Neuroscience 152 (2008) 950–958

ROLES OF DORSAL COLUMN PATHWAY AND TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 IN AUGMENTATION OF CEREBRAL BLOOD FLOW BY UPPER CERVICAL SPINAL CORD STIMULATION IN RATS

X. YANG,[a]* J. P. FARBER,[b] M. WU,[b] R. D. FOREMAN[b] AND C. QIN[b]

[a]*Department of Pain Management, Xi'an Jiaotong University First Hospital, Xi'an, Shaanxi 710061, PR China*

[b]*Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA*

Abstract—Clinical and basic studies have indicated that upper cervical spinal cord stimulation (cSCS) significantly increases cerebral blood flow (CBF), but the mechanisms are incompletely understood. This investigation was conducted to differentiate between stimulation of dorsal column fibers and upper cervical spinal cord cell bodies in cSCS-induced increases in CBF and decreases in cerebrovascular resistance (CVR). cSCS (50 Hz, 0.2 ms, 1 min) was applied on the left C1–C2 dorsal column of pentobarbital anesthetized, ventilated and paralyzed male rats. Laser Doppler flowmetry probes were placed bilaterally over the parietal cortex, and arterial pressure was monitored. cSCS at 30%, 60%, and 90% of motor threshold (MT) produced vasodilation bilaterally in cerebral cortices. Subsequently, cSCS was applied at 90% MT, and ipsilateral responses were recorded. Ibotenic acid (0.3 mg/ml, 0.1 ml) placed on dorsal surface of C1–C2 (n=7) to suppress cell body activity, did not affect cSCS-induced %ΔCBF (42.5±8.1% vs. 36.8±7.1%, P>0.05) and %ΔCVR (−19.4±4.2% vs. −15.2±5.6%, P>0.05). However, bilateral transection of the dorsal column at rostral C1 (n=8) abolished cSCS-induced changes in CBF and CVR. Also, rostral C1 transection (n=7) abolished cSCS-induced changes in CBF and CVR. Resinferatoxin (RTX), an ultrapotent transient receptor potential vanilloid type 1 (TRPV1) agonist, was used to inactivate TRPV1 containing nerve fibers/cell bodies. RTX (2 μg/ml, 0.1 ml) placed on the C1–C2 spinal cord (n=7) did not affect cSCS-induced %ΔCBF (60.2±8.1% vs. 46.3±7.7%, P>0.05) and %ΔCVR (−25.5±3.5% vs. −21.4±8.9%, P>0.05). However, i.v. RTX (2 μg/kg, n=9) decreased cSCS-induced %ΔCBF from 65.0±9.5% to 27.4±7.2% (P<0.05) and %ΔCVR from −28.0±7.6% to −14.8±4.2% (P<0.05). These results indicated that cSCS-increases in CBF and decreases in CVR occurred via rostral spinal dorsal column fibers and did not depend upon C1–C2 cell bodies. Also, our results suggested that cerebral but not spinal TRPV1 was involved in cSCS-induced cerebral vasodilation.

Key words: spinal cord stimulation, cerebral ischemia, vasodilation, transient receptor potential vanilloid type 1, resiniferatoxin.

Spinal cord stimulation (SCS) has been widely used as a clinically effective therapeutic modality to treat refractory neuropathic pain as well as ischemic pain resulting from peripheral vascular disease and angina pectoris (Cameron, 2004; Linderoth and Foreman, 1999, 2006). However, SCS has been used to a much less extent for treating cerebral vascular disturbances (Wu et al., 2008). Hosobuchi (1985) was the first to report that cervical spinal cord stimulation (cSCS) produced an increase in cerebral blood flow (CBF) in humans. Subsequent clinical observations demonstrated that cSCS decreases cerebrovascular resistance (CVR) and increases blood flow velocity, leading to an enhancement of the local-regional delivery of oxygen (Clavo et al., 2004; Mazzone et al., 1996; Meglio et al., 1991a,b). Therefore, the promising results of cSCS-induced CBF augmentation have led some clinicians to use this procedure to treat various cerebral vascular disorders. These cerebral diseases and/or pathological conditions include cerebral ischemia (Broseta et al., 1994; De Andres et al., 2007; Hosobuchi, 1991), ischemic spastic hemiparesis (Visocchi et al., 1994), focal cerebral ischemia (Meglio et al., 1991a,b; Ebel et al., 2001; Sagher et al., 2003; Sagher and Huang, 2006; Robaina et al., 2004), cerebral vasospasm (Gurelik et al., 2005; Karadag et al., 2005; Visocchi et al., 2001), stroke (Hosobuchi, 1991; Matsui and Hosobuchi, 1989; Visocchi et al., 1994, 2001), ischemic cerebral edema (Gonzalez-Darder and Canadas-Rodriguez, 1991), postapoplectic spastic hemiplegia (Nakamura and Tsubokawa, 1985), prolonged coma (Fujii et al., 1998), persistent vegetative state (Kanno et al., 1987; Funahashi et al., 1989; Kuwata, 1993), as well as migraine and post-traumatic cervicogenic headache (Dario et al., 2005). However, the underlying mechanisms of blood flow improvement are not well understood (Wu et al., 2008).

Animal models have been used to evaluate possible central and peripheral mechanisms of cSCS-induced increases in CBF. Blockade of autonomic ganglia with hexamethonium and blockade of α1-adrenergic receptors can suppress cSCS-induced increases in CBF (Patel et al., 2003; Sagher and Huang, 2000), but muscarinic receptor blockade with atropine had no effect (Garcia-March et al.,

*Corresponding author. Tel: +86-29-85324031; fax: +86-29-85323644.
E-mail address: pain67@163.com (X. Yang).

Abbreviations: CBF, cerebral blood flow; cSCS, cervical spinal cord stimulation; CVR, cerebrovascular resistance; i.t., intrathecal, intrathecally; MT, motor threshold; RTX, resiniferatoxin; SCS, spinal cord stimulation; TRPV1, transient receptor potential vanilloid type 1.

0306-4522/08$32.00+0.00 © 2008 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2008.01.009

1989). Effects on brain vasomotor areas also are presumed to be of importance for increasing CBF (Patel et al., 2004; Sagher and Huang, 2000). In the case of SCS-induced hind-limb vasodilation, the response to SCS depends on transient receptor potential vanilloid type 1 (TRPV1) containing peripheral fibers, as well as TRPV1 containing neurons in the spinal cord (Wu et al., 2006, 2007), but it is unknown whether similar pathways are relevant to cSCS effects on CBF. Spinal cord transection in rats and dorsal column section in cats at the cervicomedullary junction abolish effects of cSCS on CBF (Isono et al., 1995; Patel et al., 2004). These effects may indicate that dorsal column fibers carry cSCS input to the brain; however, the upper cervical spinal cord also contains spinal neurons with projections to supraspinal structures, including various nuclei in caudal medulla, thalamus, hypothalamus, and periaqueductal gray in rats and cats (Malick et al., 2000; Mouton et al., 2005). It is unknown whether these spinal neurons play a role in changes of CBF by cSCS. Another important limitation of the SCS data discussed above is that CVR had not been calculated for each animal in previous studies. In some cases, a decrease in resistance might be inferred from mean changes in blood pressure versus mean changes in blood flow, but the occurrence of vasodilation is best verified by taking the mean of individual changes in vascular resistance.

To clarify and expand the results of previous studies, we addressed two issues with respect to upper cSCS-induced cerebral vasodilation in pentobarbital anesthetized rats. The first goal was to clarify the pathways from cervical spinal cord to brain through which cSCS produced cerebral vasodilation. Previous studies suggested that dorsal columns transmitted the information for vasodilation to the brain, but the effect could have been related to activation of cell bodies in the upper cervical spinal cord rather than axons. To differentiate between these mechanisms, effects on cSCS-induced cerebral vasodilation after C1 transections and after application of ibotenic acid to the C1–C2 spinal cord were compared. Ibotenic acid is excitotoxic to neuronal cell bodies but does not affect axons of passage (Ren et al., 1990). Our second goal was to assess the effects of TRPV1 containing neurons and nerve fibers during cSCS-induced cerebral vasodilation, since TRPV1-related mechanisms are crucial for SCS-induced peripheral vasodilation. We used resiniferatoxin (RTX), an ultrapotent TRPV1 agonist, to deactivate TRPV1 containing neurons/fibers involved in SCS (Wu et al., 2006, 2007). RTX was applied to the C1–C2 spinal cord or administered i.v. All responses were examined with respect to changes in CBF and CVR for each tested animal. Because previous studies did not rigorously examine effects on resistance, we also verified that ganglionic blockade with hexamethonium suppressed upper cSCS-induced vasodilation. In addition, we considered whether the effects from the sympathetic chain could be excluded from the cSCS response using C6–C7 spinal transection. Some of our results have been presented in abstract form (Yang et al., 2007a,b).

EXPERIMENTAL PROCEDURES

Animal preparation

Experiments were performed in 61 male Sprague–Dawley rats (310–430 g Charles River Laboratories, Wilmington, MA, USA). The protocol for this study was approved by the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center and also followed the guidelines of animal experiments of the International Association for the Study of Pain. Animal numbers were kept to the minimum that are typically required to achieve statistically significant results. Appropriate levels of anesthesia were used to minimize suffering and the animals were killed with an overdose of sodium pentobarbital. Anesthesia was initially induced by intraperitoneal sodium pentobarbital (60 mg/kg i.p.) and was maintained throughout the experiment with constant i.v. infusion (15–25 mg/kg/h) through a catheter (PE-50) placed into the right jugular vein. Another catheter (PE-50) was inserted into the right carotid artery to monitor blood pressure (BP). A tracheotomy was performed for mechanical ventilation with a rodent ventilator (Model 683; Harvard Apparatus, Inc., S. Natick, MA, USA) using a constant-volume pump (55–60 strokes/min, 3.0–5.0 ml stroke volume). Animals were paralyzed with pancuronium bromide (0.4 mg/kg, i.p.) and muscle relaxation was maintained with supplemental doses (0.2 mg/kg/h i.v.) during the experiment. Average blood pressure was kept between 80 and 120 mm Hg, and pupils were constricted throughout experiments. Core body temperature was measured with a rectal probe and maintained between 36 and 38 °C at all times using a servo-controlled heating pad (Model 71A; Yellow Springs Instruments, Yellow Springs, OH, USA). Animals were positioned in a stereotaxic frame and the vertebral column was stabilized with clamps. A laminectomy was performed to expose the dorsal surface of the cervical spinal cord segments (C1–C7). Room temperature was maintained between 22 and 24 °C.

SCS

A silver spring-loaded unipolar ball electrode with a tip diameter of approximate 1 mm was placed on the left dorsal column 0.5 mm rostral to the C2 dorsal root entry zone to electrically activate C1–C2 spinal segments (Qin et al., 2007). The motor threshold (MT) stimulus intensity was determined in each animal at 50 Hz, 0.2 ms duration by slowly increasing the cSCS current from zero until a clear retraction of the left neck muscles was observed. Experimental cSCS (50 Hz; 0.2 ms, monophasic rectangular pulses), similar to clinical SCS, was performed for 1 min at 30%, 60%, 90% of MT in random order of stimulus intensities (Tanaka et al., 2001; Wu et al., 2006). The lowest level of stimulation at 30% of MT is used because it was closest to the threshold of SCS that produced vasodilation. The level of stimulation at 60% of MT is also used because it approximates the parameters of clinical applications of SCS (Linderoth and Foreman 1999, 2006). The level of stimulation at 90% of MT intensity is used since it is close to, but below MT. To obtain the stimulus–response relationships, different intensities of cSCS were applied at intervals $>$10 min and effects on CBF/CVR were assessed. In the experiments to examine the neural pathway and role of TRPV1, cSCS (90% MT) was reapplied after CBF/CVR recovered to control levels subsequent to spinal transection or chemical blockades ($>$20 min). Usually, cSCS was applied five to eight times in each animal. In addition, to avoid potential interactions among drugs, testing trials for different chemical blockades were conducted in different groups of animals.

Measurement of CBF

A midline incision was made to expose the parietal region of the skull. A dental drill was used to produce a 3.0-mm-diameter hole that exposed the right and left parietal cortex at 4.0 mm lateral and