Effects of spinal cord stimulation on the flexor reflex and involvement of supraspinal mechanisms: an experimental study in mononeuropathic rats

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The physiological mechanisms responsible for pain relief caused by spinal cord stimulation (SCS) are essentially unknown and recent experimental data are sparse. In the present study the authors explored the possible involvement of supraspinal mechanisms in the effects of SCS applied in rats with experimental mononeuropathy produced by sciatic nerve ligation according to the method of Bennett and Xie or that of Seltzer, et al. Confirming results of a previous study undertaken by the authors, the thresholds of the early component of the flexor reflex (latency 8–12 msec), which is mediated by A fibers, were significantly lower in the nerve-ligated than in the intact leg. In halothane-anesthetized animals the spinal cord was exposed and SCS was applied with parameters similar to those used in clinical SCS. Ten minutes of SCS produced a significant elevation of the lowered threshold of the early flexor component only in the nerve-ligated leg, and this augmentatory effect of SCS persisted for 30 to 40 minutes after cessation of the stimulation. The threshold elevation amounted to between 50% and 80% of the prestimulatory value and it was related to the intensity of SCS. The threshold of the late, C-fiber–mediated component of the flexor reflex was not influenced in either of the legs. After transection of the spinal cord at the T-6 level, there was a moderate threshold increase in both the early and late components in both legs, but the threshold of the early component in the nerve-ligated leg remained lower. Spinal cord stimulation produced an almost identical threshold increase in the early component in the nerve-ligated leg with the same time course as before the transection. There was no effect on the late component of the reflex in either leg. The results indicate that this effect of SCS in mononeuropathic rats does not necessarily involve supraspinal mechanisms; instead SCS is operative at a spinal, segmental level. In view of the similarities between the effects of therapeutic SCS on cutaneous hypersensitivity in patients with peripheral neuropathic pain and the effects demonstrated in neuropathic rats, the clinical pain relief achieved with SCS may be produced, at least partially, by intraspinal mechanisms.

KEY WORDS • spinal cord stimulation • neuropathic pain • allodynia • flexor reflex • nerve injury • rat
trodos to avoid injury to the plantar skin. Low-intensity peripheral stimuli in lightly anesthetized animals produced an early component of the flexor reflex, believed to represent the activation of Aβ-fibers, which may also be involved in the production of the withdrawal response to innocuous mechanical stimuli. The “classic” flexor reflex corresponding to C-fiber activation appears only in response to high-intensity peripheral stimuli. After nerve ligation both the early and late component of the flexor reflex displayed decreased thresholds in the nerve-ligated leg compared to thresholds before ligation and to those in the contralateral, intact leg. Spinal cord stimulation resulted in a marked threshold increase in the early component confined to the nerve-ligated leg, whereas the C-fiber–mediated, late component was unaffected in both legs.

Several hypotheses have been proposed to explain the effector mechanisms responsible for the pain alleviation produced by SCS. In principle, the inhibitory effects of SCS on the transmission of nociceptive impulses may be exerted segmentally in the spinal cord and/or at a supraspinal level. There is some evidence, both in experimental and clinical studies, to support the idea that SCS involves supraspinal mechanisms or at least a supraspinal loop. This notion appears to be compatible with the fact that in patients the perception of paresthesia–like sensations throughout the painful region is a prerequisite for effective pain relief. In view of our previous findings on the selective effect of SCS on the early, A-fiber–mediated component of the flexor reflex in mononeuropathic rats, it seemed reasonable to use the same experimental design and to study the effects of spinal cord transection to test whether the hypothetical supraspinal mechanisms are involved in the effects of SCS.

Materials and Methods

The experiments were performed using a group of approximately 60 adult female Sprague-Dawley rats weighing 200 to 300 g. The results presented in this paper are based on data derived from 15 animals that presented with mechanical allodynia after nerve ligation and displayed a reproducible early component of the flexor reflex both before and after spinal transection (see below). The study was approved by the Regional Ethical Committee for Animal Research.

Nerve Ligation

The rats were anesthetized with 3% halothane in a mixture of 50% oxygen/50% air delivered by means of an open-circuit nose mask at 1.5 L per minute. Body temperature of the animals was maintained at 37.5 ± 0.5°C with an automatic heating device. Eight of the 15 animals were operated on according to the technique described by Bennett and Xie, and seven according to Seltzer, et al. (Hereafter, these animals will be referred to as Bennett and Seltzer rats, respectively). In the former group of animals the common sciatic nerve was exposed at the level of the mid-thigh. Four loose ligatures (No. 4 chromic catgut) were tied around the nerve to minimize the risk of inducing a cumulative effect caused by repeated applications of SCS at different intensities. The stimulation unit with monophasic rectangular pulses and constant current control. The stimulation parameters were chosen to correspond as closely as possible to those used in clinical practice. The stimulation frequency was 50 Hz and the pulse width 0.2 msec. First, the stimuli intensity required to produce a segmental motor response in the lower thorax or leg stretching was assessed. In the subsequent experiment SCS was applied with different intensities ranging from 40% to 80% of the amount needed to produce such a motor response. The intensity of SCS varied between 0.36 and 1.6 mA. Inhalation anesthesia was reduced to 0.5% to 0.75% halothane, and SCS at different intensities was applied for 10 minutes. To minimize the risk of inducing a cumulative effect caused by repeated applications of SCS with different intensities, the first SCS session was always performed using an intensity corresponding to two-thirds of the motor threshold. Spinal cord stimulation was then applied with either gradually decreasing or gradually increasing intensities in a random fashion. The 10-minute SCS sessions were separated by approximately 60 minutes. The thresholds of the flexor reflex in both legs were monitored every 3rd or 5th minute before, during, and after SCS.

Spinal Cord Transection and SCS

Once the effects of SCS on the thresholds of both the early and late components of the flexor reflex response had been assessed, a second laminectomy at the level T-6 was performed. After opening the dura the spinal cord was transected with a sharp knife. The halothane concentration was again decreased and, after 30 to 60 minutes, the thresholds of the early and late components of the flex-

Behavioral Tests

A few days after the nerve-ligation procedure, the animals were tested daily or every 2nd day for the presence of allodynia/hyperalgesia in their hindpaws with the set of von Frey filaments calibrated to 1.3, 5.5, 10, 20, and 30 g pressure. Observations were made with the rats placed in a transparent plastic chamber with a mesh metal floor. Each filament was pressed against different areas of the plantar skin until the filament began to bend. The procedure was repeated six times with filaments of different stiffnesses. Thresholds were compared for the same parts of the paw with the nerve-ligated and the contralateral hindpaw. The final assessment of the thresholds was based on tests performed on 3 consecutive days prior to the main experiment. Only animals having a markedly and consistently decreased withdrawal threshold—corresponding to at least two grades of the stiffness of the filaments—in the nerve-ligated leg were selected for subsequent experiments.

Recording of the Flexor Reflex and SCS

Two to 3 weeks after nerve ligation the animals were again lightly anesthetized with halothane and placed in an experimental frame with their hind limbs extended. Stimulating plate electrodes (diameter 3–4 mm) were taped, 10 to 12 mm apart, to the plantar skin of the hindpaws. Stainless steel needle electrodes were inserted bilaterally in the biceps femoris for recording the hindlimb flexor reflex. Responses were recorded, rectified, and averaged, then transferred to a computer program to be stored and analyzed. Stimuli applied to the skin consisted of rectangular 1-msec pulses delivered at 0.2 Hz. To produce the early component of the flexor reflex response a low-intensity current of 0.6 to 1.8 mA was used, whereas to produce the late component of the response 6 to 20 mA was required. Six to eight responses to the threshold stimulation were averaged. The thresholds of both the early and late components of the response were assessed before additional surgery was performed. After infiltration of the superficial subcutaneous tissue with 0.5% lidocaine, the sciatic nerve was exposed by a laminectomy of T12–13. The dura was preserved intact and covered with prewarmed paraffin oil. Monopolar electrical stimulation was applied to the dorsal aspect of the cord using a silver sphere, 1 mm in diameter, which was suspended on a spring and lightly rested on the spinal dura. The relationship between the size of this stimulating electrode and the width of the rat spinal cord is comparable to that of a flat disc-shaped spinal electrode often used clinically and the diameter of the human spinal cord. Stimulation was performed using a photoelectrical isolation unit with monophasic rectangular pulses and constant current control. The stimulation parameters were chosen to correspond as closely as possible to those used in clinical practice. The stimulation frequency was 50 Hz and the pulse width 0.2 msec. First, the stimulus intensity required to produce a segmental motor response in the lower thorax or leg stretching was assessed. In the subsequent experiment SCS was applied with different intensities ranging from 40% to 80% of the amount needed to produce such a motor response. The intensity of SCS varied between 0.36 and 1.6 mA. Inhalation anesthesia was reduced to 0.5% to 0.75% halothane, and SCS at different intensities was applied for 10 minutes. To minimize the risk of inducing a cumulative effect caused by repeated applications of SCS with different intensities, the first SCS session was always performed using an intensity corresponding to two-thirds of the motor threshold. Spinal cord stimulation was then applied with either gradually decreasing or gradually increasing intensities in a random fashion. The 10-minute SCS sessions were separated by approximately 60 minutes. The thresholds of the flexor reflex in both legs were monitored every 3rd or 5th minute before, during, and after SCS.
or reflex were determined. Thereafter, the SCS protocol was repeated.

Statistical Analysis

The threshold values and times to return to prestimulatory threshold for all animals are presented in this paper as means ± standard error of the means. The differences between means of values obtained before, during, and after SCS and before and after spinal cord transection were examined with two-tailed Student’s t-tests for dependent and independent observations where appropriate. A probability level of less than 0.05 was considered significant.

Sources of Supplies and Equipment

The automated heating device used to maintain rat body temperature was the CMA/150, obtained from CMA Microdialysis AB, Stockholm, Sweden. Digitimer, Ltd. (Welwyn, Garden City, UK) provided the Neurolog System used to record the hindlimb flexor reflex and the Stimulator DS7, used to stimulate the skin during that experiment. During surgery, stimulation was performed with the PSIU6 provided by Grass Instruments Co. (Quincy, MA) or with the Neurolog Stimulus Isolator NL800, available from Digitimer Ltd. For storage and analysis of signals, a MacLab program was used in conjunction with a MacIntosh II vx computer.

Results

Signs of Mononeuropathy

Mononeuropathy produced by the two different methods was associated with typical changes in the posture of the nerve-ligated hindpaw. These changes often were already present 1 to 2 days after surgery and remained stable for 6 to 8 weeks, after which the animals gradually resumed a normal posture. Before nerve ligation, the thresholds for eliciting a withdrawal response to von Frey filaments corresponded to 10 to 20 g in the entire group of animals. After nerve ligation the thresholds in the nerve-ligated paw decreased to 1 to 3 g and remained unchanged in the intact paw. The magnitude of the threshold changes as well as the abnormality of paw posture scored according to Attal, et al., did not significantly differ between the Bennett and Seltzer rats. It should be added that definite signs of allodynia in the form of a consistent decrease in the withdrawal threshold occurred in approximately 80% of the nerve-ligated animals.

Flexor Reflex and Spinal Cord Transection

The flexor reflex, which was elicited by electric stimulation of the plantar skin of the hindpaw and recorded electromyographically, consisted of three components. Low-intensity stimulation produced a short-lasting, biphasic deflection with a latency of 8 to 12 msec. This component was present only in animals under light anesthesia and even then it could be absent. In approximately 20% of the intact animals we failed to produce this early component; in 20% it was lost following nerve ligation or laminectomy for SCS; and in approximately 25% of the remaining animals it was lost after spinal cord transection. At a somewhat higher stimulus intensity the early response was followed by a second component with more irregular configuration and lasting 10 to 20 msec. When the stimulus intensity was further increased (typically five to 10 times that of the threshold of the early component) a late and long-lasting, bursting type of response appeared with a latency of 200 to 250 msec. This part of the response corresponded to the “classic” flexor reflex, and its presence was less dependent on the depth of the anesthesia and surgery than the early component.

After nerve ligation the thresholds of both the early and late components were significantly decreased in the nerve-ligated leg as compared to the thresholds before surgery and to those of the intact leg after surgery. The mean threshold of the early component was 1.7 mA and that of the late component 7.8 mA in the nerve-ligated leg compared to 2.0 mA for the early and 10.4 mA for the late component in the intact leg (p < 0.01). In only two of 15 animals were the thresholds of both components the same in both legs. There were no statistically significant differences in these respects between the Bennett and Seltzer models. The configuration and latency of the early and late components were determined.
Effects of SCS on the Flexor Reflex Before and After Spinal Cord Transection

The latencies and configurations of the early and late components were not influenced by spinal cord transection. As previously reported, SCS applied to the intact spinal cord in lightly anesthetized animals produces a marked increase in the threshold of the early component of the flexor reflex in the nerve-ligated leg.\(^\text{16}\) This effect could be reproduced in all animals except one, which also failed to display a difference in the flexor reflex thresholds between the nerve-ligated and intact leg. The graphs in Fig. 1A illustrate the effect of SCS applied for 10 minutes on the mean thresholds of the early component of the flexor reflex recorded from nerve-ligated and intact legs in the present group of 15 animals before spinal transection. It appears that the maximum value of threshold elevation, the time to reach peak value, and the time to return to prestimulatory values were markedly different in the two legs and clearly related to the intensity of the SCS. Stimulation using a relatively low intensity (40% to 60% of the intensity required to produce a motor response) resulted in a moderate and successive increase in threshold values in the nerve-ligated leg. With a somewhat higher stimulus intensity (approximately two-thirds of that needed to produce a motor response) the thresholds of the early component were augmented approximately 75% by the end of the SCS period in the nerve-ligated leg, whereas the thresholds in the intact leg were only slightly influenced. The threshold augmentations were statistically significant (p < 0.01) for all SCS intensities used. There was also a marked poststimulatory effect: it generally took 30 to 40 minutes after cessation of the SCS until prestimulatory threshold levels were restored (see also Fig. 3). It is obvious that, although there was a slight or moderate threshold elevation also in the intact leg, the duration was short and the prestimulatory threshold values were generally attained within 10 minutes after the SCS application.

It should be emphasized that the effect of SCS on the flexor reflex was confined to the early component evoked in the nerve-ligated leg. The late component, whether elicited by threshold or suprathreshold peripheral stimuli, was unaffected in spite of the fact that the threshold was lower in the nerve-ligated than in the intact leg.

Figure 1B shows the effects of SCS on the early component in both legs after spinal transection. Although the thresholds in both legs were markedly increased after the transection, the selective augmentative effect of SCS on the thresholds in the nerve-ligated leg remained. In fact, the threshold elevations before and after transection were markedly similar. For example, SCS that was applied with an intensity two-thirds of that required for a motor response produced increases in the thresholds of the nerve-ligated leg amounting to 76% before and 72% after spinal cord transection. The relationship between the intensity of SCS and the magnitude of threshold elevations in both nerve-ligated and intact legs before and after spinal transection are shown in Fig. 2. It is evident that the values are highly similar. The same applies to the rates of return to prestimulatory threshold values after SCS application with different intensities before and after the transection; the marked difference between the nerve-ligated and the intact leg in that respect was in no way influenced by the surgery (Fig. 3).

It should be noted that after spinal cord transection SCS, regardless of intensity, had no effect on the late component of the flexor reflex in the legs.
Discussion

The previous report that SCS exerts a selective increasing effect on the lowered threshold of the early Aβ-mediated component of the flexor reflex in the nerve-ligated leg was confirmed by this series of experiments. We failed to observe any effect on the late component evoked by high-intensity peripheral stimuli, which may represent the activation of C fibers. Previously it was reported that in intact animals SCS does not depress the flexor reflex, which instead may even be moderately facilitated. These observations were made in intact animals and it was concluded that the nociceptive flexion reflex does not supply a suitable model for the study of the effect of SCS on pain. These findings are compatible with our observation that SCS did not influence any of the components of the flexor reflex in the intact leg in our rat models of mononeuropathy. Furthermore, the C-fiber-mediated component of the reflex in the nerve-ligated leg was not affected by the stimulation. However, it should be noted that in patients undergoing treatment with SCS a suppressive effect of the stimulation on the late component of the flexor reflex (RIII) has been reported. As discussed in a previous study, the selective effect of SCS on the low threshold, Aβ-fiber-mediated component of the flexor reflex is compatible with earlier findings in patients in whom therapeutic SCS could markedly reduce allodynia associated with spontaneous pain, whereas acute nociceptive pain, either experimentally induced or present as a result of trauma, was resistant to SCS. There is no obvious explanation why two of the rats displayed no difference in flexor reflex thresholds between the nerve-ligated and intact leg, in spite of the fact that both of them presented with decreased withdrawal threshold to mechanical stimulation with von Frey filaments. The reason may be that the plate electrodes used to evoke the flexor reflex happened to be placed outside the most allodynic area of the plantar surface of the nerve-ligated leg.

Spinal transection in the present study resulted in a moderate, but consistent, increase of the thresholds of both the early and late components of the flexor reflex. This was a somewhat unexpected finding in view of the well-known fact that spinal cord transection results in a facilitation of the nociceptive flexor reflex. In the present study the spinal cord was transected at a middle or upper thoracic level and up to 1 hour elapsed before the experiment was resumed. The augmentation of the flexor reflex thresholds is compatible with a residual spinal “shock.” However, it should be noted that the configurations, latencies, and relative relation between the thresholds of the two components were all unchanged after the transection.

In the few studies that have been designed to explore the possible sites of the inhibitory effect of SCS, acute and noxious peripheral stimuli in normal animals have been used. As mentioned above, this type of study design appears less appropriate in view of the fact that therapeutic SCS is particularly effective for chronic, neurogenic forms of pain. Inhibition produced by SCS may be exerted at two alternative or complementary levels of the central nervous system, segmentally or supraspinally. In addition, there is also the possibility of a simple block of transmission of nociceptive impulses, which could occur as a result of collision with antidromic activity evoked by SCS in ascending pathways. This explanation as well as the notion of peripheral mechanisms are, however, less likely because of the long-lasting poststimulatory effects observed in patients undergoing this treatment. Needless to say, supraspinal centers must become involved with a perception of stimulation-induced paresthesia, and it has been claimed that these sensations may have a masking effect on pain. However, it has been argued that these sensations are an epiphenomenon indicating only that the large fiber systems in the dorsal columns are orthodromically activated but do not necessarily form a link in a supraspinal loop activating inhibitory mechanisms in the brainstem and thalamus.

The development of SCS as a therapeutic modality directly evolved from an experimental model of pain inhibition according to the gate-control theory that postulates inhibition of noxious impulses at a spinal, segmental level. These ideas have been challenged and the importance of SCS-induced inhibitory effects also on a supraspinal level has been emphasized. Saadé and coworkers have reported experiments on cats subjected to various types of peripheral noxious stimuli and to SCS. These investigators observed inhibitory effects on nociceptive transmission in the dorsal horn as well as on flexor reflexes that could not be attributed to antidromic activation of the dorsal columns, which were cut caudally to the stimulating electrode. Instead they ascribed the inhibition to a supraspinal loop mediated via the dorsal column nuclei, the raphé system, and the dorsolateral funiculus. It should also be noted that in their experiments, SCS applied at a level below the transected dorsal columns resulted in a partial but consistent inhibition of the late, C-fiber-mediated component of the flexor reflex. Their results are at variance with our observation that SCS did not influence either the threshold of that late component or its amplitude when evoked with suprathreshold peripheral stimuli. It is difficult to explain the lack of accord between our results and those of Saadé and coworkers in this respect; however, it may be that they applied SCS with higher intensity than we did in the present study in which the intensity was set in relation to the motor threshold.

Recently, Rees and Roberts have summarized studies concerned with the possible function of the anterior pretectal nucleus in the modulation of nociception. This nucleus obviously receives excitatory input from low-threshold afferents via the dorsal columns, and the authors hypothesized that this structure may take part in the long-lasting pain inhibition induced by SCS. They also found that electric stimulation applied rostrally to a transection of the dorsal columns produced almost the same inhibitory effect as stimulation of the intact system, thus indicating the possible involvement of a supraspinal loop.

Some studies provide circumstantial evidence that supraspinal structures are involved in the effect of SCS, and it has been reported that SCS produced long-lasting alteration of cortical and thalamic evoked potentials and depression of activity in the medial thalamus. In the present study the selective SCS-produced threshold augmentation of the early component of the flexor reflex evoked in the nerve-ligated leg was preserved after spinal cord transection, and the late component of the reflex remained resistant to SCS. Moreover, the time course of
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this effect and its poststimulatory duration, as well as the relationship between the intensity of SCS and the magnitude of threshold augmentation, were almost identical before and after the transection. These findings indicate that the effects of SCS in rats with mononeuropathy may be independent of supraspinal mechanisms. Thus, our results provide circumstantial evidence favoring the hypothesis that the suppression of neuropathic pain in patients undergoing treatment with SCS is predominantly dependent on spinal, segmental mechanisms, although the participation of mechanisms on several neural levels is possible in the intact organism.

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References

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