Somatosympathetic Reflexes From the Low Back in the Anesthetized Cat

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Somatosympathetic reflexes from the low back in the anesthetized cat. J Neurophysiol 90: 2548–2559, 2003. First published June 11, 2003; 10.1152/jn.00999.2002. In the appendicular skeleton, substantial evidence demonstrates that somatosensory input from deep tissues including limb muscles and joints elicits somatosympathetic reflexes. Much less is known about the presence and organization of these reflexes from the axial skeleton. We determined if mechanical loading of the lumbar spine and lumbar paraspinal muscle irritation reflexively affects postganglionic sympathetic nerve discharge (SND) to the spleen and kidney. In 27 α-chloralose-anesthetized cats, the L_{2–4} multifidus muscles were injected with the inflammatory irritant mustard oil (20%, 60 μl total) and a vertebral load (100% body weight) was applied dorsal-ventral at the L_{3} spinous process. Mustard oil injection alone without vertebral loading (n = 7) increased mean splenic SND (60%), renal SND (30%), and heart rate (HR; 52 bpm). Mustard oil injection accompanied by the vertebral load (n = 7) increased mean splenic SND (55%), renal SND (16%), and HR (27 bpm). Blood pressure changes were biphasic and could not account for these changes. When the vertebral load accompanied mustard oil, the increases in splenic SND, renal SND, and HR remained elevated in a pattern significantly different from when the vertebral load was absent. Vehicle injection combined with the mechanical load (n = 3) did not change any of the autonomic responses. Similarly, mustard oil injection combined with a mechanical load did not change these responses when either the medial branches of the dorsal rami from T_{11}–L_{4} had been cut (n = 4) or when the spinal cord had been transected between the second and third cervical vertebrae (n = 6). The results indicate that inflammatory stimulation of multifidus muscle in the low back evokes a somatosympathetic reflex integrated supraspinally in the upper cervical spinal cord or higher. The reflex’s afferent arm travels in the medial branch of the dorsal rami, and its efferent arm can affect sympathetic outflow to the spleen and the kidney as well as HR and BP. A static mechanical load applied to the lumbar spine accompanying the inflammatory stimulus appears to sustain the inflammatory-induced reflex activity.

INTRODUCTION

In the appendicular skeleton, substantial evidence demonstrates that somatosympathetic reflexes arise from stimulation of receptive nerve endings in deep tissues including limb muscles and joints. Contraction of skeletal muscle activates the sympathetic nervous system via a reflex whose afferent arm consists of small diameter, thinly myelinated (group III) and unmyelinated (group IV) fibers (Kaufman and Forster 1996). Group III and group IV afferents discharge in response to dynamic exercise (Adreani et al. 1997; Pickar et al. 1994), static and intermittent tetanic contractions, and twitch contractions (Hayward et al. 1991; Kaufman et al. 1983; Mense and Stahnke 1983; Painalt 1960). These types of muscle contraction have been shown to reflexively increase renal adrenal, cardiac, and hindlimb sympathetic nerve activity in both anesthetized and decerebrate cats (Gelsema et al. 1985; Hill et al. 1996; Matsukawa et al. 1990; McMahon and McWilliam 1992; Victor et al. 1989; Vissing et al. 1994). In addition to muscle contraction, passive muscle stretch increases cardiac and renal sympathetic nerve discharge (Matsukawa et al. 1990; Matsukawa et al. 1992). These somatosympathetic reflexes elicited by small diameter afferents in skeletal muscle increase blood pressure (BP), heart rate (HR), and ventilation and are thought to contribute to cardiovascular and respiratory adjustments during exercise (Coote et al. 1971; McCloskey and Mitchell 1972).

Somatosensory input from peripheral synovial joints also contributes to sympathetic control of cardiovascular function (Barron and Coote 1973) via a reflex whose afferent arm is comprised of three types of sensory fibers innervating the joint capsule: thickly myelinated group II, as well as group III and group IV afferents (Langford and Schmidt 1983). Passive bicycle pedaling movement of the hindlimbs increases BP, HR, and ventilation in the nonanesthetized decerebrate cat (Barron and Coote 1973). The responses were significantly reduced after sectioning the nerve supply to the knee joints and were abolished after sectioning the nerve supply to the hindlimbs. In the same experiments, similar cardiovascular and respiratory responses were evoked by electrical stimulation of the articular nerve to the knee that activated sensory nerve fibers with conduction velocities of groups III and IV afferents. In the anesthetized rat, movement of the knee joint in its normal working range influences neither HR, BP, nor inferior cardiac sympathetic nerve activity unless the joint is inflamed or until the knee is rotated beyond its physiological range (Sato et al. 1984, 1985). Electrical stimulation of the articular nerve to the knee at a strength sufficient to activate group II afferents also elicits a reflex response from the inferior cardiac nerve (Sato et al. 1983), indicating that low- and high-threshold mechanical input from the knee joint reflexively affects cardiovascular and respiratory responses.
respiratory control in general and cardiac sympathetic nerve outflow specifically.

In the axial skeleton, very little is known about the reflex relationships between deep paraspinal tissues and the sympathetic nervous system. Sato and Swenson (1984) directly studied the effects of mechanical input from paraspinal tissues on efferent sympathetic nerve activity and concluded that nonnoxious stimuli applied reflexively inhibits the level of sympathetic nerve activity to the kidney and adrenal gland. With paraspinal musculature removed so that somatosensory input was derived presumably from the facet joints, intervertebral discs, and/or intervertebral ligaments, they mechanically loaded several vertebrae in the thoracic and lumbar spine by applying a laterally directed force. The short-lasting (30 s) mechanical input reflexively decreased by 25–45% the level of renal and adrenal sympathetic nerve activity. The inhibition was partially integrated supraspinally because spinalization reversed the decrease to a 40% increase.

Deep tissues of the lumbar spine, i.e., the low back, are well-innervated (Cavanaugh 1999). During activities of daily living, these paraspinal tissues are likely a constant source of mechanosensory input evoked as the spinal column continuously provides mechanical stability to support external physical loads, transfer loads and bending moments from the head and trunk to the shoulder and pelvis, and help suspend internal organs (White and Panjabi 1990). These functional demands may contribute to the fact that idiopathic low back pain is a common occurrence (Videman and Battie 1996). Because mechanical derangements of a motion segment and inflammation contribute to low back pain (Saal 1995; Schultz et al. 1989), we wanted to determine if either segmental loading or muscle inflammation in the low back has reflex consequences on autonomic activity. Because inflammation can augment mechanosensory input via sensitization of groups III and IV mechanoreceptive endings (including silent nociceptive endings) and higher order neurons in the dorsal horn (Gillette et al. 1993b; Mense and Simons 2001; Schaible and Schmidt 1996), we were also interested in the combined effects of mechanical loading and paraspinal muscle inflammation on autonomic activity. We determined whether autonomic responses were reflexive in nature, being due to activation of nerve fibers in the medial branch of the dorsal rami and to integration at spinal or supraspinal levels.

**Methods**

Experiments were performed on 27 anesthetized adult cats. All cats were treated in accordance with the Guiding Principles in the Care and Use of Animals approved by the American Physiological Society. Surgical anesthesia was induced using a mixture of O2 (5 l/min) and halothane (5%) delivered to a sealed plastic chamber. After induction, the cat was removed from the chamber, and surgical anesthesia was maintained by delivering O2 (2 l/min) and 3% halothane through a facemask placed over the cat’s nose and mouth. Catheters were placed in a common carotid artery and an external jugular vein to monitor BP or introduce fluids. The trachea was intubated, and the cat was ventilated mechanically using a Harvard Respirator (model 681). End-tidal CO2 was monitored (CWE) and kept at 5%. After the catheters had been positioned and the trachea intubated, the cat was maintained on anesthesia using α-chloralose (initial dose 35 mg/kg, additional doses given as needed). Arterial pH, pCO2, and pO2 were be monitored using an i-Stat pH/blood gas analyzer (i-Stat). Arterial blood gas values were maintained within the normal range (pH: 7.32–7.43; pCO2: 32–35 mmHg; and pO2: >85 mmHg). Arterial pH and pCO2 were corrected by infusing sodium bicarbonate and by adjusting the ventilator; pO2 was maintained by bleeding 100% O2 into the ventilator’s intake line.

**Sympathetic nerve discharge**

The left splenic and renal nerves were exposed and isolated using a retroperitoneal approach. Nerves traveling to the spleen and kidney nerves were identified and placed on bipolar platinum-iridium hook electrodes (uninsulated wire diameter: 0.005 in). The nerves were crushed distally using jeweler’s forceps to ensure recording of only efferent activity. To prevent fluid from short-circuiting the electrodes and to provide mechanical stability, the nerve-electrode interface was covered with a silicone sealant (Kwik-Cast, WPI). To further prevent mechanical movement of the nerve-electrode interface, looping the insulated portion of the electrode wire and attaching it to the overlying muscle introduced slack into the setup. The retroperitoneal opening was sewn shut to prevent the abdominal contents from desiccating. Nerve activity was passed through a high-impedance probe (H151, Grass), filtered (30–3,000 Hz), and amplified (P151, Grass). Nerve activity, BP, and HR were monitored on the video display of a TA5000 chart recorder (Gould, OH), printed to the chart recorder, and simultaneously digitized (11 kHz) by a PC-based data acquisition system (Spike 2, Cambridge Electronic Design). Data analysis was performed off-line using the data acquisition system. For each cat, postganglionic sympathetic nerve activity was differentiated from electrical noise and preganglionic sympathetic nerve activity using the ganglionic blocker hexamethonium (30 mg/kg, iv; see Data management).

**Multidus muscle**

The lumbar region was exposed by incising the skin overlying the vertebral column from L1–L6. To minimize any trauma or injury to the lumbar paraspinal tissues, the lumbar dorsal fascia was left intact. The lumbar spine was stabilized by fixing the L1 spinous process and the iliac crests in a Kopf spinal unit. Manual palpation was used to identify the spinous processes of the L2, L3, and L4 vertebrae. The multidus muscle at each segmental level originates at the caudal edge of the respective spinous process (Bogduk 1980). Multidus muscles on the left side were identified manually as they lie just lateral to the spinous processes.

**Injections (mustard oil or mineral oil) into multidus muscle**

The inflammatory irritant mustard oil 20% vol/vol, allylsulphocyanate, Fluka) or its vehicle, light mineral oil (Fisher Scientific), was slowly injected. Injections were made at two sites in each of the L2, L3, and L4 multidus muscles on the left side. Injections were identical in volume (2 injections/muscle, 10 μl/injection, 60 μl total). Injection sites were spaced approximately 5 mm apart. Injections were delivered using a 25-gauge needle and were completed within 45–60 s.

**Mechanical loading**

With the cat prone, a static mechanical load was applied to the L3 vertebra in a dorsal-ventral direction. The procedure for applying loads to the vertebra has been previously described in detail (Pickar 1999). Slight modifications were made, and the apparatus is briefly described here. Loads were applied using an electronic feedback control system (Aurora Scientific, Lever System model 310). The system is comprised of a rotary moving-coil motor, an 8-cm-long lever arm, and an electronic feedback interface. The motor controlled the mechanical load applied at the end of the lever arm, and the
amount of displacement was measured electronically. A programmable arbitrary waveform generator (model 39, Wavetek) delivered the command input signal (2 V/kg) to the electronic feedback interface. The lever arm was coupled directly to the spinous process via a vertical shaft that terminated in a pair of adjustable, rat-toothed forceps. The forceps were attached to the L3 spinous process. The forceps were narrow requiring only a thin, narrow, slit (approximately 2 mm long) along either side of the L3 spinous process for attachment. The forceps were clamped tightly onto the lateral surfaces of the L3 of T12 experiment 4 prior to the start of all protocols for 2 mm long) along either side of the L3 spinous process. Little of the multifidus muscle was detached from the vertebra using this method because most of the muscle fibers attach to the spinous process via a tendinous insertion onto the caudal edge of the spinous process. Only a small portion of the multifidus muscle inserts onto the lateral surface of the lumbar spinous processes (Bogduk 1980). During vertebral loading, motions of the adjacent vertebrae were not measured. Because the adjacent vertebrae were not fixed, they were free to produce coupled motions. The load applied dorsally was intended to simulate lumbar extension. The mechanical system delivered ramp and hold loads. The magnitude of each load was 100% of the cat’s body weight. Each load lasted 20 min.

Peripheral nerve transection (medial branch of dorsal ramus)

The medial branch the dorsal ramus innervates the medial-most paraspinal tissues, including multifidus muscles and facet joints (Bogduk 1976). In experiments where peripheral nerve transection was performed (experiment 4 in RESULTS), each medial branch from T11–L3 was exposed near the root of the left superior articular pillar of T12–L4, respectively. The medial branch was cut completely ±1 h prior to the start of all protocols for experiment 4.

Spinal cord transection (C2,3)

Skin overlying the vertebral column was incised from C2 to C4, and neck muscles overlying the C2–3 vertebral column were separated and partially removed. A C3–4 laminectomy was performed. The dura mater was visualized and cut to clearly expose the spinal cord. The narrow, blunt edge of a Freer elevator was passed dorsal-ventrally until it struck the intervertebral disc or vertebral body, and then it was passed transversely through the spinal cord completely transecting it at C2–3 level. Little bleeding occurred. In experiments where spinal cord transection was performed (experiment 5), the cut was made prior to the start of all protocols. After spinal cord transection and prior to the start of the first protocol, each cat was allowed to stabilize for 60–90 min.

Experiments and protocols

Five groups of cats were used, each group comprising a different experiment (Figs. 2 and 4–7). In all experiments, splenic sympathetic nerve discharge (SND), renal SND, HR, and BP were monitored, and the effects on these variables were analyzed. The five experiments were used to determine the following and are presented in the RESULTS in the following order: 1) the effect of intramultifidus mustard oil injection, 2) the effect of intramultifidus mustard oil injection combined with a mechanical load placed on the spine at the L3 vertebra, 3) the effect of intramultifidus mineral oil (vehicle) injection combined with a mechanical load, 4) the effect of transecting peripheral nerves innervating the multifidus muscle on the responses to intramultifidus mustard oil injection combined with a mechanical load, and 5) the effect of cervical spinal cord transection on the responses to intramultifidus mustard oil injection combined with a mechanical load. Within each experiment, two protocols were applied to each cat: a Control protocol and an Injection protocol (Fig. 1). In each cat, the Control protocol was identical to the Injection protocol, with the exception that mustard or mineral oil was injected during the Injection protocol. During the Injection protocol, the mechanical load followed the beginning of the injection by approximately 3 min. The first interval was a baseline time period represented by the first 5 min of each protocol (baseline interval). The second interval was a 20-min time period beginning at the end of the baseline period during

FIG. 1. Schematic showing the timeline used in the experimental design. Each protocol was divided into 3 time intervals for statistical analysis: 1) baseline, 2) 20-min intervention interval, and 3) 5-min post-intervention interval. In all experiments, only an injection of mustard oil or its vehicle followed by a 2-min incubation period differentiated the intervention intervals of the Injection and Control protocols. The 2 cross-hatched boxes from left to right represent the duration of the injection and incubation periods, respectively. In all experiments except experiment 1, the 20-min intervention interval was initiated by the onset of the vertebral load.
the Control protocol or approximately 180 s after the start of injection during the Injection protocol (intervention interval). This 20-min interval represented the duration of the mechanical load (except during experiment 1 where no mechanical load was applied). The third interval was a 5-min time period following the 20-min interval (post-intervention interval). The 5-min post-intervention interval represented a recovery period from the mechanical load (except during experiment 1, where no mechanical load was applied).

Data management

Nerve activity was digitized (11.1 kHz) using a PC-based data acquisition system (Spike 2, Cambridge Electronic Design). Data analysis was performed off-line using Spike 2 software. All nerve activity was full-wave rectified and integrated (τ = 10 ms). Integrated nerve activity, BP, and HR were averaged over 25-s intervals, and the mean of each interval placed in a 25-s bin (Figs. 2 and 4–7). At the end of the Injection protocol, hexamethonium was injected, and mean renal and splenic nerve discharge during a 25-s interval was measured in each cat. Integrated postganglionic sympathetic nerve discharge was obtained in each cat by subtracting the residual electrical activity following hexamethonium injection from the mean in each of the 25-s bins.

Statistical analysis

In each experiment, splenic SND, renal SND, HR, and BP were analyzed. Analyses were performed on the binned data. Each of the three time intervals within an experiment was analyzed separately. Each interval was evaluated within a protocol × time factorial, within-subject design, where “within subjects” refers to repeated measurements across time and in animals exposed to both the Control and Injection protocols. Because a fundamental assumption in repeated measures designs is that the measurements themselves are not affecting the responses, the baseline interval phase was evaluated for both protocol and protocol × time effects. Significant effects were considered reasonable arguments for violation of the assumption. If no significant protocol and/or protocol × time effects were observed in the baseline interval for any of the responses, both the intervention and post-intervention intervals were evaluated for each of the four responses using the same protocol × time factorial design. If significant protocol or protocol × time effects were observed for a response, then, for that response, the protocol × time factorial experiment was analyzed using analysis of covariance, with the covariate being the mean protocol value measured during the baseline interval for that response. Since evaluation for significant protocol and/or protocol × time effects in the baseline interval were testing for assumptions of no effects associated with repeated assessments, type I error rate was set at 0.10 to increase statistical power to identify

FIG. 2. Group data from 7 animals used in experiment 1 showing the effect of the Control (○) and Injection (●) protocols on splenic and renal sympathetic nerve discharge (SND), heart rate (HR) and blood pressure (BP). Mustard oil was injected during the Injection protocol into the L2–4 lumbar multifidus muscles (long, bold arrow). Each symbol represents the mean response ± SE in a 25-s bin (see Data management). The break in the x axis represents the duration of the injection and incubation period (see Fig. 1). Data obtained during the Control protocol are continuous but have been aligned with data from the Injection protocol for ease of presentation. Dashed vertical lines represent the boundaries between the baseline and the 20-min intervention intervals and between the 20-min intervention and the 5-min post-intervention intervals (see Fig. 1). B, body weight; N, number of cats.
assumption violations. For analyses in the intervention and post-intervention intervals, type I error rate was set at the more traditional 0.05 level.

Because inflammation can change neuronal responses to mechanosensory inputs (Gillette et al. 1993b; Mense and Simons 2001; Schaible and Schmidt 1996), experiments 1 and 2 were designed to be identical except for the presence of a vertebral load during experiment 1. An a priori decision was made to compare the pattern of interactions between the two experiments. No adjustments for multiple tests were necessary. Significance was set at the $P < 0.05$ level.

RESULTS

Experiment 1: mustard oil injection and no vertebral load

Figure 2 shows group responses (mean ± SE) in the absence of a vertebral load in seven cats. The Control protocol served as a time control because no load was applied for the approximately 30 min required to complete the protocol. The preparation remained stable over this duration because average values changed little over the 5-min baseline interval compared with the next 25 min: mean splenic SND (1.78 ± 0.41 vs. 1.85 ± 0.45 V-s), mean renal SND (2.00 ± 0.67 vs. 2.01 ± 0.68 V-s), mean HR (216.6 ± 27.5 vs. 216.3 ± 27.9 bpm), and mean BP (122.3 ± 13.8 vs. 122.4 ± 14.6 mmHg). These values were not compared statistically in the model.

BASELINE INTERVAL. Response measures were similar between the Control and Injection protocols: mean splenic SND (1.78 ± 0.41 vs. 1.85 ± 0.61 V-s, $P < 0.60$, control vs. injection), mean renal SND (2.00 ± 0.67 vs. 2.08 ± 0.70 V-s, $P < 0.35$), mean HR (216.6 ± 27.5 vs. 216.1 ± 28.5 bpm, $P < 0.88$), and mean BP (125.9 ± 13.1 vs. 122.3 ± 13.8 mmHg, $P < 0.47$).

INTERVENTION INTERVAL. Intramuscular mustard oil injection significantly increased mean splenic SND by 60% (2.39 ± 0.85 vs. 1.92 ± 0.45 V-s, $P < 0.08$) or mean renal SND (2.25 ± 0.63 vs. 2.04 ± 0.72 V-s, $P < 0.37$). Mean BP significantly decreased 38 mmHg (85.4 ± 15.2 vs. 122.8 ± 14.5 mmHg, $P < 0.002$).

Similar to the intervention interval, the protocol × time effects were significant for splenic and renal SND ($P < 0.002$ and $P < 0.05$, respectively). The interaction’s form (Fig. 2) reflected a continuation of the pattern observed during the intervention interval: values slowly decreased toward Control protocol levels. Similarly, HR slowly decreased over the interval ($P < 0.0001$), whereas the depressor response stabilized ($P < 0.30$). During the Control protocol, SND, HR, and BP remained relatively constant across time.

Experiment 2: mustard oil injection and vertebral load

Figure 4 shows group responses for each of the four responses before, during, and after the lumbar spine was passively loaded with 100% BW in seven cats. The mechanical load translated the L₃ vertebra 4.9–6.5 mm ventralward. Mustard oil was injected into the lumbar (L₂₋₄) multifidus muscles.
only during the Injection protocol and just prior to the intervention interval.

**BASELINE INTERVAL.** Baseline values were similar between protocols for mean renal SND (1.65 ± 0.34 vs. 1.53 ± 0.25 V-s, P = 0.26, control vs. injection), mean HR (206.3 ± 17.1 vs. 205.7 ± 29.6 bpm, P = 0.93), and mean BP (128.8 ± 16.3 vs. 127.3 ± 20.0 mmHg, P = 0.75). However, mean splenic SND had significantly increased by 10% (1.13 ± 0.52 vs. 1.24 ± 0.55 V-s) between the Control and Injection protocols (P = 0.04); therefore the statistical model incorporated covariate adjustments for evaluating the intervention and post-intervention intervals.

**INTERVENTION INTERVAL.** Intramuscular mustard oil injection combined with the vertebral load significantly increased mean splenic SND by 55% compared with the vertebral load alone (1.95 ± 0.55 vs. 1.26 ± 0.11 V-s, P = 0.02, injection vs. control) and increased mean HR by 27 bpm (233.8 ± 23.0 vs. 206.9 ± 19.4 bpm, P = 0.006). A weak trend toward increased mean renal SND was found (1.98 ± 0.71 vs. 1.71 ± 0.36 V-s, P = 0.14), but not for mean BP (136.4 ± 25.4 vs. 131.1 ± 15.1 mmHg, P = 0.27). In Fig. 4, note that, during the Control protocol, autonomic responses were similar during the baseline and intervention (with mechanical load) intervals.

Significantly different patterns of change across time (i.e., a protocol × time interaction) were observed for BP (P < 0.009) and HR (P < 0.001) but not for splenic SND (P = 0.71) or for renal SND (P = 0.24). During the Injection protocol, splenic and renal SND remained stable but elevated above the similarly stable values of the Control protocol (Fig. 4). The form of the interaction for BP indicated the mechanical load alone had little or no effect, but when it was combined with mustard oil injection, BP increased during the early part of the interval followed by a gradual decrease toward and eventually falling below BP levels of the Control protocol. This BP pattern explains the lack of an observed main effect. For HR, the form of the interaction again indicated mustard oil increased HR, but in contrast to BP, HR gradually increased during the first 5 min of the intervention interval, eventually remaining relatively stable through the remaining 15 min of the interval.

**POST-INTERVENTION INTERVAL.** With removal of the mechanical load, mean splenic SND and HR remained significantly elevated: mean splenic SND by 53% (1.96 ± 0.42 vs. 1.28 ± 0.10, P < 0.004, injection vs. control), and mean HR by 29 bpm (234.0 ± 20.4 vs. 204.6 ± 20.2 bpm, P < 0.002). The weak trend toward increased renal SND persisted (1.91 ± 0.54 vs. 1.72 ± 0.32 V-s, P = 0.12), and a strong trend toward
decreased BP developed (112.9 ± 27.3 vs. 128.1 ± 17.5 112.9 ± 27.3 mmHg, P < 0.06). Thus the mustard oil–induced increases in splenic and renal SND and HR observed during the intervention interval were maintained.

Similar to the intervention interval, a significant interaction effect was observed for BP (P < 0.02), but not for renal SND (P < 0.59) or for splenic SND (P < 0.55). In contrast to the intervention interval, no significant interaction effect was observed for HR (P < 0.98). The form of the BP interaction (Fig. 4) reflected a continuation of the interaction described for the intervention interval: stable BP during the Control protocol but a continuing depressor response during the Injection protocol. This pattern also explains the observed trend toward decreased mean BP.

**Experiment 3: vehicle injection and vertebral load**

Figure 5 shows group responses to injecting mineral oil in three cats. The mechanical load (100% BW) translated the L3 vertebra 3.5–4.4 mm ventrally.

**BASELINE INTERVAL.** Values were similar between the control and injection for mean renal SND (1.59 ± 0.52 vs. 1.70 ± 0.24 V-s, P < 0.67, control vs. injection), mean HR (223.3 ± 9.7 vs. 223.8 ± 14.4 bpm, P < 0.97), and mean BP (130.1 ± 6.1 vs. 131.9 ± 9.3 mmHg, P < 0.54). Mean splenic SND increased 13% between the Control and Injection protocols (1.17 ± 0.32 vs. 1.32 ± 0.31 V-s, P < 0.04), for which covariate adjustments were made.

**INTERVENTION INTERVAL.** Mineral oil injection produced no significant change in any of the response measures: mean splenic SND (1.31 ± 0.06 vs. 1.33 ± 0.06 V-s, P < 0.28, control vs. injection), mean renal SND (1.58 ± 0.42 vs. 1.77 ± 0.24 V-s, P < 0.32), mean HR (223.3 ± 11.3 vs. 224.9 ± 13.9 bpm, P < 0.82), and mean BP (133.8 ± 9.7 vs. 132.7 ± 11.1 mmHg, P < 0.33). No significant interaction effects were observed: (P < 0.39, P < 0.98, P < 0.98, and P < 0.34 for splenic SND, renal SND, HR, and BP, respectively).

**POST-INTERVENTION INTERVAL.** Removing the mechanical load produced no significant protocol main effects or interaction effects.

**Experiment 1 versus experiment 2: comparison of interaction patterns**

**INTERVENTION INTERVAL.** The pattern of interaction between experiments 1 and 2 (i.e., protocol × time × experiment) was significantly different for splenic SND (P < 0.0001), renal SND (P < 0.02), and BP (P < 0.0001), but not for HR (P <
Each interaction’s form is seen by comparing Figs. 2 and 4. The early increase in both splenic and renal SND produced by mustard oil combined with the vertebral load (experiment 2) tended to persist throughout the interval, whereas the early increase produced by mustard oil alone (experiment 1) slowly fell toward levels of the Control protocol. During the Control protocols in these experiments, SND remained relatively constant across time. Similarly, the interaction’s form for BP indicated that the increase in BP was maintained longer in experiment 2 compared with experiment 1. As with SND, BP during the Control protocol remained relatively constant across time. The elevation in HR caused by mustard oil persisted in both experiments.

POST-INTERVENTION INTERVAL. The pattern of interaction between experiments 1 and 2 was significantly different for HR \((P < 0.0001)\), but not for splenic SND \((P < 0.90)\), renal SND \((P < 0.93)\), or BP \((P < 0.56)\). The interaction’s form for HR (compare Figs. 2 and 4) was similar to that observed for splenic and renal SND during the intervention interval: the HR increase in experiment 2 tended to persist more than 25 min after injection, whereas the HR increase caused by mustard oil alone in experiment 1 tended to fall toward baseline values. HR during the Control protocol remained relatively constant across time.

Experiment 4: transection of medial branch of dorsal ramus

Mean and standard deviation (SD) were used to estimate individual differences between Control and Injection protocols for each autonomic response in four cats. Figure 6 shows mean differences with their 95% confidence intervals. The mechanical load (100% BW) translated the L3 vertebral 3.5–4.3 mm ventralward (data not shown).

BASELINE INTERVAL. Mean values were similar between the Control and Injection protocols. No significant protocol main effects were present for splenic SND \((1.58 \pm 0.37 \text{ vs. } 1.97 \pm 0.48 \text{ V-s}, P < 0.19 \text{ control vs. injection})\), renal SND \((1.81 \pm 0.78 \text{ vs. } 1.91 \pm 0.81 \text{ V-s}, P < 0.45)\), or BP \((95.3 \pm 11.8 \text{ vs. } 95.8 \pm 11.0 \text{ mmHg}, P < 0.76)\). Mean HR increased 20 bpm between the Control and Injection protocols \((194.3 \pm 15.3 \text{ vs. } 214.2 \pm 25.3 \text{ bpm}, P < 0.10)\) for which covariate adjustments were made.

INTERVENTION INTERVAL. Mustard oil did not produce significant changes in any of the response measures or any significant interaction effects except for HR \((P < 0.0006)\). The interaction’s form indicated that HR slowly and slightly increased during the Control protocol, whereas it remained relatively constant during the Injection protocol. This effect is seen in Fig. 6 as an increasing negative mean difference during the intervention interval.

POST-INTERVENTION INTERVAL. Removing the mechanical load produced no significant protocol main effect or interaction effect for any of the four autonomic responses.

Experiment 5: spinal cord transection

Spinal cord transection between the second and third cervical segments was completed 60–90 min prior to the start of the experiment. Similar to Fig. 6, Fig. 7 shows the mean differences between protocols \(\pm 95\%\) confidence intervals in six cats. The mechanical load (100% BW) translated the L3 vertebral 6.8–8.0 mm ventralward (data not shown).

BASELINE INTERVAL. All response measures were significantly different between protocols except renal SND \((P < 0.11)\). To be conservative, all responses were adjusted for the covariate. Small reductions in mean BP were present at the start of the Injection protocol compared with the Control protocol \((91.7 \pm 17.3 \text{ vs. } 96.5 \pm 18.1 \text{ mmHg}, P < 0.03)\) and in mean HR \((207.4 \pm 28.1 \text{ vs. } 214.4 \pm 25.2 \text{ bpm}, P < 0.07)\). Mean splenic SND was reduced 28% \((2.12 \pm 0.33 \text{ V-s}, P < 0.02)\), but mean renal SND was increased 44% \((0.95 \pm 0.75 \text{ vs. } 0.66 \pm 0.454 \text{ V-s}, P < 0.11)\).

INTERVENTION INTERVAL. Mustard oil produced no significant change in any of the adjusted response measures. No significant interaction effects were observed for splenic SND or BP. A significant interaction effect was observed for renal SND \((P < 0.0001)\) and HR \((P < 0.0001)\). The interaction’s form indicated that for both renal SND and HR each slowly and slightly increased during the Control protocol and slowly and slightly decreased during the Injection protocol. This effect is
The pattern of mustard oil–induced increases over time was different depending on the presence of a vertebral load. In the load’s presence (experiment 2) mustard oil–induced increases in splenic SND, renal SND, and the initial increase in BP were maintained longer, whereas the increases slowly fell toward levels of the Control protocol in the load’s absence (experiment 1). HR showed a similar pattern even during the post-intervention interval.

These changes were evoked specifically by mustard oil because injection of its vehicle, mineral oil, did not affect any of the autonomic responses. In addition, the responses were mediated neurally and not by a circulating factor evoked by the injection. They were initiated by a reflex whose afferent arm traveled in the medial branch of the dorsal ramus because cutting this nerve abolished the mustard oil–induced increases. A supraspinal component within or cranial to the upper cervical spinal cord contributed to the reflex pathway because transecting the spinal cord at C2–3 abolished the increases.

The magnitude of the mustard oil–induced increases in mean renal but not splenic SND were substantially lower in experiment 2 versus experiment 1 (renal SND: 16 vs. 30%; splenic SND: 55 vs. 60%, respectively). Similarly, the mustard oil–induced increase in HR during experiment 2 was approximately one-half of the increase during experiment 1 (27 vs. 55 bpm). These observations might suggest that vertebral loading during paraspinal muscle irritation differentially affects reflex control of HR and renal and splenic sympathetic outflows, mitigating the mean increases in the former two. The possibility exists that the 16% increase in renal SND was not statistically significant due to variability in sympathetic nerve recordings and inadequate power for this response variable from the conservative number of cats used in this study. The functional significance of even small increases in SND to a target organ has not yet been determined. Nonetheless, the mechanical load in combination with mustard oil sustained the smaller, non-statistically significant changes in renal SND in a pattern similar to the pattern of changes in splenic SND, HR, and BP.

Previous studies in the rat indicate that innocuous mechanical stimuli applied to the thoracic or lumbar spine reduce or are without cardiovascular or sympathetic effects (Sato and Swenson 1984; Budgell et al. 1995). In rats, short-lasting (30 s) lateral flexion of the lower thoracic or upper lumbar spine with its paraspinal muscles removed reflexively produces short-lasting decreases in BP, HR, renal SND, and adrenal SND. The approximately 50–85% decrease in SNDs was graded with the magnitude of the mechanical load (Sato and Swenson 1984). While these laterally applied mechanical loads ranged between 0.5 and 3 kg and were considered innocuous, they represent loads of 166–1,000% BW (assuming an average rat BW of 300 g). However, it is not known if loads of this magnitude moved the facets joints outside their physiological range, a criteria that has been used previously to define a noxious load, for example, in the knee joint (Sato et al. 1984). In a previous study of the rabbit lumbar spine, axial loads were considered noxious when they reached 500% BW (Avramov et al. 1992). Using an alternative method for delivering an innocuous mechanical stimulus, Budgell et al. (1995) injected a small volume (20 μl) of physiological saline into the lumbar and thoracic facet joints or midline interspinous tissues, which produced small but statistically significant decreases in arterial blood pressure lasting 30–45 s. The injections had no measur-
able effect on adrenal nerve activity or catecholamine secretion. Our observations in the cat support the conclusion that a relatively long innocuous shear bending force applied to a lumbar vertebra is without effect on resting HR, BP, or sympathetic output to the spleen or kidney.

In contrast to these mechanical studies, noxious chemical stimuli applied to the thoracic tissues increase sympathoexcitation, but the relationship may depend on the paraspinal tissue stimulated. Injecting the C-fiber stimulant capsaicin into the midline interspinous tissues increases adrenal nerve activity (70–80%), catecholamine secretion (140% on average), and BP (30%) (Budgell et al. 1995; Budgell et al. 1997). However, similar injections into the lumbar facet joint produce a depressor response (Budgell et al. 1995). When the inflammatory mediator bradykinin is injected into the abdominal aorta, splenic and renal SND reflexively increase (104 and 18%, respectively) (Weaver et al. 1984). The differential magnitudes were thought to arise from stimulation of sensory receptors in axial muscles of the lumbar region and from abdominal viscera because both femoral arteries were catheterized, occluding circulation to hindlimb muscles. Consistent with this interpretation, the current experiments demonstrated that inflammatory stimulation of lumbar multifidus muscles differentially and reflexively increased the magnitudes of splenic and renal SND.

Injecting the inflammatory irritant mustard oil in the multifidus muscle increased splenic and renal SND and HR for ≥20 min. Intra-articular mustard oil produces tissue inflammation, intensely activates high-threshold C-fibers, and facilitates the somato-somatic flexion reflex (Woolf and Wall 1986). The facilitation outlasts the C-fiber input and is due to excitability changes in dorsal horn neurons (Cook et al. 1987; Woolf and Wall 1986). Similarly, intramuscular (masseter muscle) mustard oil injection produces prolonged increases spontaneous activity and alters mechanical thresholds of brain stem trigeminal neurons (Hu et al. 1992). Together, these findings suggest that the long-lasting somato-sympathetic reflex responses evoked by mustard oil injection into the lumbar multifidus muscle could be maintained by neuroplastic changes in the CNS. Surprisingly though, the long-lasting increases in our experiments were not accompanied by increased response magnitudes when mechanical stimulation accompanied the mustard oil injection compared with mustard oil alone. Possible reasons could include segmental interactions in excitatory and inhibitory pathways activated by the combined afferent input from paraspinal tissues exposed and not exposed to mustard oil, as well as from modulation via pathways within or cranial to the upper cervical spinal cord, demonstrated in this study as necessary for expression of the reflex response.

The mechanical force applied to the lumbar spine was non-specific and potentially loaded a variety of paraspinal tissues including joints ligaments and muscles and intervertebral discs. Electrophysiological studies indicate that populations of groups II, III, and IV afferents with receptive endings in the facet joint capsule (Avramov et al. 1992; Cavanaugh et al. 1989; Pickar and McLain 1995; Yamashita et al. 1990), in tissues surrounding the facet joint (Cavanaugh et al. 1989; Pickar and McLain 1995; Yamashita et al. 1990), and in lumbar paraspinal muscles (Pickar and McLain 1995; Yamashita et al. 1993a,b) can have low mechanical thresholds. The magnitude of their discharge can be graded with the direction of load applied to the facet (Pickar and McLain 1995). Substance P decreases by 30% the von Frey threshold of afferents in and around the lumbar facet joints, suggesting that paraspinal inflammation reduces mechanical thresholds of the receptive endings (Yamashita et al. 1993). Indeed, carrageenan-induced inflammation of the lumbar facet joint capsule and adjacent tissues (after removing the overlying multifidus muscles) increases the background discharge and mechanical sensitivity of groups II, III, and IV afferents and recruits previously silent groups II, III, and IV afferents with receptive endings in and around the facet joint capsule (Ozaktay et al. 1994). In peripheral skeletal muscle, the presence of inflammatory metabolites (e.g., bradykinin and metabolites of the cyclooxygenase pathway) increases the mechanical sensitivity of groups III and IV muscle afferents to muscle contraction (reviewed in Kaufman and Forster 1996). However, to our knowledge, neither the presence of inflammatory-induced changes in mechanoreceptor sensitivity to passive muscle stretch nor the presence of silent mechanonociceptors in skeletal muscle have yet been determined.

Mustard oil injection combined with the mechanical load either maintained the autonomic responses (SND and BP) or prolonged the response following load removal (HR) compared with mustard oil alone. This interaction may reflect the hyperconvergence of mechanosensory input from lumbar paraspinal tissues: dorsal horn neurons with receptive fields in the lumbar paraspinal tissues, including muscle and facet joints, ligaments, and periosteum receive more convergent input than dorsal horn neurons with receptive fields in the limbs (Gillette et al. 1993b). Lamina 1 spinal cord neurons are known to project to the intermediolateral cell column as well as to supraspinal autonomic centers including nucleus tractus solitarius, ventromedial medulla, and midbrain parabrachial nucleus (discussed in Craig 1993), and paraspinal afferents clearly terminate on lamina I spinal cord neurons. Tracing studies reveal that small diameter primary afferents from multifidus muscle and facet joints produce substantial collateral labeling in laminae I and II, as well as in the deeper laminae V-VIII and X (Gillette et al. 1993a). Electrophysiological studies show that wide dynamic range and nociceptive specific neurons in the superficial dorsal horn of the L1–3 spinal segments share receptive fields with deep and superficial tissues of the lumbar spine, the hip, and proximal leg (Gillette et al. 1993b). It is reasonable to suggest that the wide dynamic range neurons, receiving innocuous mechanical input from groups III and IV neurons (Avramov et al. 1992; Cavanaugh et al. 1989; Pickar and McLain 1995; Yamashita et al. 1990) summate with noxious chemical input due to the inflammatory irritant mustard oil.

While the increases in HR and SND after mustard oil injection likely evoked the initial rise in BP (Figs. 2 and 4), the mechanism underlying the biphasic change in BP is not clear. Two possibilities require consideration. The spleen might have contributed to the depressor effect by storing more blood volume, but storage is inconsistent with the increase in sympathetic discharge (Greenway et al. 1968). In addition, the volume of blood stored by the spleen in cat is small (Webb-Peploe 1969). A more likely explanation is a substantial vasodilation controlled by sympathetic nerves whose activity was not recorded in the current experiments. The delayed decrease in BP is consistent with a baroreceptor-mediated vasodilation that overrode the sustained and maintained somatosympathetic reflexive increases in HR and splenic and renal SND. While
baroreflex mechanisms may have modulated the magnitude and temporal profile of the mustard oil–induced increases in HR and splenic and renal SND, they cannot account for the increases themselves because the increases are different in direction from baroreflex effector responses.

Comparing BP levels during the baseline intervals in the nerve transected preparation of experiment 4 (Fig. 6) with baseline intervals in the nerve intact preparations of experiments 1–3 (Figs. 2, 4, and 5, respectively) suggests that resting primary afferent input from paraspinal tissues may contribute tonically to the maintenance of BP. BP was approximately 30 mmHg lower during experiments where the dorsal ramus had been cut even with transection occurring \( \geq 1 \) h prior to the start of the Control protocol in each cat. While BPs are typically lower after spinal cord transection, the apparent tendency after cutting the innervation of the medial most paraspinal tissues has not been reported previously.

Do the changes in sympathetic nerve activity have any potential physiological significance? Sympathetic neural innervation to the kidney affects numerous physiological responses including renal blood flow, renin release, and salt and water retention by the renal tubules (DiBona 1994; Koepke and DiBona 1985). Antiuretic and antinaturosis may be responses to inflammatory stimuli enabling the organism’s use of its fight or flight resources for responding to environmental threats implied by the noxious stimulus. Splenic sympathetic nerve activity is an important source of immune system modulation and represents a direct link between the CNS and splenic lymphocytes (Madden et al. 1994; Wan et al. 1993). Increased splenic SND can have suppressive effects on cellular immune responses including reduced natural killer cell cytotoxicity (Katafuchi et al. 1993). Suppression of the immune response in response to inflammation of the lumbar tissues may be a mechanism that restrains the immune system’s response to the internal injury. These somato-autonomic reflexes may also have pathophysiological consequences yet to be recognized.

Patients with disorders of the vertebral column have physical function status scores that are worse compared with the U.S. population norm and with individuals having other disease conditions (Fanuele et al. 2000). Although comorbidities often accompany spinal disorders, the spinal disorder itself appears most responsible for the low scores (Fanuele et al. 2000). Of spinal disorders, low back pain in particular will likely affect \( \geq 75\% \) of the U.S. population at some time in their lives (Videman and Battie 1996). Mechanical and inflammatory changes are thought to contribute substantially to this clinical problem (Saal 1995; Schultz et al. 1989). Our results raise the possibility that sensory feedback from paraspinal tissues may impact adaptive and homeostatic mechanisms via reflex pathways that affect autonomic regulation.

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DISCLOSURES

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