Pressor reflex evoked by static muscle contraction: role of nitric oxide in the dorsal horn

L. BRITT WILSON, JOHN ENGBRETSON, AND ANGELA D. CREWS
Department of Physiology, University of South Alabama
College of Medicine, Mobile, Alabama 36688

Wilson, L. Brit, John Engbretson, and Angela D. Crews. Pressor reflex evoked by static muscle contraction: role of nitric oxide in the dorsal horn. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1639–R1646, 1999.—In this study, we tested the hypothesis that nitric oxide (NO) production in the dorsal horn is involved in producing the pressor reflex elicited by static contraction of skeletal muscle. Cats were anesthetized with α-chloralose (80 mg/kg) and urethane (100 mg/kg), and a laminectomy was performed. With the exception of the L1 dorsal root, the dorsal and ventral roots from L3 to S2 were sectioned on one side and static contraction of the ipsilateral triceps surae muscle was evoked by electrically stimulating the peripheral ends of the L7 and S1 ventral roots. Dialysis of the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 50 mmol/l syringe concentration, based upon dose-response data) into the dorsal horn at L5 and S1 failed to attenuate the peak change in mean arterial pressure (MAP) evoked by static contraction (ΔMAP in mmHg: 57 ± 5 before and 50 ± 6 after 2 h of L-NAME). However, this dialysis of L-NAME reduced the magnitude of the initial pressor response as the MAP at 10 s of the contraction fell from 27 ± 4 to 17 ± 4 mmHg. On the other hand, 2 h of L-arginine dialysis (50 mmol/l) shifted the curve representing the time course of the pressor response upward and increased the peak pressor response to static contraction from 51 ± 9 to 68 ± 9 mmHg. A 2-h dialysis of D-NAME (50 mmol/l), the inactive enantiomer of L-NAME, had no effect on the time course or the peak pressor response (ΔMAP in mmHg: 78 ± 12 before and 72 ± 15 after). These data suggest that NO production in the dorsal horn has a modulatory influence on the pressor reflex evoked by static contraction of skeletal muscle and that increasing the level of NO in the dorsal horn enhances the excitability of dorsal horn cells to muscle afferent input.

cats; exercise pressor reflex; excitatory amino acids; spinal cord; muscle afferent neurons; blood pressure

Previous studies have shown that static contraction of skeletal muscle can reflexly increase heart rate (HR) and mean arterial blood pressure (MAP), and these changes are commonly called “the exercise pressor reflex” or “the pressor reflex” (4, 12, 27). The increases in cardiovascular function associated with this reflex are mediated by a contraction-induced activation of group III and IV muscle afferent fibers (12). These afferent fibers respond to the mechanical and metabolic changes that occur within the contracting muscle (9, 10). Most, if not all, of these muscle afferent neurons synapse in the dorsal horn of the spinal cord (18). Thus the dorsal horn of the spinal cord serves as the site of the first synapse for the reflex pressor response evoked by static contraction of skeletal muscle (27).

N-methyl-D-aspartic acid (NMDA) receptors are found on neurons throughout the central nervous system including the dorsal horn of the spinal cord. Recently, Adreani et al. (1) showed that intrathecal blockade of NMDA receptors blunts the pressor reflex evoked by static contraction of skeletal muscle. We have demonstrated that administration of an NMDA antagonist directly into the dorsal horn, via microdialysis, attenuates the pressor reflex (7, 26). This attenuation occurred over several spinal segments, despite limiting the afferent input mediating the pressor reflex to a single segment. Furthermore, NMDA receptor blockade diminished the curve depicting the time course of the pressor response, particularly the initial (first 10 s) change in MAP (7, 26). Thus the pressor reflex evoked by static contraction is partially dependent on activation of NMDA receptors in the dorsal horn of the spinal cord.

The NMDA receptor is unique in that it is both ligand gated and voltage gated (32). The endogenous ligands for the NMDA receptor are the excitatory amino acids glutamate and aspartate (32). Activation of the NMDA receptor opens an ion channel that principally increases calcium flux into the cell (32). In turn, the increased intracellular calcium can stimulate second messenger systems that can modulate the excitability of the cell (13). One such second messenger is nitric oxide (NO) (13, 16, 24). NO is produced when L-arginine is converted to L-citrulline via nitric oxide synthase (NOS), an enzyme that is located in the superficial dorsal horn (2). Current evidence suggests that the influx of Ca2+, the result of NMDA receptor stimulation, activates NOS, thereby liberating NO (13, 16, 24). Thus NO may be one of the cellular mechanisms mediating NMDA-induced effects. Because NMDA receptor activation in the dorsal horn partially mediates the pressor reflex (1, 7, 26), we hypothesized that NO production in the dorsal horn is also involved in producing this reflex.

The purpose of this study was to test this hypothesis. Specifically, we recorded the cardiovascular responses to a static contraction before and during the dialysis of the NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME). In addition, we tested the effect of L-arginine dialysis into the dorsal horn. Because L-arginine is the substrate for NO production, it was presumed that the production of NO would increase if substrate availability was increased.
METHODS
Surgical preparation. Adult cats of either sex (mean body wt of 3.0 ± 0.2 kg) were anesthetized by inhaling an isoflurane (5%)-oxygen (1-3 l/min) mixture. Polyethylene catheters were inserted into an external jugular vein and a common carotid artery. The inhalation anesthetic was removed, and anesthesia was maintained by an intravenous injection of α-chloralose (80 mg/kg) and urethane (100 mg/kg). The trachea was exposed, and an endotracheal tube was inserted into the airway. If a corneal reflex appeared, or HR and/or blood pressure increased, then additional α-chloralose (5–10 mg/kg) was provided. The animals were mechanically ventilated (MDI). Arterial blood gases were periodically measured and maintained within normal limits (pH 7.35 to 7.40; PCO2, 35 to 40 Torr; PO2, >80 Torr) by adjusting the ventilator, administering sodium bicarbonate intravenously, and/or providing supplemental oxygen. Body temperature was continuously monitored using a rectal probe (Yellow Springs Instrument, series 400) and maintained between 36.0–38.0°C by a heating pad and a heat lamp.

A laminectomy was performed, exposing the spinal cord, and the animal was placed in a spinal unit (Kopf) to stabilize the vertebral column. The dura of the spinal cord was opened, allowing visual identification of the L5–S2 spinal roots. With the exception of the L7 dorsal root, the L5–S2 spinal (dorsal and ventral) roots were unilaterally severed. A cavern was formed around the exposed neural and muscular tissue by suturing skin flaps to brass bars, and this was filled with warm (37°C) mineral oil. The knee joint of the hindlimb containing the triceps surae muscle, ipsilateral to the cut spinal roots, was secured by attaching the patellar tendon to a post. Muscle tension was determined by cutting the calcaneal bone and attaching the Achilles tendon to a force transducer (Grass model FT10). Because the positions of the force transducer and the knee were fixed, all muscle contractions were isometric.

For these experiments, microdialysis was used to deliver agents to the dorsal horn region. The microdialysis probe (Bioanalytical Systems model CMA-10) has a diameter of 500 µm at the membrane, which is 3 mm in length. A microdialysis probe was inserted into the dorsal horn at L5 a minimum of 4 mm from the most rostral aspect of L7. Likewise, a second probe was inserted into the dorsal horn at S1 a minimum of 4 mm from the most caudal aspect of L7. This placement of the probes ensures against spillover into the L7 dorsal horn when dialyzing into L5 and S1 (14, 20). The probes were inserted so that the entire membrane was submerged in spinal cord tissue. Because it is hypothesized that activation of NMDA receptors is the signal, or a least one signal, causing NO production to increase during static contraction, the L5 and S1 sites were chosen, because we have previously shown that blockade of NMDA receptors in these regions attenuates the pressor reflex (7). Furthermore, this previous work was performed using the same experimental preparation that is described for these studies. For all experiments, the probes were continuously perfused (5 µl/min) with an artificial extracellular fluid containing 0.2% bovine serum albumin, 0.1% bacitracin, and the following ions (in mM): 6.2 K+, 134 Cl−, 2.4 Ca2+, 150 Na+, 1.3 P−, 13 HCO3−, and 1.3 Mg2+. This solution was made fresh for each experiment. After inserting the probes, the cat was allowed to stabilize for at least 2 h.

For all of the following protocols, contraction of the triceps surae muscle was induced by electrically stimulating the peripheral ends of the cut L7 and S1 ventral roots at two to three times the motor threshold, 40 Hz, and 0.1-ms duration for 1 min. Prior to each contraction, the resting tension on the muscle was set at 1 kg, which represents L0 (optimal length) in this preparation (7, 8, 14, 20). Because the L5–S2 spinal (dorsal and ventral) roots were unilaterally severed, with the exception of the L7 dorsal root, all of the afferent information mediating the pressor reflex from the triceps surae muscle entered the spinal cord via the L7 dorsal root (4, 12, 29).

Protocol I: Dose response of L-NAME. After a minimum of two contractions of the triceps surae muscle in which the pressor reflex was reproducible, doses of 0.1 mmol/l, 1.0 mmol/l, 10 mmol/l, and 50 mmol/l of the NOS inhibitor, L-NAME, were microdialyzed sequentially into the dorsal horn at L5 and S1 (n = 5). Each dose was dialyzed for ~1 h, at which time the muscle was contracted while recording the cardiovascular changes.

Protocol II: Single dose of L-NAME. Because the aforementioned dose-response protocol is rather long, our priori design is to test a single dose, based upon the dose-response curve, using a separate group of cats, thereby minimizing any possible confounding effect of time and/or drug accumulation. Thus protocol II was designed to determine the effect of dialyzing 50 mmol/l L-NAME into the dorsal horn at L5 and S1 on the reflex cardiovascular responses to static contraction (n = 7). When at least two reproducible responses to contraction were obtained, the dialysis of L-NAME was initiated. After ~1 h of this dialysis, the reflex responses to static contraction were determined. The contraction was repeated after L-NAME had been dialyzed for ~2 h. Next, the contraction was repeated after a 20% lidocaine solution had been dialyzed for ~1 h. This was done as a functional test for placement of the probe. The data from a given animal were excluded if the pressor response to static contraction was not reduced by at least 50% relative to control.

Protocol III: Single dose of D-NAME. The purpose of this protocol was to ensure that any alteration in the pressor reflex during L-NAME dialysis was not the result of a nonspecific action of L-NAME and/or an effect of time. To test this, the protocol described in the preceding paragraph (protocol II, single dose of L-NAME) was performed, except D-NAME (50 mmol/l) was dialyzed into the dorsal horn of the L5 and S1 spinal segments of four cats. D-NAME is the inactive enantiomer of L-NAME, and 50 mmol/l was chosen because it is equimolar to the L-NAME concentration.

Protocol IV: Single dose of L-arginine. The protocol described above (protocol II, single dose of L-NAME) was repeated, except an equimolar dose of L-arginine (50 mmol/l) was dialyzed into the dorsal horn of the L5 and S1 spinal segments of seven cats. L-Arginine is the substrate for NOS.

Measured and calculated variables. Arterial blood pressure was measured by connecting the carotid artery catheter to a pressure transducer (Statham model P23ID), and muscle tension was measured using the force transducer. The arterial blood pressure and tension variables were continuously monitored on a four-channel chart recorder (Astromed model 7400) and a personal computer (Biopac acquisition system). The data were analyzed off-line using the acquisition software. MAP and HR were obtained from the arterial blood pressure signal. Baseline values were determined by averaging at least 30 s of data immediately prior to muscle contraction. Peak values represent the peak level that each variable attained during the 1 min of the muscle perturbation, regardless of when it occurred during the contraction. The "peak change" or "maximal change" in a given variable represents the difference in the peak and baseline values. Time course MAP data were measured as the level of MAP at 10-s intervals from the onset to the end of the contraction. To help protect against inconsistencies associated with blood pressure oscillations, MAP values at the 10-s intervals represent...
the mean of the MAP signal beginning 1 s before through 1 s after the 10-s time point. For example, MAP at 10 s represents the mean value of blood pressure from 9–11 s from the onset of the contraction; MAP at 20 s represents the mean value of blood pressure from 19–21 s from the onset of the contraction, etc.

Data analysis. Data are expressed as means ± SE. The time course of the pressor response was determined as the change in MAP at 10-s time points, compared with baseline, during the 1-min contraction and was done for control (predrug), 1 h, and 2 h of drug dialysis. The time course data were analyzed using a three-way ANOVA, with the three factors being contraction time (0–60 s at 10-s intervals), duration of dialysis (control, 1 h, and 2 h), and animal (considered a random effect variable) (31). The three-way ANOVA was also used to compare baseline and peak hemodynamic and tension data. The peak changes in MAP, HR, and tension produced by the contraction were analyzed using a two-way ANOVA, with the factors being the change in the variable and animal. Tukey’s test was performed when a significant F value was found. For all analyses, P < 0.05 was used as the level of statistical significance.

RESULTS

Protocol I: Dose response of L-NAME. The effects of microdialyzing increasing doses of L-NAME into the dorsal horn for ~1 h on the pressor response to static contraction are shown in Fig. 1A. Although there was a trend for the two highest doses (10 and 50 mmol/l) to reduce the peak pressor response, it failed to reach statistical significance. On the other hand, there was a dose-dependent reduction in the magnitude of the initial (first 10 s) MAP changes (Fig. 1B). This reduction in the initial MAP changes, but not the peak change, is similar to our recent results for NMDA blockade in the L6 dorsal horn (26). The peak tensions developed at each dose were not statistically different (in kg: control = 11.8 ± 1.2; 0.1 mmol/l = 12.9 ± 0.8; 1.0 mmol/l = 13.2 ± 0.6; 10 mmol/l = 12.2 ± 1.4; and 50 mmol/l = 12.9 ± 0.7).

Protocol II: Single dose of L-NAME. On the basis of aforementioned dose response data, we tested the effect of microdialyzing 50 mmol/l L-NAME into the dorsal horn on the reflex cardiovascular responses elicited by static contraction. Table 1 denotes the baseline, peak, and peak change in MAP, HR, and tension before and during the dialysis of L-NAME. Prior to the administration of L-NAME, static contraction increased MAP and HR, and these increases were unaffected after 1 and 2 h of L-NAME. Also, there were no differences in the baseline hemodynamic and tension data across the various time points.

Although L-NAME had no effect on the peak changes in MAP and HR evoked by static contraction, it did suppress the curve depicting the time course of the pressor response, particularly the initial phase of the response. An original record from one cat depicting this reduction is shown in Fig. 2. Note in Fig. 2 that the rate at which MAP rises is blunted after administration of L-NAME. The mean data for the time course of the changes in MAP during the static contraction are shown in Fig. 3A. Statistical analysis of these data showed a significant main effect for time (0–60 s, P = 0.0001), indicating that MAP increased during the 1-min static contraction. Also, there was a significant main effect for duration of drug dialysis (control, 1 h, and 2 h; P = 0.0289) and a significant interaction (P = 0.0420) between time and duration. There was a statistically significant reduction, compared with control, for the 10–40 s time points after 2 h of L-NAME dialysis. Thus L-NAME caused a downward shift in the curve representing the time course of the pressor response to static contraction. Figure 3B illustrates the time course for developed tension. There was a significant main effect for time (P < 0.0001), but not duration of drug (P = 0.7094) or interaction (P = 0.9263). Thus it is unlikely that the suppression of the curve depicting the time course of the pressor response was due to reduced developed tension.

Protocol III: Single dose of D-NAME. The baseline, peak, and peak change in MAP, HR, and tension before and during the dialysis of D-NAME are provided in Table 1. Similar to L-NAME, there were no differences in the baseline, peak, or peak changes in the hemodynamic and tension data across the duration of dialysis time points. However, in contrast to L-NAME, D-NAME
had no effect on the time course of the pressor response to static contraction (Fig. 4A). There was a significant main effect for time ($P < 0.0001$), but not duration ($P = 0.4778$) or interaction ($P = 0.8935$). The time course for developed tension was also unaltered during d-NAME dialysis, and the statistical analysis showed a significant effect of time ($P < 0.0001$), but not duration ($P = 0.4181$) or interaction ($P = 0.7058$). Thus dialysis of d-NAME had no effect on the pressor reflex.

Protocol IV: Single dose of L-arginine. If static contraction of skeletal muscle stimulates NOS, then providing more substrate may increase the amount of NO produced. To test this, we evoked a pressor reflex before and during the dialysis of 50 mmol/l L-arginine into the dorsal horn. Prior to L-arginine dialysis, static contraction increased MAP by 51 ± 9 mmHg and HR by 18 ± 4 beats/min (Table 1). These contraction-induced changes in MAP and HR were significantly accentuated to 68 ± 9 mmHg and 25 ± 5 beats/min after 2 h of L-arginine dialysis, but not after 1 h (63 ± 7 mmHg and 23 ± 4 beats/min, respectively). An example of an L-arginine-induced enhancement of the pressor reflex from one cat is illustrated in Fig. 5. There was no difference in

Table 1. Hemodynamic and tension data in response to static contraction before and during L-NAME, d-NAME, or L-Arg dialysis into the dorsal horn

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 h</th>
<th>2 h</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Peak</td>
<td>Peak Δ</td>
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<tr>
<td>L-NAME (n = 7)</td>
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<tr>
<td>MAP, mmHg</td>
<td>88 ± 5</td>
<td>144 ± 7†</td>
<td>57 ± 5</td>
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<tr>
<td>HR, beats/min</td>
<td>208 ± 13†</td>
<td>24 ± 5</td>
<td>208 ± 14</td>
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<tr>
<td>T, kg</td>
<td>3.1 ± 0.3</td>
<td>12.5 ± 0.9</td>
<td>11.5 ± 0.9</td>
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<tr>
<td>d-NAME (n = 4)</td>
<td></td>
<td></td>
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<tr>
<td>MAP, mmHg</td>
<td>101 ± 14</td>
<td>180 ± 13†</td>
<td>78 ± 12</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>203 ± 7</td>
<td>230 ± 8†</td>
<td>23 ± 3</td>
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<tr>
<td>T, kg</td>
<td>1.0 ± 0.1</td>
<td>9.0 ± 1.7</td>
<td>9.0 ± 2.1</td>
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<tr>
<td>L-Arg (n = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>84 ± 4</td>
<td>135 ± 9†</td>
<td>51 ± 9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>180 ± 7</td>
<td>198 ± 8†</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>T, kg</td>
<td>1.0 ± 0.2</td>
<td>12.1 ± 0.2</td>
<td>11.9 ± 0.1</td>
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Values are means ± SE; n = no. of experiments. MAP, mean arterial pressure; HR, heart rate; T, tension; L- and d-NAME, N(Ο)-nitro-L(or D)-arginine methyl ester. * $P < 0.05$ compared with corresponding control value. † $P < 0.05$ baseline vs. peak. ‡ $P < 0.05$ compared with control.

Fig. 2. Original record from one cat showing changes in MAP and tension in response to a static contraction before (A) and after (B) 1 h of L-NAME (50 mmol/l) dialysis into the dorsal horn. Note that the rate of rise in MAP (first 20 s) is less after L-NAME.

Fig. 3. Time course of changes in MAP (A) and tension (B) evoked by static contraction of the triceps surae muscle before and during dialysis of 50 mmol/l L-NAME into the dorsal horn. † $P < 0.05$, 2 h compared with control; n = 7.
baseline MAP across the time points for duration of dialysis, but there was a rise in the baseline HR (Table 1).

Similar to the peak changes, the curve representing the time course of the pressor response was enhanced by L-arginine (Fig. 6). Statistical analysis showed significant main effects for time ($P < 0.0001$) and drug duration ($P = 0.0137$), and there was a significant interaction ($P < 0.0001$) for time and drug duration. Post hoc analysis revealed that the 20–60 s time points for both 1 h and 2 h were significantly elevated compared with control. This occurred despite a reduction in the 10-s time point in developed tension at 2 h (Fig. 6, interaction $P < 0.0001$). Thus dialysis of L-arginine into the dorsal horn accentuated the pressor reflex evoked by static contraction of skeletal muscle.

Fig. 4. Time course of changes in MAP (A) and tension (B) evoked by static contraction of the triceps surae muscle before and during dialysis of 50 mmol/l D-NAME, the inactive isomer of L-NAME, into the dorsal horn; $n = 4$.

Fig. 6. Time course of changes in MAP (A) and tension (B) evoked by static contraction of the triceps surae muscle before and during dialysis of 50 mmol/l L-arginine into the dorsal horn. *$P < 0.05$, 1 h compared with control. †$P < 0.05$, 2 h compared with control; $n = 7$.

Fig. 5. Original record from one cat showing the cardiovascular responses and tension produced by static contraction before (A) and −1 h after (B) dialysis of 50 mmol/l L-arginine into the dorsal horn. Administration of L-arginine into the dorsal horn enhanced the reflex cardiovascular increases.
DISCUSSION

The purpose of this study was to examine the possibility that NO production in the dorsal horn plays a role in producing the pressor reflex evoked by static contraction of skeletal muscle. The results of this study showed that inhibition of dorsal horn NOS by L-NAME diminished the curve depicting the time course of the pressor response, but had minimal effects on the peak changes in MAP and HR. It is unlikely that this effect on the MAP changes is related to time and/or a nonspecific action of L-NAME, since dialysis of an equimolar concentration of L-NAME (for the same amount of time) failed to alter the pressor response. Furthermore, developed tension was the same before and during the dialysis of L-NAME, indicating that the reduction in the curve representing the time course of the pressor response was not due to less activation of muscle afferents. These results suggest that NO production in the dorsal horn is involved in producing the blood pressure changes evoked by static contraction of skeletal muscle. In addition, administering L-arginine into the dorsal horn, and presumably increasing NO production, enhanced the pressor and tachycardic responses elicited by muscle contraction. This suggests that enhancing NO production in the dorsal horn increases the excitability of dorsal horn cells to muscle afferent input, which in turn accentuates the contraction-induced rise in cardiovascular function.

NOS, the enzyme responsible for producing NO, is found in cells within the dorsal horn of the spinal cord (2). Although the precise signal for dorsal horn NO production during a static contraction is unknown, one likely candidate is activation of NMDA receptors. Several studies have provided evidence that NMDA stimulation in neural tissue increases NO in the dorsal horn (13, 16, 24). Also, the NMDA-induced excitation of dorsal horn cells is attenuated by NOS inhibitors (3, 21). Activation of NMDA receptors in the dorsal horn is involved in producing the pressor reflex evoked by static contraction (1, 7, 26). Thus stimulation of NMDA receptors within the dorsal horn by muscle afferents may be the signal that increases NO production during a static muscle contraction.

NMDA receptors have also been implicated in causing long-term increases in dorsal horn cell excitability that are characteristic of hyperalgesic states (13, 16, 23). For example, NMDA receptor blockade reduces the hyperexcitability of dorsal horn neurons induced by peripheral inflammation (19). In addition, activation of spinal NMDA receptors leads to thermal hyperalgesia (11). Because of the connection between NMDA receptors and NO production, NO has been implicated in producing hyperexcitability of dorsal horn cells in a variety of hyperalgesic models (11, 13, 16, 17, 25). Recently, Wu et al. (30) showed that an intradermal injection of capsaicin, an intervention that produces hyperexcitability of dorsal horn neurons, causes NO release in the dorsal horn. Thus the NMDA-NO cascade appears to be involved in producing a hyperexcitable state in dorsal horn neurons.

The hyperexcitability of dorsal horn neurons described in the preceding paragraph is typically slow to develop (minutes) and has a long duration (hours to days; see Refs. 11, 13, 16, and 30). However, stimulating afferent neurons with a brief train of repetitive stimuli at amplitudes sufficient to activate unmyelinated fibers results in hyperexcitability of dorsal horn cells (5, 6). This enhanced excitability, termed "windup," occurs very rapidly and previous work suggests that an NMDA-NO cascade is involved producing this phenomenon (5, 6, 13, 16). Static contraction of skeletal muscle causes a repetitive activation of muscle afferent neurons, some of which are unmyelinated (9, 10), and thus may induce rapid changes in the excitability of dorsal horn cells via activation of an NMDA-NO cascade, i.e., windup or something analogous. In turn, this alteration in dorsal horn excitability may play an important role in the time course and/or the peak increases in MAP evoked by static contraction of skeletal muscle. Administration of L-NAME diminished the rate of rise in MAP, suggesting a reduced excitation of dorsal horn cells to the initial volley of muscle afferent input. Administration of L-arginine, the substrate for NO, shifted the curve representing the time course of the pressor response upward and enhanced the peak cardiovascular changes. Mellor et al. (15) showed that the spinal administration of L-arginine causes a rapid facilitation of sensory transmission. Considered together, these data suggest that NO increases the excitability of dorsal horn cells to peripheral input and that this alteration can occur rapidly and produce functional responses, e.g., changes in cardiovascular function.

If indeed the contraction-evoked activation of NOS occurs as a result of NMDA receptor activation, then the results of the current study suggest that the role of NMDA in producing the pressor reflex is not solely dependent upon this pathway. Blockade of NMDA receptors in the dorsal horn shifts the curve depicting the time course of the pressor response downward and decreases the peak changes in MAP and HR (1, 7, 26). If NMDA receptor activation exerts its action via NO production only, then we would expect that L-NAME would attenuate the pressor reflex in a manner that is qualitatively similar to NMDA receptor blockade. However, this did not occur, suggesting that NMDA receptor activation in the dorsal horn increases cardiovascular function via a mechanism(s) that is at least in part independent of NO production. Direct depolarization of neuronal cell membranes is the most likely alternate action (32). On the other hand, activation of other receptor systems can stimulate NO production. Previous work suggests that NO is a component of the actions evoked by activation of non-NMDA or neurokinin (NK)-1 receptors (3, 21). Since these receptors are also involved in producing the pressor reflex at the level of the dorsal horn (8, 28), we cannot discount their possible involvement in causing NOS stimulation. However, similar to NMDA blockade, inhibition of non-NMDA or NK-1 receptors decreases the peak cardiovascular responses, indicating that enhanced NO.
production is not the sole mechanism by which these receptor systems operate as it pertains to the pressor reflex.

The contractions evoked in the current study are maximal or close to maximal. As a result, we would expect maximal activation of muscle afferent neurons (9, 10). Although L-NAME reduced the curve depicting the time course of the pressor response, it failed to attenuate the peak MAP and HR changes. This suggests that high-intensity muscular work increases NO production in the dorsal horn, but NO has only a modulatory influence on the cardiovascular responses and is thus not required for peak expression of the reflex. Further work is necessary to determine whether NO is involved in evoking cardiovascular responses produced by contractions of less intensity. On the other hand, administration of L-arginine increased the peak MAP and HR responses. This suggests that NO production is increased by providing more substrate and that NO increases the excitability of dorsal horn cells to muscle afferent input. These results also suggest that NO production during a static contraction is substrate limited. It is possible that during control conditions, the contraction induces a rapid increase in NO production, which in turn is partially responsible for the rapid rise in MAP. However, the elevated NO production quickly wanes (10–20 s after the onset of tension development) because of a lack of substrate, even though the muscle is still contracted. As this occurs, the rate of rise in MAP diminishes, eventually plateauing or decreasing slightly (see Figs. 3, 4, and 6). Preventing this loss of substrate allows blood pressure to rise to a higher level, e.g., L-arginine data (Fig. 6), because NO production does not fall. Without this increase in NO production, e.g., L-NAME data (Fig. 2), MAP still rises to about the same peak level, it just takes longer to get there. If so, then NO appears to play a very intricate and interesting role in modulating dorsal horn excitability and thus the output of a multisynaptic reflex.

Alternatively, we cannot dismiss the possibility that L-NAME failed to attenuate the peak cardiovascular responses because an insufficient amount of spinal tissue was exposed to the drug. In the current study, the afferent input mediating the pressor reflex was limited to the L7 dorsal root. However, we have shown that despite limiting the afferent input to L7, transduction of the pressor reflex occurs over at least three (L6–S1) spinal segments (7, 8, 26, 28). In the current study, we dialyzed L-NAME into the dorsal horn at L6 and S1, regions in which blockade of NMDA, non-NMDA, or NK-1 receptors attenuates the peak pressor and tachycardic responses (7, 8, 28). Furthermore, dialysis of an equimolar concentration of L-arginine into these sites enhanced the pressor reflex, and a previous study suggests that the EC50 values for L-arginine and L-NAME are similar (22). Thus it seems unlikely that an inadequate amount of spinal tissue was exposed to L-NAME. Nevertheless, further work is needed to test this possibility.

In summary, the results of the current study provide evidence that static contraction of skeletal muscle increases NO production in the dorsal horn of the spinal cord. This increased NO production appears to have an excitatory influence on the rate at which MAP rises in response to this static contraction. We have also shown that administering L-arginine, which presumably increased NO production, enhanced the reflex cardiovascular responses to static contraction. Considered together, the results of this study suggest that NO production in the dorsal horn is involved in evoking the pressor reflex and support the hypothesis that NO modulates the excitability of dorsal horn cells to muscle afferent input.

Perspectives

The reflex increases in MAP and HR evoked by static contraction of skeletal muscle represent the integrated, end-organ responses to changes in the activity of the autonomic nervous system. This somato-autonomic coupling is initiated by activation of muscle afferent neurons that synapse on cells in the dorsal horn of the spinal cord. There is considerable evidence to indicate that the responsiveness of dorsal horn cells to peripheral input exhibits plasticity, i.e., it is not fixed but can undergo changes. It follows then, that the autonomic changes, and thus the resultant cardiovascular responses, produced by muscular activity are not fixed. Exaggerations or reductions in dorsal horn responsiveness would lead to vastly different cardiovascular changes during physical activity. A “plastic” pressor reflex has important functional implications for many facets of human physiology and behavior. For example, peripheral inflammation produces chronic hyperexcitability of dorsal horn neurons, i.e., secondary hyperalgesia. If this enhanced excitability occurs in dorsal horn cells that comprise part of the pressor reflex pathway, then an exaggerated pressor reflex is likely to result. If this occurs in patients with underlying cardiovascular disease, such as coronary artery disease, then physical activity may evoke a cardiovascular crisis. On the other hand, can the excitability of dorsal horn cells be modified in such a way as to optimize blood flow distribution and changes in HR and MAP during physical activity and thus maximize performance? These are just a couple of examples concerning the physiological implications associated with changes in dorsal horn excitability and the pressor reflex. Clearly, much more work is needed.

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Address for reprint requests and other correspondence: L. B. Wilson, Dept. of Physiology, Univ. of South Alabama College of Medicine, 307 Univ. Blvd., Mobile, AL 36688 (E-mail: bwilson@usamail.usouthal.edu).

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