The use of L-NAME, a non-specific NOS inhibitor, in our study prevents us from ascribing our effects to a specific NOS isoform(s). Results from previous studies using specific NOS inhibitors demonstrate that NOS isoforms differ in their contribution to HPA regulation (8, 9) and that the contribution of different NOS isoforms to HPA regulation can change following repeated stressor exposure (4, 10). In this study, L-NAME was administered to animals for several weeks. Previous work had demonstrated that the effect of L-NAME on the ACTH response can be observed with both acute (2 min prior) and chronic (4 d) administration of L-NAME (29). In a previous study that examined the effects of NO in modulating the HPA response it was concluded that NO does not affect the ACTH response indirectly through its action as a vasoconstrictor (19). This conclusion was based on the fact that use of i.c.v. L-NAME did not alter mean arterial blood pressure. It is not surprising that local changes in brain NO bioavailability did not alter systemic blood pressure. However, a lack of change in systemic blood pressure does not necessarily indicate that vasomotor tone in brain arteries was unaffected. Indeed, our results show that L-NAME treatment does increase vasomotor tone in the hypothalamus, resulting in decreased hypothalamic blood flow. Therefore, this may be a mechanism by which NO modulates HPA axis function. This observation suggests that studies altering NO bioavailability in the hypothalamus should consider the fact that blood flow in this region may be altered when NOS synthesis is inhibited.

**PERSPECTIVES AND SIGNIFICANCE**

Our results identify NOS activity as a modulator of the HPA axis response during acute exercise. The demonstration that chronic NOS inhibition increases the ACTH
response to exercise but decreases the ACTH response to restraint suggests that NO release from NOS functions in a context-specific manner to inhibit or activate neuroendocrine activity. Thus, NO is an important modulator of the neuroendocrine component of the HPA axis during exercise in large animals.
ACKNOWLEDGEMENTS

The authors would like to thank Dave Harah, Cory Weimer, Miles Tanner, Sean Newcomer, Kevin Eklund, Ann Melloh, Pam Thorne and Robert Johnson for their assistance.

GRANTS

This research was supported by NIH Grants RR-18276, HL-36088, HL-52490 and AR-048523.

DISCLOSURES

There are no conflicts of interest to disclose.
REFERENCES


FIGURE LEGENDS

Figure 1.
Circadian rhythm for ACTH (top) and cortisol (bottom) in female pigs ($n = 4$).
There was a significant main effect of time on ACTH and cortisol and post-hoc analysis revealed significant differences between individual points (ACTH: *different from 0700 h, **different from 0500, $P < 0.05$; cortisol: *different from 1100 h, **different from 0500 and 1100 h, $P < 0.05$).

Figure 2.
Plasma ACTH (top) and cortisol (bottom) response to manual blood collection (left) and treadmill activity (right). Pen refers to samples collected while animals remained in their pen. Female pigs were removed from pen and placed on scale or treadmill. Treadmill standing is pre-exercise while treadmill jogging is after the completion of 10 min of treadmill jogging. All samples were collected via vascular access ports. Bars represent mean ± SE, *$P < 0.05$ vs. pen; **$P < 0.05$ vs. treadmill standing; $n = 10$, pen; $n = 4$, scale; $n = 12$, treadmill standing and treadmill walking.

Figure 3.
The effect of chronic NOS inhibition on tissue vascular conductance in conscious standing pigs ($n \geq 4$). L-NAME treatment significantly reduced vascular conductance in the frontal cortex, the hypothalamus and the adrenal but did not significantly change vascular conductance in the pituitary. *$P < 0.05$ vs. control.
Figure 4.

Effects of chronic NOS inhibition on eNOS (endothelial), iNOS (inducible) and nNOS (neuronal) mRNA message in carotid artery endothelial cells. Results are expressed as number of NOS mRNA molecules per molecules of GAPDH mRNA as outlined in text. *$P < 0.05$ versus control; $n = 7$ per group.

Figure 5.

The effect of chronic NOS inhibition on the ACTH (A), cortisol (B), blood pressure (C) and blood lactate (D) response to treadmill exercise. Black filled bars represent control animals and open bars represent L-NAME treated animals. Blood samples were collected prior to initiation of exercise (Pre), during the exercise test upon completion of 5 min stages (4.8, 8 and 11.3 km/h) and once exhaustion was reached (Max). There was a significant main effect of exercise on ACTH, cortisol, blood pressure and blood lactate. There was a significant main effect of L-NAME on the ACTH response to exercise (*$P < 0.05$; $n \geq 7$ per group). There was not a significant main effect of L-NAME on the cortisol, blood pressure or blood lactate response to exercise.

Figure 6.

The effect of chronic NOS inhibition on the plasma ACTH (top) and cortisol (bottom) response to restraint. Animals were removed from pen, placed on back and blood samples were collected within 5 min. Data displayed as mean ± SE, *$P < 0.01$ for L-NAME ($n = 11$) vs. control ($n = 8$).
Table 1. Effects of chronic NOS inhibition on plasma nitric oxide, mean arterial pressure and tissue blood flow in pigs.

<table>
<thead>
<tr>
<th></th>
<th>NOx†</th>
<th>MAP</th>
<th>Frontal Cortex</th>
<th>Hypothalamus</th>
<th>Pituitary</th>
<th>Adrenal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>µM</td>
<td>mmHg</td>
<td>ml/min/100g</td>
<td>ml/min/100g</td>
<td>ml/min/100g</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>12.2 ± 1.9</td>
<td>81 ± 5</td>
<td>126 ± 17</td>
<td>96 ± 4</td>
<td>247 ± 67</td>
</tr>
<tr>
<td>L-NAME</td>
<td>6</td>
<td>6.8 ± 0.7*</td>
<td>119 ± 8*</td>
<td>83 ± 9*</td>
<td>63 ± 5*</td>
<td>192 ± 26</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure. *P < 0.05 vs. control. †NOx, nitric oxide metabolite (n = 19, control; n = 14, L-NAME). Values presented as mean ± SE.
Frontal Cortex

Control L-NAME

Vascular Conductance (ml/min/100g/mmHg)

0.0
0.5
1.0
1.5
2.0

Control L-NAME

Hypothalamus

Control L-NAME

Vascular Conductance (ml/min/100g/mmHg)

0.0
0.4
0.8
1.2
1.6

Control L-NAME

Pituitary

Control L-NAME

Vascular Conductance (ml/min/100g/mmHg)

0.0
1.0
2.0
3.0
4.0

Control L-NAME

Adrenal

Control L-NAME

Vascular Conductance (ml/min/100g/mmHg)

0.0
1.5
3.0
4.5
6.0

Control L-NAME