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Spinal cord stimulation modulates intraspinal colorectal viscereroreceptive transmission in rats

C. Qin [a,*], R.T. Lehew [b], K.A. Khan [b], G.M. Wienecke [b], R.D. Foreman [a]

[a] *Department of Physiology, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73104, United States*
[b] *Department of Anesthesiology, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73104, United States*

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Abstract

Previous studies have shown that spinal cord stimulation (SCS) of upper lumbar segments decreases visceromotor responses to mechanical stimuli in a sensitized rat colon and reduces symptoms of irritable bowel syndrome in patients. SCS applied to the upper cervical spinal dorsal column reduces pain of chronic refractory angina. Further, chemical stimulation of C1–C2 propriospinal neurons in rats modulates the responses of lumbosacral spinal neurons to colorectal distension. The present study was designed to compare the effects of upper cervical and lumbar SCS on activity of lumbosacral neurons receiving noxious colorectal input. Extracellular potentials of L6–S2 spinal neurons were recorded in pentobarbital anesthetized, paralyzed and ventilated male rats. SCS (50 Hz, 0.2 ms) at low intensity (90% of motor threshold) was applied to the dorsal column of upper cervical (C1–C2) or upper lumbar (L2–L3) ipsilateral spinal segments. Colorectal distension (CRD, 20 mmHg, 40 mmHg, 60 mmHg, 20 s) was produced by air inflation of a latex balloon. Results showed that SCS applied to L2–L3 and C1–C2 segments significantly reduced the excitatory responses to noxious CRD from 417.6 ± 68.0 to 296.3 ± 53.6 imp ($P < 0.05$, $n = 24$) and from 336.2 ± 64.5 to 225.0 ± 73.3 imp ($P < 0.05$, $n = 18$), respectively. Effects of L2–L3 and C1–C2 SCS lasted 10.2 ± 1.9 and 8.0 ± 0.9 min after offset of CRD. Effects of SCS were observed on spinal neurons with either high or low-threshold excitatory responses to CRD. However, L2–L3 or C1–C2 SCS did not significantly affect inhibitory neuronal responses to CRD. C1–C2 SCS-induced effects were abolished by cutting the C7–C8 dorsal column but not by spinal transection at cervicomedullary junction. These data demonstrated that upper cervical or lumbar SCS modulated responses of lumbosacral spinal neurons to noxious mechanical stimulation of the colon, thereby, proved two loci for a potential therapeutic effect of SCS in patients with irritable bowel syndrome and other colonic disorders.

Keywords: Visceral hypersensitivity; Irritable bowel syndrome; Colorectal distension; Spinal cord

1. Introduction

During the last three decades, spinal cord stimulation (SCS) has become a therapy used for treating certain chronic pains, such as neuropathic pain and ischemic pain (Cameron, 2004; Meyerson and Linderoth, 2000a,b). Various neurophysiological and neurochemical mechanisms underlying the beneficial effects of SCS have also been proposed (Linderoth and Foreman, 1999, 2006). In general, electric stimulation to the dorsal column, which contains large diameter afferent fibers, inhibits transmission of nociceptive information at the spinal segmental level. This finding implicates elements of the gate-control theory (Melzack and Wall, 1965), although activation of supraspinal circuits may also be involved (El-Khoury et al., 2002). Several experimental studies have been performed to explore effects of SCS on spinal neuronal processing of noxious somatic inputs. For example, SCS mainly suppresses excitatory responses of spinothalamic tract neurons (STT) and spinal neurons to noxious somatic stimuli in monkeys (Foreman et al., 1976) and cats (Lindblom et al., 1977), attenuates dorsal horn neuronal hyperexcitability in rats with mononeuropathy (Yakhnitsa et al., 1999), and inhibits long-term potentiation of spinal dynamic range neurons in rats (Wallin et al., 2003).

The mechanisms evoked by SCS have primarily addressed relief of somatic pain, particularly neuropathic pain. However, few studies have examined effects of SCS on viscereroreceptive transmission in the spinal cord. Indeed, SCS is used in patients to treat chronic refractory angina (Eliasson et al., 1996;

* Corresponding author. Tel.: +1 405 271 2226x56272; fax: +1 405 271 3181.
E-mail address: chao-qin@ouhsc.edu (C. Qin).

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Hautvast et al., 1998). Persistent esophageal pain also has been treated with SCS (Jackson and Simpson, 2004). Chandler et al. (1993) report that SCS in monkeys reduces excitatory responses of thoracic STT neurons to electrical stimulation of cardiopulmonary sympathetic afferent fibers and to intracardiac injection of bradykinin. Recently, experimental studies have shown that SCS suppresses or attenuates the nociceptive visceromotor reflex produced by colorectal distension in rats with acute or chronic colonic hypersensitivity produced with acetic acid or trinitrobenzenesulfonic acid (Greenwood-Van Meerveld et al., 2003, 2005). Based on these animal studies, Krames and Mousad (2004) demonstrated in a case study that SCS might be a potential therapy for the treatment of patients with irritable bowel syndrome. However, the effects of SCS on spinal neuronal responses to colorectal distension have not been examined.

Commonly SCS is applied to the segments that elicit paresthesias in areas where patients experience the pain (Linderoth and Foreman, 1999, 2006; Meyerson and Linderoth, 2000a). However, in one human study SCS applied distant to the site of origin of refractory angina in upper cervical spinal segments also reduced the pain symptoms (Gonzalez-Darder et al., 1991). Animal studies have also shown that chemical activation of cell bodies in the C1–C2 spinal segments of spinal cord significantly suppresses excitatory responses of thoracic spinal neurons receiving noxious inputs from heart and esophagus (Qin et al., 2004), and lumbosacral spinal neurons with noxious colorectal input (Qin et al., 1999). These observations suggest that SCS applied to upper cervical segments might also affect lumbosacral spinal neuronal responses to noxious colorectal stimulus. The present study in rats was designed to examine and compare the effects of SCS applied to upper cervical and lumbar segments on responses of lumbosacral spinal neurons to noxious colorectal distension (CRD). The results showed that SCS at both locations using clinical stimulation parameters significantly reduced excitatory responses to noxious CRD in L6–S2 spinal neurons. A preliminary report has been published in abstract form (Foreman et al., 2005).

2. Methods

Experiments were performed on 26 male Sprague–Dawley rats (Charles River Inc.) weighing between 320 and 480 g. Protocols were approved by the Institutional Animal Care and Use committee of the University of Oklahoma Health Sciences Center and followed guidelines of the American Physiological Society and the International Association for the Study of Pain. Animals initially were anesthetized with sodium pentobarbital (50 mg/kg ip). The right carotid artery and left jugular vein were cannulated to monitor blood pressure and to infuse pentobarbital (15–25 mg/(kg h)) during the experiment, respectively. After tracheal cannulation, a constant volume pump was used to provide artificial ventilation (55–60 strokes/min, 3.0–5.0 ml stroke volume). Paralysis of animals was established with pancuronium bromide (0.2 mg/(kg/h), i.v.). Body temperature was kept between 37 and 38 °C using a thermostatically controlled heating blanket and overhead infrared lamps.

Laminectomies were performed to expose L6–S2 spinal segments for recording spinal neurons. L2–L3 and C1–C2 spinal segments also were exposed for placing electrodes for spinal cord stimulation (SCS). Animals were mounted in a stereotaxic headholder and spinal clamps attached to a metal frame were fixed at caudal thoracic and sacral vertebrae. The dura mater of exposed spinal segments was carefully removed. A small well was made on the L6–S2 segments with dental impression material and filled with agar (3–4% in saline) to improve recording stability. Carbon-filament glass microelectrodes were used to record extracellular action potentials of single spinal neurons in a region from midline to 2 mm lateral and 0–1.2 mm deep from the dorsal surface of L6–S1 segments. A spring-loaded platinum-ball electrode (0.5–1.0 mm in diameter) was applied to the dorsal column ipsilateral to neuronal recording sites at either upper lumbar (L2–L3) or upper cervical (C1–C2) segments for SCS. The electrical current at the onset of small contractions in paraspinal muscles was defined as the motor threshold (Tanaka et al., 2001). Average motor thresholds of SCS (50 Hz, 0.2 ms) at C1–C2 and L2–L3 were 0.35 ± 0.8 and 0.40 ± 0.09 mA, respectively. Spinal cord stimulation was performed for 3–5 min at an intensity of 90% motor threshold before animals were paralyzed. In some cases, the short-term SCS (10 s) was used to compare the effects on spontaneous activity and responses of spinal neurons to CRD before and after spinal transection at rostral C1 segment (cervicomedullary) and cutting C7–C8 dorsal column. Spinal transections were gently performed by using a sharp surgical blade. In addition, in a few cases, ibotenic acid (1 mg/ml) was absorbed onto filter paper pledgets (2 mm × 2 mm) and placed on the dorsal surface of the C1–C2 to inactivate cell bodies. Effects of C1–C2 SCS on spontaneous activities and nociceptive responses to CRD were assessed 20 min after ibotenic acid was applied to the dorsal surface of C1–C2. Ibotenic acid is an excitatory neurotoxin and a glutamate analog that destroys neuronal perikarya, but spares axons and non-neuronal cells (Ren et al., 1990; Marini et al., 2000).

Innocuous and noxious CRD (20 mmHg, 40 mmHg, 60 mmHg, 20 s) were produced by air inflation of a 4–5 cm long latex balloon that was inserted into the descending colon and connected to a sphygmomanometer (Qin et al., 1999). Intracolonic pressure of 60 mmHg for 20 s was used as a search stimulus. To induce CRD, the intracolonic pressure rapidly reached the required level at a rate of 20–40 mmHg per second and then the pressure was consistently kept at this level by monitoring the pressure gauge and adjusting it with an air pump to make sure the pressure remained constant throughout the stimulation period. Neurons responding to CRD at 60 mmHg for 20 s were tested with this stimulus two to three times to make sure responses were consistent and repeatable. Raw traces of neuronal activity were stored in a computer with Spike-2 software (CED, Cambridge, UK) and evaluated using rate histograms (bin width 1 s). Spontaneous activity of neurons was counted for 10 s before the onset of CRD to obtain impulses per second (imp/s). Neuronal responses to CRD were calculated as total impulses (imp) of a change in activity from the onset of increased or decreased activity until the evoked activity returned to control. Latency and duration of responses to CRD also were measured. Based on the intracolonic pressure that produced a neuronal response, L6–S2 neurons excited by CRD were divided into the following two subgroups: low-threshold (LT) neurons responded to intracolorectal pressure 20 mmHg; high-threshold (HT) neurons responded to 40 mmHg pressure of CRD (Qin et al., 2003). For testing SCS effects, two consistent responses to noxious CRD (60 mmHg, 20 s) were first obtained as pre-control responses and then a noxious CRD was performed during SCS (3–5 min). After SCS offset, noxious CRD at 3–8 min intervals were administered until the CRD response recovered (>80% of pre-control responses). Recovery time of the effects of SCS was identified as the period from offset of SCS to the return of the CRD responses to control levels. Descriptive data are reported as means ± S.E. Statistical comparisons were made using Student's paired *t*-test and Chi-square analysis. Comparisons of data were considered statistically different if $P < 0.05$.

Somatic receptive fields of spinal neurons also were characterized. For innocuous stimulation of cutaneous receptive fields of spinal neurons, a camel-hair brush or light pressure from a blunt probe was used, whereas for noxious stimulation pinching the skin and muscles with blunt forceps was used. Neurons were classified as follows: wide dynamic range (WDR) neurons responded to innocuous stimulation of skin and had greater responses to noxious pinching of the somatic field; high-threshold (HT) neurons responded only to noxious pinching of the somatic field; and low-threshold (LT) neurons responded primarily to innocuous stimuli. If a cutaneous receptive field was not found, movement of tail (MT) was tested.

To mark spinal recording sites of neurons that responded to CRD, an anodal electrolytic lesion (50 μA dc, 20 s) was made after a neuron was studied. At the end of the experiment, the animal was euthanized with an overdose of pentobarbital sodium (200 mg/kg). The lumbosacral spinal cord was removed