Electrical Stimulation of Large Myelinated Afferents Inhibits Responses of Rat Spinal Dorsal Horn Neurons to Noxious and Innocuous Cutaneous Stimulation

Masayoshi Tsuruoka, Yong-Ning Wang* and Yoichiro Matsui

Department of Physiology, School of Dentistry, Showa University, 1-5-8 Hatano-dai, Shinagawa-ku, Tokyo 142, Japan (Chief: Prof. Yoichiro Matsui) *Present address: Department of Neurobiology, Institute of Experimental Medicine, Capital Institute of Medicine, You An Men, Beijing 100054, China

Abstract: The inhibitory effects of electrical stimulation of large myelinated afferents on spinal dorsal horn neurons were investigated. In rats anesthetized with thiamylal sodium, responses of 153 dorsal horn neurons to noxious heating or innocuous mechanical stimulation (light brushing) of the tail were recorded from the sacral and coccygeal levels of the spinal cord by extracellular microelectrodes. Of these neurons, 45 were low-threshold mechanoreceptive (LTM), 47 were nociceptive-specific (NS), and 61 were wide-dynamic-range (WDR) neurons. In 22.2% of the LTM neurons, responses to innocuous mechanical stimulation were inhibited by electrical stimulation of large myelinated afferents applied to the ipsilateral hindlimb for 5 min. This conditioning stimulation (large myelinated afferent stimulation, LMAS) also inhibited noxious heating responses in 19.2% of the NS neurons. The inhibitory effect of LMAS on WDR neurons was nonselective in that both responses to light brushing and noxious heating were inhibited. Of the WDR neurons, 27.3% were inhibited by LMAS. These results indicate that inhibition produced by LMAS is exerted on all 3 classes of spinal dorsal horn neurons.

Key words: large myelinated afferent stimulation, dorsal horn neurons, inhibition, rat

The spinal cord dorsal horn is involved in the somatosensory mechanisms. Dorsal horn neurons are functionally classified on the basis of their cutaneous receptive field properties as low-threshold mechanoreceptive (LTM), nociceptive-specific (NS), or wide-dynamic-range (WDR) neurons. LTM neurons respond to weak mechanical stimuli and exhibit no discharge increase in response to noxious stimuli. NS neurons receive inputs exclusively from nociceptors. WDR neurons are activated by weak mechanical stimuli, but these neurons respond maximally to intense and potentially tissue-damaging stimulation. The activity of dorsal horn neurons can be inhibited by stimuli applied to the same dermatome as a receptive field of the neuron. This segmental inhibition can be produced by activation of large myelinated afferents as well as by activation of nociceptive afferents. The activity of dorsal horn neurons is also inhibited by electrical stimulation with high intensity current or noxious cutaneous stimuli applied to an area of the body remote from the excitatory receptive fields of the neurons. This type of inhibition is known as the "diffuse noxious inhibitory controls" (DNIC). Characteristics of DNIC are that these are effective only on WDR neurons, but not on LTM and NS neurons.

Recently, we found that nociceptive responses of WDR neurons are inhibited by electrical stimulation of large myelinated afferents applied to an area of the body remote from the excitatory receptive fields of the neurons. This inhibition is totally different from other spinal inhibition. Concerning this type of inhibition, effects on LTM and NS neurons still remain unclear, because only the effects on WDR neurons were examined in a previous study. The present study, there-
fore, was designed to examine the effect of electrical stimulation of large myelinated afferents on LTM and NS dorsal horn neurons. In addition, effects on nonnociceptive responses of WDR neurons is also not known. In the present study, the inhibitory effects of electrical stimulation of large myelinated afferents were compared between nociceptive and nonnociceptive responses of WDR neurons.

Materials and Methods

Thirty-eight female Wistar albino rats (240–310 g body weight) were anesthetized with thi-amyal sodium (80 mg/kg i.p. initially) and maintained during surgery with supplementary 8 mg/kg doses. Cannulae were inserted into the trachea and the left femoral vein. The spinal cord, from the sacral to the coccygeal levels (S3-Co2 segments), was exposed by laminectomy, and the vertebra was rigidly held in a frame. After the dura was removed, the spinal cord was covered with warm mineral oil. The animals were paralyzed with gallamine triethiodide (10–20 mg/kg i.v.) and maintained on artificial respiration. The body temperature was maintained between 38 and 39°C with a heating pad controlled by a rectal thermistor, and electrocardiogram monitoring was used throughout the experiment to aid in assessing the condition of the animal.

Neuronal activity in the dorsal horn was recorded extracellularly with glass micropipettes filled with 0.5 M sodium acetate containing 2% pontamine sky blue (resistance 5–10 MΩ). Electrical and natural stimulation were applied to the ipsilateral side of the tail. After neurons were searched by electrical stimulation (rectangular constant current pulses, 7 mA, 0.1 ms) of the tail, they were tested for responsiveness to natural cutaneous stimulation to identify the neuron type. Natural cutaneous stimulation was either light brushing (innocuous mechanical stimulation) or noxious heating. Light brushing was delivered at 2 strokes per second by a reciprocating motion of a badger-hair brush. Noxious radiant heating was by a projection lamp focused through a condensor lens. A fine copper-constantan thermocouple was glued to the center of the heat spot to record the skin temperature during radiant heat stimulation. The mean skin temperature of the tail before heating was $28.4 \pm 0.7°C$ (mean ± S.D., $n=38$). The applied heat was kept below 55°C to avoid tissue damage. The neuronal responses were recorded on magnetic tape, and played back on an oscilloscope and through a window discriminator so the discharge rate could be recorded by a pulse analyzing system (Nihon Kohden SEN-6104).

Large myelinated afferent stimulation (LMAS) was applied to the hindlimb ipsilateral to the noxious heat stimulation. A pair of stainless steel needles were inserted into the skin on the hindlimb, and large myelinated afferents were stimulated transcutaneously with 0.1 ms rectangular constant current pulses at 50 Hz. The stimulus intensity was 3 times the threshold. LMAS was delivered for 5 min since the minimum threshold duration of 5 min for application of LMAS was required for producing the inhibitory effect. These parameters of LMAS were the same as those described in a previous study. The common peroneal nerve was the same as those described in a previous study.

The recording sites of the neuronal events were marked by electrophoresis (20 mA, 5 min) of pontamine sky blue from a microelectrode. At the end of each experiment, the animals were perfused intracardially with 10% formalin. The recording sites were verified from reconstruction of the electrode tracks in frozensections.

Results

The activity of 153 dorsal horn neurons was recorded from the sacral and the coccygeal levels of the spinal cord. Forty-two neurons were in laminae I–II, 50 in laminae III–IV, and 61 in laminae V–VI. Of these 153 neurons, 45 were LTM neurons, and 47 and 61 of the remaining 108 nociceptive neurons were NS and WDR neurons, respectively.

Forty-five LTM neurons tested responded to
light brushing of the tail with the MDF of 90–125 impulses/s. With 18 LTM neurons, neuronal discharges in response to light brushing were measured every 5 min for 45 min. In this control trial, the MDF did not change substantially during 45 min without LMAS. With 27 neurons, effects of LMAS were examined. When the MDF were measured immediately after the cessation of 5 min LMAS, in 6 of 27 neurons, it decreased from the 95% confidence interval of the control trial. The MDF reduced 34.2 ± 6.4 impulses/s (mean ± S.D., n = 6) as compared with the value before LMAS (control value). This change in the MDF from the control value was statistically significant compared to the control (p < 0.01, two-sided paired t-test). An example is shown in Fig. 1. In the remaining 21 neurons, the MDF was within the range of the 95% confidence interval even after LMAS was applied. There were no neurons in which responses were facilitated.

In responses of NS neurons to noxious heating, the threshold temperature for firing was in the range of 45.8–48.2°C, and discharge frequencies increased to 70–110 impulses/s with an increase of temperature during heating up to 55°C. In the control trial which was tested with 21 neurons, the MDF did not alter substantially during the control trial. The application of LMAS resulted in the decrease of the MDF from the 95% confidence interval of the control trial in 5 of 26 neurons. The MDF of 5 inhibited neurons reduced 29.5 ± 4.8 impulses/s (n = 5) as compared with the control value. An increase of the MDF was not observed. An example is shown in Fig. 1.

WDR neurons responded to noxious heating with maximum discharges of 70–130 impulses/s; the threshold temperature for firing was in the range of 44.7–49.4°C. With 29 WDR neurons, the MDF in responses to noxious heating was measured to calculate the 95% confidence interval of the control trial. WDR neurons also responded to light brushing with discharges of 60–
110 impulses/s. Twenty-one neurons were tested for the control trial. In both responses to light brushing and noxious heating, the change of substance of the MDF was not seen during the control trial. With 22 WDR neurons, the effects of LMAS were compared between responses to noxious heating and those in light brushing. In 6 neurons, the MDF in both responses reduced from the 95% confidence interval when LMAS was applied (Fig. 2). The reduction of the MDF in response to noxious heating and light brushing was 42.4±5.1 impulses/s ($p<0.01$, $n=6$) and 48.2±11.2 impulses/s ($p<0.01$, $n=6$). There was no statistical difference between the inhibitory effect on nonnociceptive responses and that on nociceptive responses. In the remaining 16 neurons, neither of the MDF in responses decreased from the 95% confidence interval even after LMAS. Table 1 summarizes the proportion of neurons inhibited by light brushing or noxious heating. The inhibition produced by LMAS was exerted predominantly on WDR neurons rather than LTM and NS neurons.

The time-course of inhibitory effects of LMAS was similar among the 3 classes of dorsal horn neurons. As shown in Fig. 3, the MDF decreased by LMAS recovered to the control level at 10 min after the cessation of LMAS. In each class of dorsal horn, the MDF at 10 min after the cessation of LMAS was not significantly different from the control value ($p>0.1$).
Discussion

In the present study, we established a standard for the valuation of the inhibitory effect to investigate the proportion of inhibited neurons. The inhibitory effect was estimated by the decrease of the MDF from the 95% confidence interval of the control trial. A 95% confidence interval is often used statistically for the interval estimate of the median of population. Furthermore, the 95% confidence interval has been used for the estimation of effectiveness of some clinical treatment, such as acupuncture effect in man. Therefore, it is reasonable to use a 95% confidence interval for the valuation of the inhibitory effect of LMAS.

The inhibition produced by LMAS was exerted on all 3 classes of dorsal horn neurons, although it was predominant on WDR neurons rather than LTM and NS neurons. It is obvious from this result that the inhibitory effects are not related to the response characteristics of dorsal horn neurons. Furthermore, in WDR neurons, LMAS inhibited both nonnociceptive and nociceptive responses. These findings indicate that LMAS lead to a decrease of sensitivity of all somatic sensations including pain. One of characteristics of DNIC is that the inhibition is exerted only on WDR neurons, but not LTM and NS neurons. The results from DNIC and the present study suggest that the neural mechanisms in the central nervous system are different between two inhibitory effects produced by peripheral nerve stimulation.

In each class of dorsal horn neurons, the complete inhibition, which is the reduction of the MDF in response to zero, was not observed in the condition of LMAS in the present study. Concerning the effects of LMAS on the jaw opening reflex and the tail flick reflex in the rat, it has been reported that the inhibitory effect depends on the stimulus frequency of LMAS. The degree of inhibition of the MDF in response, therefore, may increase with the increase of stimulus frequency of LMAS. However, this assumption could not be cleared in the present study since effects of LMAS delivered at various frequencies were not examined quantitatively.

The inhibition produced by LMAS may contribute in part to the analgesia produced by counter-irritation procedures, such as transcutaneous electrical nerve stimulation and electroacupuncture because nociceptive responses of NS and WDR neurons are inhibited by LMAS. Maixner et al. have shown that WDR neurons, and not NS neurons, are involved in the encoding process by which monkeys perceive the intensity of noxious stimuli near the detection threshold. Their study suggests that inhibition of nociceptive responses of WDR neurons participate in the pain relieving process. In the present study, the inhibition produced by LMAS was more effective on WDR neurons than NS neurons. In this context, it is possible that the inhibition produced by LMAS is in part involved in the alleviation of pain.

Although it remains unclear what role this type of inhibition plays in the processing of sensory information under natural conditions, results of the present study suggest that electrical stimulation of large myelinated afferents not only excites neurons in both the spinal cord dorsal horn and dorsal column nuclei to transmit sensory information to higher centers, but also inhibits responses of dorsal horn neurons, if applied for at least 5 min.

![Figure 3: The time-course of inhibitory effects of LMAS on 3 classes of dorsal horn neurons.](image-url)
References

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