Cerebellar Cortical Stimulation Increases Spinal Visceral Nociceptive Responses

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INTRODUCTION

The cerebellum is endowed with a highly regular circuitry often regarded as a micro-processing system (Arbib 1987; Eccles et al. 1967; Middleton and Strick 1998). This system plays a recognized role in motor phenomena (Dow and Moruzzi 1958; Fields and Willis 1970; Gilman et al. 1981; Holmes 1922, 1939; Palay and Chan-Palay 1982) but a controversial role in pain (Spiegel 1982). Nevertheless, cerebellar modulation of spinal responses to noxious stimuli has not, to our knowledge, been investigated yet.

In this study, we have examined the influence of cerebellar cortical stimulation on spinal nociceptive neurons that responded to noxious visceral and somatic stimuli. Our results indicate that stimulation of the cerebellar vermis may lead to a pro-nociceptive effect at the level of the spinal cord.

METHODS

Thirteen adult male Sprague-Dawley rats were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with the guidelines of the National Institutes of Health and the International Association for the Study of Pain. The rats were anesthetized with pentobarbital sodium (60 mg/kg ip). Supplementary infusion of pentothal sodium (5 mg · kg−1 · h−1) was maintained through catheterization of the jugular vein. Respiration was assisted by a tracheal cannula, and body temperature was kept at 37°C using a heating blanket. A laminectomy and a craniotomy exposed the lumbosacral spinal cord and the posterior cerebellum, respectively. The rat was then fixed on a stereotactic apparatus (Kopf Instruments), and a balloon was gently inserted through the rectum for visceral stimulation.
by CRD. The dura over the cerebellum and the spinal cord was carefully excised to permit stimulation and recording, respectively.

**Visceral stimulation**

CRD is a well-documented visceral stimulus (Al-Chaer et al. 1996a, 1998; Su and Gebhart 1998). The balloon was constructed from a latex glove finger attached to a 4-cm-long tygon tubing that was connected to a manual pump and a pressure transducer to monitor stimulus intensity. CRDs consisted of consecutive inflations to pressures ranging from 20 to 80 mmHg applied in increments of 20 mmHg for 20 s every 4 min. CRDs of 60 or 80 mmHg using this balloon are considered noxious (Al-Chaer et al. 2000).

**Somatic stimulation**

The cutaneous stimuli that were applied consisted of brushing (BR) the receptive field (RF) using a camel hair brush, applying pressure (PR) to a fold of skin using a large arterial clip, or pinching (PI) with a small arterial clip exerting a force of approximately 550 g/mm². All these stimuli are considered as nonnoxious except PI, which is distinctly painful if applied to a fold of human skin (Dougherty et al. 1992). Twenty seconds of background activity was recorded preceding each stimulation. BR, PR, and PI were applied consecutively for 10 s separated by 10 s of no stimulation.

**Extracellular recording**

Extracellular single-unit recordings were made stereotaxically from the lumbosacral spinal cord (1–1.7 mm from the surface, near the midline) using a tungsten microelectrode (0.5-μm diameter tip, 12-MΩ impedance). The extracellularly recorded action potentials were evoked by either CRD or somatic stimuli applied to the skin, then amplified, fed into a window discriminator, and displayed on an oscilloscope. The outputs from the window discriminator and from the amplifier were led into a data-collection system (CED 1401+) and a computer for data compilation as “rate histograms” using the Spike 2 software program. The responses to CRDs were calculated as the differences between the mean rate of firing during a stimulus and that during the 20 s baseline recording that preceded each CRD. The responses to cutaneous stimuli were calculated similarly as the differences between the mean rate of firing during a stimulus and that during the 10 s that preceded each stimulus.

**Cerebellar stimulation**

A tungsten microelectrode was guided vertically by a micromanipulator into the posterior cerebellar vermis (lobule VI) until the first layer of spontaneously active cells was encountered (1–2 mm from the vermal surface). The electrode was then disconnected from the recording equipment and connected instead to a Master 8 stimulator for the generation of current pulse trains (0.2-ms pulses at 333 Hz, 100–150 μA). Electrical stimulation started at 2 s before the start of a stimulus and lasted for either 10 s during each CRD or 5 s during each somatic stimulation. Therefore electrical stimulation was terminated at 10 or 5 s before the end of a CRD or a somatic stimulation, respectively. This paradigm was advantageous in allowing for observation of the effect of electrical stimulation alone on the baseline (2 s prior to CRD), and arguing against an electrical stimulus artifact (during CRD).

For chemical stimulation, a 10-μl Hamilton microsyringe was filled with a 0.1 M solution of D,L-homocysteic acid (DLH) and advanced stereotaxically into the same region as described above for electrical stimulation. Ten seconds before each CRD, 2 μl DLH was injected, whereas only one 3-μl injection was applied 10 s before the start of each somatic stimulation session.

**Histology**

Small lesions were made by passing DC (15–20 μA for 5 s) through the microelectrode to mark the recording site in the spinal cord (Fig. 1A) and the stimulation site in the cerebellum (Fig. 1B). The tissue was then preserved in 10% neutral formalin, then prepared for sectioning (50-μm thick) using a microtome. Sections were stained using neutral red.

**RESULTS**

Fifteen units were isolated. All units were activated by CRD, while seven were also activated by applying cutaneous stimuli on the hindlimbs or the pelvic region. The isolated neurons had a low level of background discharges (0–10 spikes/s) and were classified as “nociceptive” based primarily on their responses to CRD at intensities higher than 40 mmHg but also on their responses to noxious cutaneous stimuli where applicable. Accordingly, all cells were classified as nociceptive even though some also responded to nonnoxious stimulation.

**Neuronal response characteristics**

Cerebellar stimulation caused significant increases in the responses of cells to all intensities of CRD (Figs. 2B and 3). Facilitation occurred in nine neurons using only chemical injections compared with six using only electrical current. However, when the percent increase in the responses to graded visceral stimuli was compared between the two neuronal populations (chemical versus electrical stimulation) using a *t*-test, the two groups were not found to be significantly different (*P* > 0.5). Therefore data from both groups were pooled in Fig. 3. However, variable effects were observed on the responses to somatic stimuli (Table 1). For example, the responses to PR and PI applied on the hindlimb of one rat were increased (Table 1, rat I and Fig. 2C), but only the responses to BR were increased in another rat while those to PR and PI were decreased (Table 1, rat II). Applied alone, cerebellar stimulation did not seem to have an effect on the background discharges of cells (Fig. 2C4). In addition, some responses to graded CRDs and somatic stimuli were tested twice before cerebellar stimulation to rule out any sensitization effect that may have been
caused by repetitive noxious stimulation. Repeated noxious stimulation did not alter the response pattern (examples of repetitive visceral and somatic stimuli are shown in Fig. 2, A and C, I and 2, respectively). In one rat, responses to graded CRDs applied alone 20 min after cerebellar stimulation with DLH were still significantly increased compared with the initial responses, indicating that the cerebellar effects may be long lasting (data not shown).

**DISCUSSION**

The brain regions involved in the processing of nociceptive information are often described as sensory regions because pain is first and foremost a sensory experience, and in parallel, a motor function has been attributed to the cerebellum. However, many objections have been raised to the conceptual schism between sensory and motor phenomena (Fetz 1992), especially in the cerebellum (Bloedel and Bracha 1995; Houk 1997; Schmahmann 1991, 1997; Schmahmann and Pandya 1997), which seems to play a role in both cognitive (Fiez 1996; Schmahmann and Sherman 1997) and nociceptive information processing (Wu and Chen 1991).

In this study, we have shown that stimulation of the cerebellar cortex increases the responses of spinal nociceptive neurons to noxious, as well as nonnoxious, CRDs and often modulates their responses to different somatic stimuli. The disparity in the effects of cerebellar stimulation on visceral versus somatic spinal inputs may be due to the disparity in the effects of cerebellar stimulation on visceral versus somatic spinal pathways (for example, Sluka et al. reported in 1997 that...
intradermal injection of capsaicin in monkeys caused a significant increase in the responses of spinothalamic cells to weak mechanical stimuli but not to noxious mechanical or heat stimuli). Furthermore it is not clear whether the increase in the responses to nonnoxious stimuli observed in this study implies that the same stimuli may provoke a nociceptive reaction based solely on these data. If so, then this reaction should produce allodynia in the unanesthetized state, whereas increases in the responses to noxious stimuli should result in hyperalgesia. The neurons were isolated from the midline zone of the spinal cord near the central canal. This region contains cells of origin of a newly identified component of the postsynaptic dorsal column (PSDC) pathway that largely conveys visceral input to the dorsal column nuclei (Al-Chaer et al. 1996a,b). In fact, the PSDC was suggested to contribute, at least in part, to the somatic nociceptive input to the cerebellum (Ekerot et al. 1991). However, it is not clear whether both visceral and somatic information is relayed via the same pathway to the cerebellum.

When the effects of cerebellar stimulation are appraised at the spinal or peripheral levels, they are described as “rather complex” (Eccles et al. 1967, p. 257). This complexity is due to the fact that even focal electrical stimulation excites not only Purkinje cells, the sole output from the cerebellar cortex, but also granule cells and their axons, basket, stellate, and Golgi cells and climbing and mossy fibers, as well as mossy and climbing fiber collaterals that may activate the inferior olive and many subcortical nuclei. In this study, the chemical injection of DLH may have limited the activation to cortical cells around the injection site by only depolarizing cell bodies of Purkinje cells and cerebellar interneurons but not axons coursing en passant (Goodchild et al. 1982), such as climbing or mossy fibers. The effects of electrical and chemical stimulation in this study were not significantly different.

The enhancement of nociceptive responses observed in this study is consistent with the analgesic effects previously described following cerebellar cortical lesions (Chambers and Sprague 1955a,b; Russel et al. 1894; but also refer to Bloedel and Bracha 1995). Moreover, an interesting observation made by Siegel and Wespic (1974) points to an alteration of nociceptive thresholds by electrical stimulation of the cerebellum in monkeys. These authors found that a profound and long-lasting analgesia was produced on activation of the brachium conjunctivum. However, stimulation of lobulus simplex (lobule VI) and other regions of the posterior cerebellum resulted in decreased thresholds for withdrawal from a noxious stimulus. In another study in which the effect of cerebellar lesions on the potency of morphine-induced analgesia was tested in rats, it was reported that lesions of the anterior cerebellum markedly decreased the duration of analgesia caused by systemic administration of morphine (Dey and Ray 1982). However, prolongation of the analgesic effects following posterior cerebellar lesions was also noted. In light of these and our findings, it is likely that the anterior cerebellum may be exerting an overall anti-nociceptive effect. By contrast, the posterior cerebellum decreases the latency of withdrawal from noxious stimuli and enhances spinal nociceptive responses.

Cerebellar modulation of spinal cord nociceptive responses and pain-behavior must depend on descending pathways to the spinal cord (Mendlin et al. 1996; Palay and Chan-Palay 1982; Voogd and Glickstein 1998). Anatomical interconnections exist between the cerebellum and the reticular formation of the brain stem, nucleus raphé magnus, locus ceruleus, and the periaqueductal gray region, all considered to be involved in descending inhibition or “sensory gating.” In this case, stimulation of the cerebellar cortex may inhibit the deep cerebellar nuclei projecting to these brain stem centers, thus relieving the spinal nociceptive neurons from tonic inhibition. If cellular responses to CRD and somatic noxious stimuli can be used as indices for pain, then there seems to be a cerebellar role in the modulation of pain.

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