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The Spine Journal ■ (2013) ■

THE SPINE JOURNAL

Basic Science

The influence of cervical spinal cord compression and vertebral displacement on somatosympathetic reflexes in the rat

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Received 17 January 2013; revised 30 June 2013; accepted 20 August 2013

Abstract

BACKGROUND CONTEXT: One theory within chiropractic proposes that vertebral subluxation in the upper cervical region induces spinal cord compression sufficient to alter spinal cord efferent output. We report on the feasibility of three different experimental approaches to test this theory.

METHODS: A high threshold electrical-evoked somatosympathetic reflex was recorded in adrenal or renal nerves of 10 anaesthetized adult male rats before and after (1) graded pressure was applied directly to the C1/C2 spinal cord segment in eight rats by the use of either direct compression or inflation of an extradural balloon and (2) displacement, less than a dislocation applied posterior to anterior, to the C2 vertebra in two rats. The latency and amplitude of the pre- and postintervention reflex responses were compared.

RESULTS: The reflex amplitude was not significantly changed by pressure (26 mmHg) from an extra-dural balloon or direct compression of the dura mater onto the dorsal spinal cord. Additional pressure, at least sufficient to occlude the dorsal vessels, induced a significant reduction in the amplitude of the reflex, and this reduction persisted for 20 minutes after removal of the pressure (Dunn's method for all pairwise multiple comparison Q stat=3.437; critical value for k=6 with α=0.05 is 2.936). Maximal vertebral (C2) displacement (4 mm), without dislocation did not induce significant changes compared with the control period.

CONCLUSIONS: Although this feasibility study suggests it is unlikely that upper cervical vertebral subluxation, displacement less than a dislocation, compromises the sympathetic outflow in the adrenal or renal nerves, further vertebral displacement studies are necessary to formally test this.

Keywords: Rat; Somato-sympathetic reflex; Spinal cord; Vertebral subluxation; Chiropractic

Introduction

Central to the use of spinal manipulative therapy by chiropractors is the theory that vertebral subluxation, that is, displacement of a vertebra less than a dislocation, compromises health [1]. One proposed consequence of such vertebral displacement, particularly in the upper cervical vertebral column, is alteration of the descending neural activity from the brain caused by compression of the thecal sac or spinal cord and thereby dysfunction in dependent organs [2,3]. It is well established that compression sufficient to damage the spinal cord can not only result in a loss of somatic sensation and voluntary motor function but also loss

FDA device/drug status: Not applicable.

Author disclosures: ***MJB:*** Grant: Australian Spinal Research Foundation (D, Paid directly to the institution). ***BSB:*** Nothing to disclose. ***PSB:*** Grant: Australian Spinal Research Foundation (D, Paid directly to the institution); Trips/Travel: University of Western Sydney, Australia (A), Australian Spinal Research Foundation (A), Chiropractor's Association of Australia (A), Chiropractic and Osteopathic College of Australasia (A); Grants: Australian Spinal Research Foundation - Project Grant A (D, Paid directly to the institution), Australian Spinal Research Foundation - Project Grant B (C, Paid directly to the institution), University of Newcastle - Linkage Pilot Grant (B, Paid directly to the institution), Australian National Health and Medical Research - Project Grant (F, Paid directly to the institution), Australian National Health and Medical Research - Equipment Grant (D, Paid directly to the institution).

The disclosure key can be found on the Table of Contents and at www.TheSpineJournalOnline.com.

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1529-9430/$ - see front matter
http://dx.doi.org/10.1016/j.spinee.2013.08.013

of descending regulation of the autonomic outflow, resulting in autonomic dysreflexia and significant disease [4]. In contrast, there is only indirect evidence that health is compromised by cervical spinal cord dysfunction in the absence of spinal cord damage or demonstrable pathoanatomical lesions such as spondylotic spinal stenosis or ossification of the posterior longitudinal ligament. It has been shown that after some whiplash events in which there is an absence of an identified pathoanatomical lesion, peripheral sympathetic vasoconstriction reactivity may be reduced [5]. Interestingly, although a causal relationship has not been established, a more recent study has suggested that this reduced cutaneous sympathetic reactivity is associated with posttraumatic stress disorder after the whiplash event [6].

The spinal cord compression component of the chiropractic vertebral subluxation theory, and consequent justification for the use of spinal manipulative therapy of the cervical vertebral column, would predict that (1) interference with spinal cord function can occur with quite modest degrees of compression, (2) such compression occurs with displacement of vertebrae less than a dislocation, and (3) the effects of spinal cord interference are ameliorated by the removal of the vertebral displacement. We are unaware of any study that has specifically tested this paradigm. Therefore, we have undertaken a feasibility study to determine the relative amount of spinal cord compression necessary to modulate nerve activity from the spinal cord to organs and to determine whether vertebral displacement, less than a dislocation, can modulate nerve activity. Here we define dislocation as a loss of apposition of the facet surfaces of one or both zygapophysial joints of adjacent vertebra.

We have chosen the rat as a model to investigate this chiropractic paradigm because there is good evidence that the vertebral canal in the upper cervical region of the rat is similar to that of the human [7]. Furthermore, it is known that electrical stimulation of peripheral nerves in anaesthetized cats and rats elicits at least three reflex components in sympathetic efferent peripheral fibers [8,9]. The first is a short latency response involving spinal neural circuitry and referred to as the ''early A-reflex'' because it is elicited by relatively low (electrical) threshold stimulation that activates Aβ somatic afferents. This is followed by a longer latency ''late A-reflex,'' resulting from increased electrical stimulation that activates Aβ and Aδ somatic afferent nerves and involves supraspinal projections and processing in the medulla [10,11]. The third component has an even longer latency and is referred to as the ''C-reflex,'' because it is elicited by electrical stimulation of unmyelinated C-fibers that have the greatest threshold for electrical stimulation and the slowest conduction velocities [12]. A ''very late A-reflex'' response, involving projections to even more rostral centers, can also be elicited in lightly anaesthetized animals [9,11]. In this study we explored the vertebral subluxation ''compression'' hypothesis [2] by eliciting the late A-reflex in anaesthetized rats. Then, we either compressed the upper cervical spinal cord in a controlled manner or displaced the upper cervical vertebra to determine if these procedures modulate the evoked sympathetic efferent reflex activity in adrenal and renal nerves. Preliminary data have been published elsewhere [13].

Methods

Experiments were performed on 10 adult male Wistar rats ages 10 to 13 weeks, weighing 445 ± 54 g (mean ± SD). The study involved a nonrecovery protocol in which the rats were anesthetized with urethane. All procedures were performed in accordance with protocols approved by the Institute's Animal Care and Ethics Committee and conformed to the National Code of Practice for the use of animals in experiments.

Surgical preparation

Each rat was anesthetized by intraperitoneal injection of urethane (1.3 g/kg). Once anesthesia was achieved, as judged by the absence of withdrawal and corneal reflexes, a rectal probe was used to determined body temperature, which was then maintained at 37 to 37.5°C with the use of a heating blanket and infrared lamp. The right jugular vein was catheterized for administration of additional anesthetic (1/10 of initial dose) and fluids as necessary. The left carotid artery was catheterized to allow continuous measurement of blood pressure (BP-1; World Precision Instruments, Sarasota, FL, USA), and the rat's trachea was intubated and the rat allowed to breath spontaneously. The rat's head was then fixed in a stereotaxic frame (model 1730; Kopf Instruments, Tujunga, CA, USA). The spinous process of the first thoracic vertebra was exposed and held fixed in space with a clamp attached to the stereotaxic frame. Signals from the blood pressure transducer were passed to a data-acquisition system (A-D conversion 10 kHz, Spike 2; CED, Cambridge, UK) connected to a computer for monitoring and recording.

Branches of the left sciatic nerve were exposed, isolated from adjacent tissue, placed on silver-wire stimulating electrodes, and covered with warm paraffin oil. A square wave (duration 0.2 ms) stimulus was applied to determine the voltage stimulus threshold for muscle twitch. The nerve was then ligated and cut so only the central cut end remained on the silver wire stimulating electrode, which was then covered with warm paraffin oil. A left retroperitoneal surgical approach was used to expose the adrenal or renal nerves, which were gently teased apart with fine forceps to allow recordings of whole nerve or filaments of these nerves. The isolated nerves or filaments were ligated with fine suture and cut, and their central cut ends were placed on a bipolar silver-wire hook electrode and covered with warm paraffin oil. Signals from these nerves were amplified (×1000; model 1700 amplifier, A-M Systems, Carlsborg, WA, USA), passed to an oscilloscope for online observation

and to the data acquisition system (model 1401, A-D conversion 20 kHz; CED) and computer for off-line analysis. Thus, each animal was prepared so that sensory stimulation could be provided through the sciatic nerve, and sympathetic reflex responses could be recorded from the adrenal or renal nerves.

Interventions to produce cord compression

Three separate interventions were used to induce changes in spinal cord pressure. In each instance the dorsal aspect of the neck was first dissected by making a midline incision separating the neck muscles along the ligamentum nuchae and then reflecting the superficial and middle layer of neck muscles laterally. The deep upper cervical (C1–C4) intervertebral muscles were removed to expose the caudal region of the occiput, the posterior arch of C1, and the spinous process and lamina of the C2 vertebra. In addition, in two rats, the atlanto-occiptial ligament was exposed and a 4-mm incision was made in the ligament. A deflated small purpose-built balloon (3×4 mm), attached to a catheter (PE10; Atom Medical, Tokyo, Japan) containing H_2O, was then inserted between the posterior arch of the C1 vertebra and the dura mater. The surgical field was then filled with warm paraffin and covered with gauze.

In the other six rats, a laminectomy was performed on the C1 and C2 vertebrae to expose the spinal cord, leaving the dura mater intact. A small (2.3×2.8 mm) rubber pad attached to a force transducer (max 100 g; TBM 4M, World Precision Instruments) was then positioned above the C2 spinal cord segment with the aid of a stereotaxic microdrive (1760-61; Kopf). In two of these six rats, a clamp attached to a microdrive (DCM3301R; World Precision Instruments) was attached to the C2 spinous process, allowing posterior to anterior displacement (±0.1mm) of the C2 vertebra. A small, warm, paraffin-soaked gauze was then placed over the surgical site and kept moist throughout the experiment.

Nerve recordings and study protocols

Once the anesthetized rat was prepared and deemed to be physiologically stable, as judged by stable blood pressure (systolic pressure >80 mmHg), heart rate and regular ventilation with no breath sounds, a nonintervention (control) recording was made of the electrically evoked nerve activity. Somatosympathetic reflex activity was elicited with trains of five square wave electrical pulses (pulse duration 0.5 ms, interval 0.5 ms) delivered (1 Hz) to the sciatic nerve at constant voltages equivalent to 15 times the threshold for muscle twitch as previously determined (as described previously) for each respective rat. To ensure nerve recordings were arising from sympathetic efferents, we recorded activity in filaments of adrenal or renal nerves, and whole nerves, during 100 to 500 consecutive sciatic nerve stimuli (1–2 Hz). The adrenal and renal whole nerve recordings obtained during sciatic nerve stimuli trials were averaged off-line using a computer-based data-analysis system (Spike 2; CED). Fig. 1, Top depicts the experimental set up used in this study.

In the two rats prepared for extradural balloon insertion at C1, a control recording was made of left adrenal nerve activity immediately before we inserted the balloon under the C1 vertebra. A stimulus trial and recording were then repeated after placement of the uninflated balloon, immediately after pressurizing the balloon by connecting it to a 360-mm column of H_2O (26.5 mmHg) and then immediately after deflating the balloon (0 mm H_2O; 0 mmHg). A

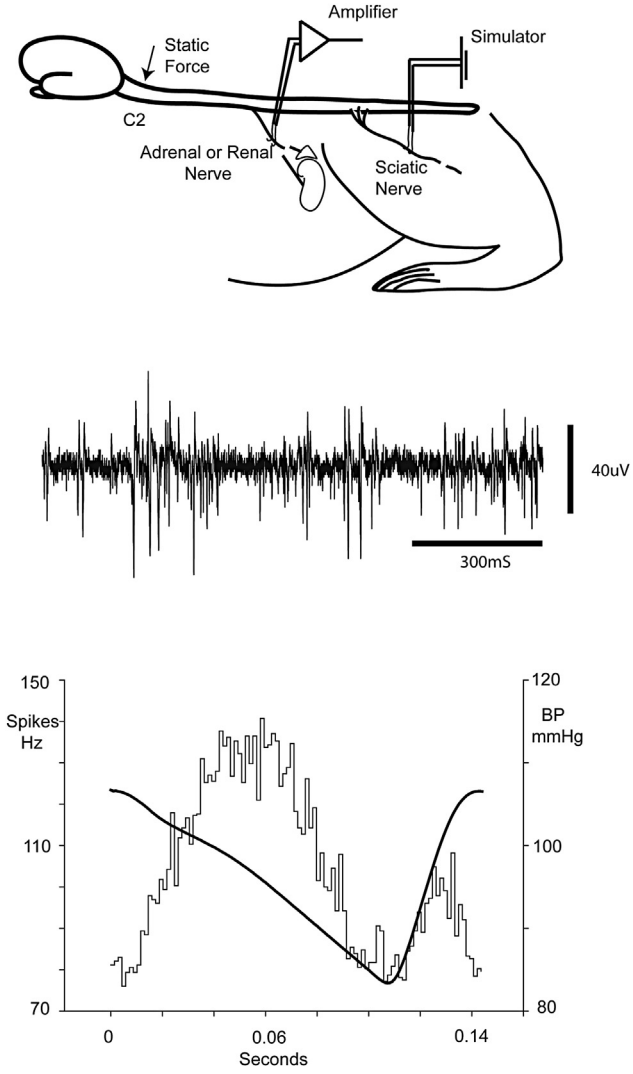

Fig. 1. (Top) Schematic of the experimental set up used to evoke and record the somatosympathetic reflex in this study. (Middle) Recording of spontaneous nerve (spike) activity in an adrenal nerve filament of one rat in this study. Note the bursting unitary (spike) activity typical of sympathetic activity. (Bottom) Histogram (skyline plot) of the frequency (values on left y-axis) of nerve spikes occurring in the adrenal nerve of one rat averaged over 100 cardiac cycles shown as the averaged blood pressure (bolded line) commencing with systole (t=0; values on right y-axis). Note the modulation of spike frequency and that the peak frequency occurs as blood pressure begins to fall (0.04–0.06s) evidencing the spike activity is cardiac (baroreceptor) modulated.

final stimulus trial and recording was performed 20 minutes after removing the balloon.

A control recording was made during sciatic nerve stimulation in each of the six rats (four adrenal and two renal nerves) in which the dura-intact spinal cord had been exposed by laminectomy. With the aid of a dissecting microscope, the pressure pad was then lowered over the C2 spinal cord segment to just touch the dura mater. Sciatic stimulation trials and adrenal or renal nerve recordings were repeated sequentially under the following conditions; (1) with sufficient pressure to displace the dura mater onto the dorsal surface of the spinal cord, and then *either* (2) sufficient pressure applied to occlude the blood vessels on the dorsal surface of the spinal cord without deforming the dorsal surface of the spinal cord (n=2), (3) sufficient pressure to occlude the dorsal blood vessels and visibly deform the dorsal surface of the spinal cord (n=2), or (4) sufficient pressure to compress the spinal cord so all visible blood vessels were occluded (n=2). Each of these positions was held for 30 seconds before stimulating the sciatic nerve and recording the adrenal or renal nerve activity. The pressure pad was then removed, and the sciatic nerve stimulation and adrenal, or renal, nerve recordings were repeated up to 20 minutes after the removal of the pressure foot pad.

After a recording of a ''control'' evoked response in the left renal nerve of the rat in which the spinous process of C2 vertebra had been clamped to the manipulator but not displaced, and after we noted the stereotaxic coordinates of the C2 spinous process, the spinous process (vertebra) was displaced from posterior to anterior with the manipulator (approx. 0.25 mm/s). This was done as we visually inspected the cervical vertebral column with a dissecting microscope (10X; OPMI 11, Zeiss, Jena, Germany). The displacement was stopped once the induced (coupled) motion between C3 and C4 vertebrae ceased. A sciatic nerve stimulus trial was then repeated while we recorded the renal nerve activity. Three minutes after inducing the displacement, the spinous process was returned to its predisplacement stereotaxic position. A sciatic nerve stimulus trial and renal nerve recording was made and then repeated 10 minutes later.

In two animals, at the conclusion of the experiments, the spinal cord was transected at the C1/C2 level, and sciatic nerve stimulation and adrenal or renal nerve recordings were repeated. All rats were euthanized at the end of the respective recording sessions by anesthetic overdose (urethane 3 g/kg intravenously).

Analysis

A threshold level detection analysis program (Levels, Spike 2, CED) was used to detect single unit (spike) activity in recordings from nerve filaments. Peak systolic blood pressure was used to automatically trigger counts and relative firing frequency of these units. Peristimulus histograms, triggered by the sciatic nerve stimulus, were also constructed. Whole nerve recordings were first analyzed to determine the signal-to-noise ratio in each rat by dividing the amplitude of the averaged prestimulus nerve recording by the amplitude of the peak of the stimulus triggered averaged evoked reflex response in the respective control recording. Because a signal-to-noise ratio of less than 4:1 would have only allowed detection of a 50% modulation, we only analyzed data from those rats in which the signal-to-noise ratio was ≥4:1. The latency and peak amplitude of the stimulus-triggered averaged reflex response were measured in each recording. For reasons inherent in recording electrically evoked reflex responses in whole nerve from different animals, such as differences in the baseline recording (ie, DC offset) and differences in the stimulating and recording electrode characteristics in each experiment, the analysis in this study is limited to a descriptive study as has been done by others recording the electrically evoked somato-autonomic reflex [14]. In particular, all amplitude (peak to peak) values of whole nerve recordings obtained during and following the three interventions (vertebral movement, balloon pressure or pressure pad) were normalized to the peak to peak amplitude of the evoked reflex in the preintervention or ''control'' recording obtained in the respective rat. This was done by dividing the peak to peak amplitude of the intervention recording by the peak to peak amplitude from the control recording. However, a Kruskal-Wallis one-way analysis of variance on ranks and a post hoc all pairwise multiple comparison procedure (Dunn's method) were performed using a commercial statistical package (SigmaPlot, version 11; SYSTAT, Chicago IL) to test whether there was a statistically significant ($p<.05$) difference in the median amplitude of the evoked somatosympathetic reflex in experiments using the pressure pad applied to the exposed but intact dura. All descriptive group data sets are reported as mean±SD.

Results

Nerve activity and evoked responses

Recordings from nerve filaments of adrenal and renal nerves exhibited bursts of unitary spike activity (Fig. 1, Middle). The peak systolic blood pressure-triggered histograms of this activity evidenced that this bursting activity was phase locked to blood pressure (Fig. 1, Bottom), as is often characteristic of sympathetic motor neurons. The electrical stimulus of the sciatic nerve at 15× threshold for muscle twitch induced a pressor response (4.8±3.6 mmHg), which remained throughout the stimulus period. The signal-to-noise ratio of the whole nerve-evoked responses from two rats, one involving renal nerve and vertebral displacement and the other involving adrenal nerve and the use of an extradural balloon, had signal-to-noise ratios of <4:1 and are therefore not included in the data set reported here (see the Methods).

The poststimulus time histograms derived from adrenal (n=5) and renal (n=3) nerve filaments revealed increased spike activity with two peaks at latencies of 96.6±8 ms and 175.4±12.4 ms, respectively. There was also an apparent increase in activity at a much longer latency (onset at approx. 350 ms), although this did not have a clear peak (Fig. 2, Top). In contrast to the nerve filament recordings, the whole nerve averaged recordings exhibited a complex response to the sciatic nerve stimulation with onset latency of 101.5±19.4 ms and duration of 112.2±31.3 ms. A whole nerve averaged recording of an evoked response is shown in Fig. 2, Bottom. The whole nerve evoked response was immediately abolished in the two rats in which the C1/C2 spinal cord was transected, confirming its dependence on supraspinal centers (Fig. 3).

Influence of vertebral displacement and cord compression

In contrast to the response to electrical stimulation of sciatic nerve, the blood pressure remained stable during the vertebral displacement and cord compression trials. The Table summarizes the relative amplitude of the whole nerve evoked responses after each of the interventions performed on the eight rats in which the signal-to-noise ratios were ≥4:1.

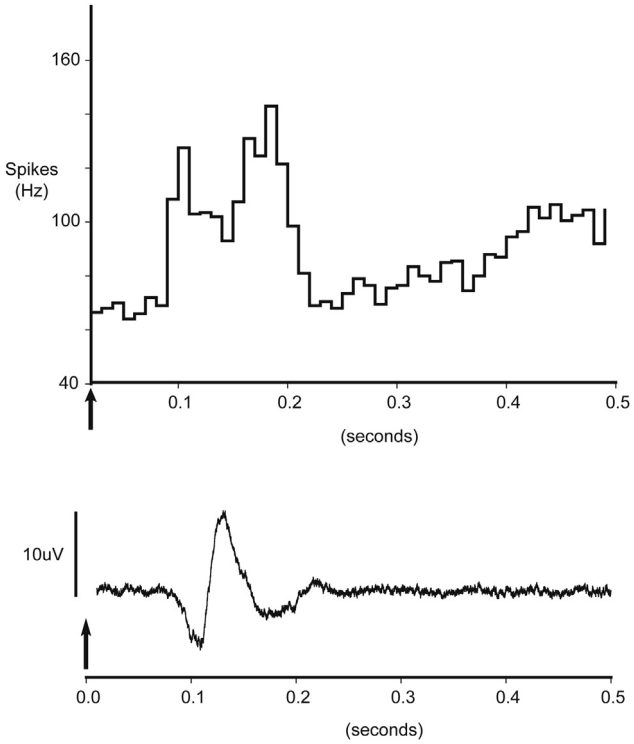

Fig. 2. (Top) Histogram (skyline plot) of the spike frequency of the averaged (n=100) somatic (sciatic nerve electrical stimulation)-evoked nerve activity (spikes) in an adrenal nerve. Note the two early evoked responses (approx. 98 and 180 ms) and the late (>300 ms) increase in activity after the stimulus (arrow). (Bottom) Averaged (n=100) whole adrenal nerve recording exhibiting the evoked (field) potential (somatosympathetic reflex response) resulting from electrical stimulation (elicited at arrow) of the sciatic nerve.

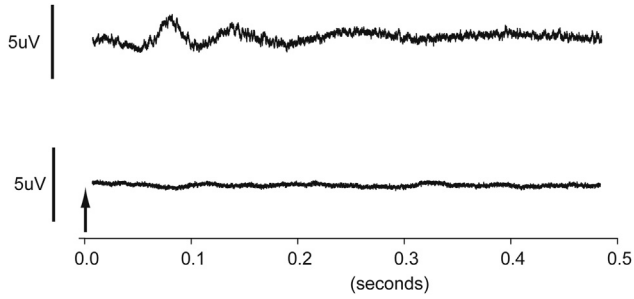

Fig. 3. The top panel shows the averaged (n=250) whole adrenal nerve recording exhibiting the evoked (field) potential (somatosympathetic reflex response) resulting from electrical stimulation of the sciatic nerve after spinal cord compression. The bottom panel shows the loss of the evoked (field) potential after complete transection of the spinal cord at the level of C2. Note the amplitude of the evoked potential in upper trace (compared with Fig. 2, Bottom) is relatively small as the recording was made after the application of spinal cord compression.

At autopsy, the small extradural balloon placed under the posterior arch of C1 in one rat was found to be positioned at the lateral margin of the spinal cord, raising concern that pressure was applied asymmetrically to the spinal cord in this instance. Consequently the data from this rat were excluded from the study. In the second rat in which the small extra-dural balloon was successfully placed under

Table
Relative amplitude of the whole nerve–evoked responses after each of the interventions performed

Expt	Nerve	Control	Dura on cord	Pressure	Recovery
B	A	1	NA	0.93*	0.95++++
P1	R	1	0.75	0.57†	0.65+
P2	A	1	1.13	0.25‡	0.32+
P3	R	1	0.56	0.48§	0.63++
P4	A	1	0.40	0.31‡	0.25+++
P5	A	1	0.71	0.60§	0.52++++
P6	A	1	0.67	0.41†	0.18++++
VD	R	1	NA	0.41‖	0.67++

Note: Normalized amplitude, relative to control, of the averaged (n>100) evoked somatosympathetic reflex in each of eight rats (Expt) recorded from the adrenal (A) or renal (R) nerves without the application of pressure (control) and then during the application of pressure at the level of the C1–C2 vertebra with either an extradural balloon (B), direct application of pressure to the surgically exposed, dura-intact, spinal cord with a pressure pad (P1–P6) or vertebral displacement (VD), sufficient to displace the dura onto the spinal cord (dura on cord), then during the application of additional pressure (pressure) (see footnotes), and then again up to 20 minutes after removal of the applied pressure (recovery) (see footnotes). Note a recording of an evoked reflex was not applicable (NA) when performing the extradura balloon or vertebral displacement as the dura mater was not visualized in these preparations. Each + represents 5 minutes after removal of pressure.

* Pressure=26.5 mmHg.

† Ischemic (all vessel occlusion on microscopic inspection during compression).

‡ Pressure=22 mmHg (2g).

§ Pressure=13.7 mmHg (1.2g).

‖ 4-mm anterior to posterior displacement.

the posterior arch of C1, there was only a 7% reduction in amplitude of the nerve response, even after a 20-minute application of 26.5 mmHg of pressure to the spinal cord. Twenty minutes after reducing the balloon pressure to 0 mmHg, the peak to peak amplitude of the evoked somatosympathetic reflex was 5% less than that of the control (prepressure) amplitude.

The application of direct pressure to the surgically exposed dura mater sufficient to displace the dura mater onto the dorsal surface of the spinal cord reduced (0.62 ± 0.14 of the control) the amplitude of the evoked somatosympathetic reflex in five rats relative to the amplitude of the control. In one rat, however, the amplitude increased by 13%. Thus, the group changes (0.7 ± 0.224 of the control) in the amplitudes of the evoked somatosympathetic reflexes were not found to be statistically different from the amplitudes of the matched controls. In contrast, the application of additional pressure to the dorsal surface of the spinal cord that were, on visual inspection, sufficient to occlude the dorsal vessels, induce deformation of the dorsal surface of the spinal cord or compress the spinal cord (see the Table footnotes: ischemic, 13 mmHg and 22 mmHg, respectively) resulted in a significant reduction in the amplitude of the evoked somatosympathetic reflex that was still apparent up to 20 minutes after the removal of the pressure (Dunn's method for all pair-wise multiple comparison Q stat=3.437; critical value for k=6 with $\alpha=0.05$ is 2.936).

Inspection with a microscope during the ventral displacement of the intact C2 vertebra in the one rat in which this was attempted confirmed that coupled motion of the two vertebrae caudal to C2 occurred with 4mm of posterior to anterior displacement of the C2 spinous process and without loss of facet apposition. This displacement induced a 59% reduction in the peak to peak amplitude of the electrically evoked somatosympathetic reflex. Ten minutes after the return of the vertebra to its predisplacement position the evoked somatosympathetic reflex had recovered by 26%.

Discussion

In this study we conducted in vivo pilot experiments to test the chiropractic theory that vertebral displacement, less than a dislocation, induces sufficient pressure on the spinal cord to alter its efferent output [2]. To determine the effect of transient graded pressure on the efferent output from the spinal cord distal to the applied pressure, we first evoked a long loop somatosympathetic reflex in the renal or adrenal nerves and determined the characteristics (latency and amplitude) of this evoked reflex response. We then tested whether these characteristics were modulated by the application of direct pressure to the dura matter in the upper cervical spinal cord, in the same animal. That pressure was applied either with a small balloon or a pressure pad. Then, in one rat, we characterized the evoked somatosympathetic reflex before and after transient displacement of the C2 vertebra.

The characteristics of the electrically evoked response in our study were consistent with those reported by others studying the somatosympathetic reflex in the splanchnic, adrenal, lumbar white rami, and lumbar sympathetic nerves in the cat and rat [9–12,15,16]. The high threshold electrical stimulation consistently induced a pressor response, evidencing activation of C-fiber afferents necessary for this long loop (late) sympathetic reflex response [11]. Furthermore, the phase locking of the increase in unit (spike) activity in the central cut ends of the adrenal and renal nerve filaments during the falling phase of blood pressure (Fig. 1, Bottom) evidenced that our unitary recordings represented sympathetic efferent nerve activity. Our reflex responses had latencies that were comparable to those recorded by others in adrenal nerves and in the splanchnic nerve from which the adrenal nerve arises [11,15]. That these high threshold electrical evoked reflex responses were then abolished by transecting the spinal cord above C2 at the conclusion of the experiment was further evidence that we had elicited long loop somatosympathetic reflexes [9,11,14,16].

In this study, we have demonstrated in the rat that the amplitude evoked somatosympathetic reflexes can be depressed when direct pressure is transiently (<3 minutes) applied to the dorsal surface of the spinal cord. In contrast to others who have used a small balloon in the thoracic region of rats [17], we were unable to reliably apply pressure symmetrically to the upper cervical spinal cord using an inflatable balloon. This was critical in the present study as it is known, at least in cats, that the descending projections from the rostral ventrolateral medulla, carrying the supraspinal component of the somatosensory reflex, course bilaterally in the dorsolateral and lateral funiculi of spinal cord white matter [18,19]. Using an alternate approach, we were able to apply known pressures directly to the dura mater and the spinal cord in a controlled manner by using a direct pressure pad method. Although compression of the dura mater onto the cord induced a reduction in the amplitude of the evoked reflex, this finding was not statistically significant. Additional pressure sufficient not only to occlude the dorsal vessels of the spinal cord but also to deform the spinal cord was necessary to induce a statistically significant decrease in the amplitude of the somatosympathetic reflex. This reduction in amplitude did not fully recover even 20 minutes after the pressure had been removed. These observations are consistent with previous studies in the dog that have shown that mechanical compression of the neural tissue, rather than ischemia, is responsible for reduction in amplitude of evoked spinal cord (dorsum) potentials during acute (<50 minutes) cord compression [20]. However, alterations in spinal cord function following chronic (weeks) cord compression are likely to involve disturbances associated with reduced blood flow and ischemia [21].

We modeled the chiropractor's vertebral subluxation on the basis that to ensure maximum vertebral displacement without inducing a dislocation, we needed to displace the vertebra (C2) sufficiently to induce coupled motion in the adjacent vertebral motion segment (C3/C4). We confirmed, by visual inspection with a dissecting microscope, that the induced displacement of the C2 vertebra was sufficient to produce coupled motion in the C3/C4 vertebral motion segment but not sufficient to dislocate the C2 vertebra. That is, the facets of the zygapophysial joints remained in apposition. Interestingly, this induced a decrease in the amplitude of the somatosympathetic reflex similar to that which occurred with the application of direct pressure to the exposed dura mater. It is important to note that although this displacement did not dislocate the C2 vertebra, the 4-mm displacement represents almost 100% of the anterior to posterior diameter of the vertebral canal in upper cervical region of young adult rats (3.62±0.31 mm; mean±SD) similar in age and weight to those used in this study [7].

Currently no data exist on the kinematics of the vertebral column of the rat and the functional changes this induces in the diameter of the vertebral canal. However, it is likely that the imposed 4-mm displacement reduced the diameter of the vertebral canal by much less than 4 mm because this displacement includes that necessary to first induce the coupled motion of the adjacent, freely moving, vertebral motion segments. A previous study showed in humans that approximately a 5% (1-mm) change in upper cervical vertebral canal diameter measured during neck extension could occur after a simulated whiplash event and that did not dislocate the vertebral motion units [22]. This suggests, by extrapolation, that the functional vertebral canal diameter in the rats in this study may have been reduced in the order of 0.2 mm in diameter. We have previously shown in the rat that vertebral displacement less than a dislocation, involving a static offset 20° rotational displacement of the C2 vertebra, can induce a statistically significant increase in mean cerebrospinal pressure from 6.1±0.7 to 6.6±0.7mmHg [23]. However, to induce significant changes to the amplitude of the evoked somatosympathetic reflex in the dura mater-exposed experiments in the current study, we needed to apply much greater pressure (>13mm Hg) after already compressing the dura mater onto the spinal cord.

Although the vertebral displacement induced a 59% decrease in amplitude of the evoked reflex in the renal nerve of the single rat reported here, we also showed that displacement of the dura mater onto the dorsal aspect of the upper cervical spinal cord similarly modulated the amplitude of the evoked reflex in renal and adrenal nerves (range 0.4–1.13). Importantly, however, these changes were not statistically different from those recorded in the control circumstance in which no pressure was applied. Only when additional pressure was applied to the extent that it occluded the blood vessels on the dorsal surface of the spinal cord and deformed the spinal cord itself was a statistically significant modulation of the evoked somatosympathetic reflex induced in the dura-exposed experiments. These findings suggest, at least in this one instance of C2 displacement, which notably involved maximum displacement of the C2 vertebra without dislocation, that the modulation of the amplitude of the electrically evoked somatosympathetic reflex was within the range shown to occur in control (no extrinsically applied pressure) circumstances. This raises doubt about the biological plausibility of the compression hypothesis [2] that vertebral displacement less than a dislocation (vertebral subluxation) in the upper cervical spinal cord is likely to induce sufficient pressure to result in significant modulation of the efferent outflow in the renal nerve.

However, to formally test this it would be necessary to undertake a pre- and post-C2 vertebral displacement study involving at least 12 rats to ensure sufficient sensitivity (power=0.8 or 80% chance) in the study (assuming a paired t test analysis) to detect at least a 20% change in amplitude of the evoked somatosympathic reflex with 95% confidence (α=0.05) since this feasibility study has demonstrated that even when no pressure is applied to the spinal cord the expected standard deviation of the amplitude of the reflex is 0.224.

This study has demonstrated that it is possible to undertake an in vivo investigation of the role of compression in the chiropractor's upper cervical vertebral subluxation hypothesis. Although the current study suggests it is unlikely that vertebral displacement less than a dislocation in the upper cervical vertebral column compromises the sympathetic outflow to the adrenal and renal nerves as measured by changes in the amplitude of electrically evoked somatosympathetic reflex, further vertebral displacement studies are necessary to formally test this.

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