Cardiovascular responses to vaginocervical stimulation in the spinal cord-transected rat

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The present study ascertained whether increases in heart rate (HR) and systolic blood pressure (SBP) produced by vaginocervical stimulation (VS; 500 g force) persist in the unanesthetized rat after chronic spinal cord transection at selected levels. Three groups were used: spinal cord transection at T7 (n = 10) or L5 (n = 10) or a sham-operated control group (Sh, n = 10). In the Sh group, VS increased significantly both HR, by 95 ± 14.3 beats/min (bpm) (22 ± 3.7% above baseline), and BP, by 37 ± 5.7 mmHg (37 ± 7.7% above baseline), confirming earlier findings. In the T7 group, VS significantly decreased HR by 107 ± 21.4 bpm (27 ± 4.1% below baseline) and increased BP by 41.3 ± 12.9 mmHg (32 ± 8.3% above baseline). In response to VS, HR increased in every rat in the Sh group and decreased in every rat in the T7 group. In the L5 group, VS failed to significantly affect HR or BP. In the present study, specific levels of spinal cord transection produced differential HR and BP responses to VS in the rat. A model is presented addressing the component responses of autonomic dysreflexia that can occur, contingent on the level of spinal cord injury, in women during parturition or sexual intercourse.

blood pressure; heart rate; spinal autonomic reflexes; autonomic dysreflexia; baroreceptor reflex

AUTONOMIC DYSREFLEXIA (AD) occurs in up to 83% of persons with spinal cord injury above T6 (5). AD is characterized by cardiovascular symptoms that include a marked increase in blood pressure (BP) and decrease in heart rate (HR) in response to visceral stimulation. The stimuli that can trigger a bout of AD include bladder or gastrointestinal distension, irritated urinary bladder due to cystitis or kidney stone formation, cystoscopy, parturition, and sexual intercourse (1, 7, 10, 12, 18, 21, 24). If not treated promptly, the hypertensive episode may produce cerebral and subarachnoid hemorrhage, seizures, and renal failure and may lead to death (20).

Visceral stimulation increases sympathetic outflow from the spinal cord and results in increased vascular resistance and elevated arterial BP. Elevation in BP at the carotid sinus and aortic arch then produce a reflexive slowing of the heart via vagal efferent activation, in addition to a reflex descending inhibition of sympathetic activation in the spinal cord (24). These mechanisms function in the intact individual to maintain cardiovascular tone within normal limits (24).

Level of injury is an additional factor in the cardiovascular response to visceral stimulation. Level of injury may limit ascending activation of input from peripheral sensory pathways, e.g., the vaginocervical sensory input that enters the spinal cord via the pelvic and hypogastric nerves (2, 3, 29). These nerves have also been shown to innervate the uterus and cervix in the female rat (26), and recent studies have provided evidence of a functional afferent vagal pathway that could convey vaginocervical activity directly to the brain, bypassing the spinal cord (4, 9, 16). Thus a better understanding of the specific afferent and efferent nerves involved in cardiovascular responses to visceral stimulation would provide insight into the autonomic sequelae underlying the pathophysiology of AD.

Vaginal stimulation produces a marked and immediate elevation of HR and BP in rats and women (6, 33). The pelvic and hypogastric nerves are known to convey significant afferent activity from the vagina, uterus, and cervix to the spinal cord (2, 3, 15, 28, 29) and likely mediate the vaginocervical stimulation (VS)-induced increase in HR and BP. In the rat, the pelvic and hypogastric nerves enter the spinal cord at L6–S1. The hypogastric nerve also enters at T13–L3 (23, 25, 31). In rats with complete spinal cord transection between C7 and T1 (i.e., above the level of entry of the hypogastric and pelvic nerves), bladder distension, as early as 1 day after surgery, triggers acute hypertensive episodes (27), thus providing an animal model of AD. By contrast, in humans, the latency of the onset of AD is usually several weeks (19).

The present study was designed to ascertain the effect of selected levels of spinal cord transection and of subdiaphragmatic vagotomy on the VS-induced elevation in systolic BP (SBP) and HR.

Two different levels of transection were used: 1) L5, which is above the level of entry into the spinal cord of the pelvic nerve, but below the level of entry of components of the hypogastric nerve, and 2) T7, which is above the levels of entry into the spinal cord of both the pelvic and hypogastric nerves. A preliminary report of these findings has been published in abstract form (30).

MATERIALS AND METHODS

Subjects

Female Sprague-Dawley rats weighing 250–300 g were used in the present study. Rats were housed at 23°C and maintained on a reversed-light cycle (lights off at 1000, on at 2200). Food and water were supplied ad libitum. All rats were ovariotomized at the time of the surgical procedure and

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received estradiol benzoate (EB) dissolved in sesame oil (10 µg EB·100 g body wt⁻¹·day⁻¹ sc) for 1–3 wk before testing. EB was administered to potentiate responses to VS (8, 17).

Experimental Design

Thirty rats (n = 10/group) underwent either spinal cord transection at T7 (T7X) or at L5 (L5X) levels or a sham surgical procedure (Sh). After a recovery period of at least 1 wk, bilateral subdiaphragmatic vagotomy was performed and each group was further divided into vagotomized and sham-vagotomized subgroups, for a total of six subgroups (n = 5/group).

Surgical Procedure

Spinal cord transection. With the use of a Zeiss surgical microscope and pentobarbital sodium anesthesia (45 mg/kg body wt), the intervertebral space between vertebrae T5 and T6 or T13 and L1 was visualized to expose the T7 and L5 levels of the spinal cord, respectively, according to the anatomical schema of Hebel and Stromberg (14). The cord was transected with the use of microscissors inserted into the spinal cord between vertebrae T5 and T6 or T13 and L1. At either site, the microscissors were angled rostrally and then caudally, making two cuts ~5 mm apart. The segment thus produced was completely removed via aspiration pump using 18- and 23-gauge blunted hypodermic needles. The cavity was packed gently with a small piece of Gelfoam to control bleeding. The overlying muscle and skin were sutured, and the rat was placed on a thermostatically controlled (via rectal probe) heating pad to maintain body temperature. The sham operation consisted of exposing and visualizing the spinal cord at either the T7 or L5 levels. No transection was performed in the sham operation.

Subdiaphragmatic vagotomy. Before surgery, the rats were food-deprived overnight to reduce stomach volume. Under surgical microscopic control and pentobarbital sodium anesthesia, the abdominal cavity was opened by a midline incision. The liver was gently reflected to the right and the esophageal-stomach junction was located as a landmark (11). Stomach and esophagus were gently retracted caudally with the use of a blunt hook placed around the junction. Both the anterior and posterior vagal trunks, lying along the esophagus, were visualized with the aid of the surgical microscope and were transected caudal to the diaphragm. Sham vagotomy consisted of exposing and gently retracting the stomach and esophagus to visualize both vagus nerve trunks.

Vaginocervical Mechanostimulation

Mechanical probing (500 g force) was applied against the cervix using a force-calibrated dynamometer (Wagner Instruments, model FD 500, Greenwich, CT) that was modified by shaping the tip of its metal plunger to accept the rubber tip of a 1-ml plastic tuberculin syringe plunger.

Autonomic Response to VS

After ~1–3 wk of postsurgical recovery, the rats were anesthetized with methoxyflurane and a PE-10 Silastic catheter filled with 2% heparin in normal saline (20 USP U/ml) was implanted into the femoral artery. Each rat was then restrained using a soft, light, flexible cloth harness, and was allowed to recover fully from the anesthesia. Electrodes for monitoring HR were inserted subcutaneously, and the arterial catheter was connected to a pressure transducer. Both the electrodes and pressure transducer were coupled to a polygraph (Grass, model 79 D) to monitor changes in HR activity and SBP before, during, and after VS.

Statistical Analysis

All values are expressed as group mean ± SE. The HR and SBP values were sampled during a 6-s period from 18 until 12 s before VS ("pre-VS" baseline value) and during a 6-s period from 12 until 18 s after the onset of VS ("during-VS" value). For both HR and SBP, mean percent change from baseline was calculated by subtracting the no-stimulation (pre-VS) from the stimulation (during-VS) value, dividing this difference by the pre-VS value, and multiplying by 100. Comparisons within groups (pre-VS compared with during-VS) were made using t-tests for dependent measures. Comparisons among groups were made using analysis of variance (ANOVA) and subsequent paired comparisons (Scheffé’s test). Baseline values were analyzed using ANOVA and subsequent Tukey’s protected t-tests. Analyses of frequency distribution of increases and decreases in HR in response to VS were made using χ² tests. In cases in which the sample was small or not normally distributed, the Mann-Whitney U test or Spearman rank-order correlation test was used [GB-STAT for the Macintosh, Version 5.4.6 (Dynamic Microsystems, Silver Spring, MD)]. A level of P ≤ 0.05 was considered statistically significant in all tests.

RESULTS

Because significant differences between vagotomized and sham-vagotomized rats were not found (P > 0.05, Mann-Whitney U test), data for these groups were combined within each spinal cord treatment.

Effect of Spinal Cord Transection on VS-Induced Increase in HR

Figure 1 summarizes the effects of spinal cord transection on VS-induced changes in HR. In the Sh group, VS produced a significant increase (22 ± 3.7%) in HR over baseline values (P < 0.01). In the L5X group, VS did not...
scheffe’s comparisons, all control group (1-way ANOVA; F2,24 differed significantly from each other and from the graph recordings in Fig. 3, A-C. T7X and L5X groups differed significantly from each other and from the control group (1-way ANOVA; F2,24 = 45.89, P < 0.0001; Scheffe’s comparisons, all P values < 0.01). A χ² analysis of the numbers of rats showing a change (+ or −) in HR in response to VS demonstrated a significant effect of level of spinal cord transection (χ² = 27.98, P < 0.0001). That is, the proportion of rats showing a decrease in HR in response to VS in the T7X group (10/10) was greater than that in the L5X (1/3) or in the Sh control group (0/9) (P values < 0.001). No significant difference on this measure was found between the L5X and the Sh groups.

Effect of Spinal Cord Transection on VS-Induced Increase in SBP

Figure 2 summarizes the effects of spinal cord transection on VS-induced changes in SBP. In the Sh and T7X groups, respectively, VS produced a 37 ± 7.7 and a 32 ± 8.3% increase in SBP over the corresponding baseline values (P values < 0.05; multiple t-tests; pairwise comparisons). The two groups did not differ significantly from each other on this measure. By contrast, VS failed to affect significantly the SBP in the L5X group. Representative individual SBP responses in the three groups are shown in the polygraph recordings in Fig. 3, A-C. No significant differences in baseline values were found between any pairs of groups (2-way ANOVA, all P values > 0.05).

Although, as discussed above, there were no within-spinal cord treatment significant differences between the vagotomized and sham-vagotomized rats in the effect of VS on HR or SBP, the difference in VS-induced change in HR between the T7X-vagotomized rats and the T7X-sham-vagotomized rats approached significance, with the latter tending to show the greater decrease (Table 1). On the basis of a small sample, the difference in VS-induced SBP change also approached significance, the vagotomized, sham-spinal cord surgery rats tending to show a greater increase than the sham-vagotomized, sham-spinal cord surgery rats (Table 2).

In the sham-vagotomized, sham-spinal cord surgery rats in which both HR and SBP data were obtained (n = 5), there was a significant negative correlation (P = 0.037, Spearman rank order correlation) between percent increase in SBP and percent increase in HR. In these rats, the greater the increase in SBP, the lesser the increase in HR. In the spinal cord-transected rats, there was no significant correlation between percent changes in HR and SBP. In all cases, however, the HR and SBP increased in response to VS. The same tendency was observed in the vagotomized rats, but there were too few cases to analyze statistically.

DISCUSSION

In the present study, in the Sh group, VS produced a significant and marked increase in both HR and SBP, thus confirming earlier reports (6). By contrast, in the T7X group, VS produced an increase in SBP and a marked decrease in HR. In the L5X group, VS did not significantly affect HR or SBP.

We interpret these findings as follows. In intact rats, afferent activity conveyed to the spinal cord by the pelvic nerves, and perhaps to a minor extent by the hypogastric nerves, activates sympathetic output above T6 to accelerate the HR (Fig. 3A). The pelvic nerve enters the spinal cord at L6–S1 (22, 31); L5X therefore blocks ascending activity initiated via the pelvic nerve. L5X eliminates the ability of VS to elevate HR by blocking ascending activity initiated via the pelvic nerve (Fig. 3B). It is possible that the hypogastric nerves normally contribute to the VS-induced increase in HR, and, because the L5X procedure destroys some of the hypogastric nerve terminals, the absence of a VS-induced increase in SBP after L5X may be due in part to destruction of hypogastric nerve terminals. By contrast, T7X blocks the ascending activity above the transection that is initiated via the pelvic nerves and, thereby, blocks the VS-induced activation of the sympathetic outflow above this level. However, the local sympathetic outflow generated by the pelvic, and perhaps the hypogastric nerves below T7, generates an increase in SBP in response to VS that, in turn, activates the baroreceptor reflex and thereby decreases the HR (Fig. 3C).

There is evidence that some afferent fibers that innervate the cervix and uterus travel in the vagus nerve and enter the brain stem through the nodose ganglion (26). In the present study, bilateral transection of the subdiaphragmatic vagus nerves did not...
significantly affect VS-induced changes in HR. This observation indicates that vagal nerve afferents do not convey genital sensory information that is relevant to HR.

However, in the present study, in the intact rats, the greater the increase in SBP in response to VS, the lesser was the increase in HR in response to VS. This may reflect the net effect of a dual mechanism: 1) the stimulatory drive of VS on BP and HR, and 2) the inhibitory baroceptive drive of the increased SBP on HR. The net result of these two drives is that the greater the VS-induced BP increase, the greater is the
perspective, the effect of level of injury of the spinal cord on its afferent and efferent neural pathways should be taken into consideration to account for cardiovascular responses to visceral stimulation.

**Perspectives**

The anatomic and functional characteristics of these effects are comparable to those in persons who exhibit AD. This is a potentially fatal condition characterized by marked hypertensive crises that are triggered by cutaneous and visceral stimuli occurring in individuals with complete spinal cord lesions above T6 (5, 12, 13, 24). The sequelae can be accounted for as follows. In cases of spinal cord injury in humans at T6 or above, the compensatory descending inhibitory regulation cannot reach sufficiently caudally in the spinal cord to adequately inhibit the reflexive sympathetic outflow, thereby allowing the reflexive BP to increase unchecked. In such individuals who experience an increase in BP due to sympathetic activation, the inhibitory effect of the vagal efferent pathway to the heart is not opposed by an acceleratory drive in response to mechanical stimulation, and the HR decreases. In the case of spinal cord injury below T6, the remaining thoracic spinal cord component caudal to the injury does not produce sufficient reflexive sympathetic outflow to result in an increase in BP. In the intact rat, it is likely that the baroreceptor reflex is functional but that the acceleratory drive from VS overcomes the vagal efferent inhibitory effect, yielding a net increase in HR.

The unique contribution of this study is that it demonstrates the differential effect of level of spinal cord injury on the induction of components of AD in response to genital stimulation in the rat. It suggests a functional neuroanatomic basis for the AD induced by sexual intercourse and parturition in women with spinal cord injury, an understanding of which could prove useful in the medical treatment of this potentially fatal condition.

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Table 1. Effect of vagotomy on VS-induced change in heart rate

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<thead>
<tr>
<th>Vagus Nerve Treatment</th>
<th>P Value (Mann-Whitney U Test)</th>
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<tbody>
<tr>
<td>Spinal Cord Treatment</td>
<td>Vagotomy</td>
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<tr>
<td>---</td>
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</tr>
<tr>
<td>Sham</td>
<td>23.0 ± 6.8 (4)</td>
</tr>
<tr>
<td>T7X</td>
<td>−23.1 ± 5.5 (7)</td>
</tr>
<tr>
<td>L5X</td>
<td>1.3 ± 1.6 (4)</td>
</tr>
</tbody>
</table>

Values are means ± SE percent change from baseline during vaginocervical stimulation (VS); n values in parentheses. T7X, L5X, transection at T7 and L5, respectively.

The suppressive effect on the VS-induced HR acceleration. The same tendency was observed in the vagotomized rats with intact spinal cords, although there were too few observations to analyze statistically. This negative correlation between SBP and HR was not observed in spinal cord-transected rats, perhaps because the ascending acceleratory drive was eliminated. It is not surprising that subdiaphragmatic vagotomy failed to affect significantly the cardiovascular responses, because the baroreceptor-cardioinhibitory response is mediated by the vagus at the supradiaphragmatic level. Thus afferent activity from the genital system via the vagus nerve evidently does not play a significant role in the HR and SBP responses to VS.

In our model, the magnitude of the increase in SBP in response to VS in the T7X group did not differ from that in the Sh group. Given the paroxysmal nature of the hypertensive episodes in AD, we expected to see a greater increase in SBP in those rats with spinal cord transection at T7 than in Sh rats as a consequence of both a loss of autonomic regulation from higher centers and alterations in the neurotransmitters and neuropeptide content within the spinal cord (24). It is possible that the relatively high degree of force applied to the cervix in the present study (i.e., 500 g) produced a ceiling SBP elevation in each group. Previous reports have shown that, in intact rats, the minimal force of VS required to elicit the maximal increase in both HR and BP is 200 g (6). Thus failure to detect differences in VS-evoked increase in SBP between T7X and intact rats may be due to the strong acceleratory drive from VS that, by strongly elevating SBP in intact rats, eliminated differences between the two groups. The relatively high force of VS used in the present study was intentional to maximize any VS-induced responses present after spinal cord transection. We had reported previously that spinal cord transection at T2 reduced by 50% the ability of VS to elevate tail flick latency to radiant heat (32).

In conclusion, VS can induce episodes of combined hypertension and bradycardia in the T7X rat model, thus providing a simple and reliable means of inducing components of AD. On the basis of the present findings, the effect of level of injury of the spinal cord on its afferent as well as efferent neural pathways should be taken into consideration to account for cardiovascular responses to visceral stimulation.

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Table 2. Effect of vagotomy on VS-induced change in systolic blood pressure

<table>
<thead>
<tr>
<th>Vagus Nerve Treatment</th>
<th>P Value (Mann-Whitney U Test)</th>
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</thead>
<tbody>
<tr>
<td>Spinal Cord Treatment</td>
<td>Vagotomy</td>
</tr>
<tr>
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</tr>
<tr>
<td>Sham</td>
<td>59.0 ± 16.0 (2)</td>
</tr>
<tr>
<td>T7X</td>
<td>28.4 ± 11.5 (5)</td>
</tr>
<tr>
<td>L5X</td>
<td>0 ± 0 (1)</td>
</tr>
</tbody>
</table>

Values are means ± SE percent change from baseline during VS; n values in parentheses.