Synaptic responses of substantia gelatinosa neurones to dorsal column stimulation in rat spinal cord *in vitro*

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1. To study the mechanism of dorsal column stimulation-induced depression of nociceptive transmission in the spinal cord, synaptic responses evoked in dorsal horn neurones by dorsal column and dorsal root stimulations were examined in a horizontal spinal cord slice of the adult rat. Intracellular recordings were made from substantia gelatinosa (SG) neurones.

2. All SG neurones examined received monosynaptic inputs and/or polysynaptic inputs from both dorsal column and dorsal root. Aδ fibres were probably responsible for the synaptic responses. The responses evoked by dorsal column stimulation were similar to those evoked by primary afferent Aδ fibre stimulation.

3. Monosynaptic excitatory postsynaptic potentials (EPSPs) evoked by dorsal column Aδ fibres were depressed by 6-cyano-7-nitroquinoxaline-2,3-dione, suggesting that these fibres released L-glutamate or a related amino acid as a transmitter.

4. In 38 of 101 SG neurones, dorsal column stimulation evoked an initial EPSP followed by fast and/or slow inhibitory postsynaptic potentials (IPSPs). These IPSPs reversed polarity at a membrane potential of -73 ± 2 mV. The fast IPSPs observed in 16 of the SG neurones (42 %) that received inhibitory inputs were depressed by strychnine, while the slow IPSPs observed in 22 SG neurones were depressed by bicuculline. In a few cells, a long-lasting slow IPSP with a much slower time course was detected; this IPSP was insensitive to strychnine and bicuculline, and reversed polarity at a membrane potential near −90 mV.

5. Repetitive stimulation of the dorsal column depressed the amplitude of monosynaptic EPSPs evoked by dorsal root stimulation.

6. The responses of SG neurones to dorsal column stimulation had configurations and durations similar to responses to dorsal root stimulation, and may be mediated largely by the same Aδ fibres. However, a C fibre-mediated response could not be detected in SG neurones from dorsal column stimulation, although dorsal root stimulation could evoke C fibre-mediated monosynaptic EPSPs in 18 of 88 SG neurones (20 %).

7. These observations suggest that SG neurones receive abundant Aδ but not C fibre inputs from the dorsal column and that dorsal column stimulation inhibits primary afferent transmission in the spinal cord both by reducing transmitter release from primary Aδ fibres and by hyperpolarizing SG neurones.

Many fine afferent fibres that carry nociceptive information terminate in the superficial dorsal horn of the spinal cord, particularly in the substantia gelatinosa (SG, lamina II of Rexed) (Rexed, 1952; Maxwell & Réthelyi, 1987; Sugiura, Terui & Hosoya, 1989; Yoshimura & Jessell, 1989*a*, 1990). SG neurones form local interneuronal circuits that are thought both to integrate afferent information from different fibre classes and to modify the output of projection neurones located in lamina I and in deeper regions of the dorsal horn (Kumazawa & Perl, 1978; Light, Trevino & Perl, 1979).

Melzack & Wall (1965) proposed the gate control theory for the dorsal horn circuitry responsible for pain transmission. In their model, SG inhibitory neurones, activated by large afferent fibres, act as a gate to control the efficiency of

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transmission between primary afferent and nociceptive projection cells. The theory has led to the hypothesis that the stimulation of myelinated dorsal column fibres may suppress the nociceptive transmission of projection cells. The hypothesis is supported by anatomical and clinical observations. Anatomical studies have provided evidence that a subset of fine myelinated and unmyelinated fibres enter the dorsal column and terminate in the SG (Horch, Burgess & Whitehorn, 1976; Light & Perl, 1979; Sugiura *et al.* 1989). Clinical observations show that dorsal column stimulation is a useful method for pain relief (Nashhold & Friedmann, 1972; Shimoji *et al.* 1977). It would be expected that there is a mechanism in the SG that allows activity in myelinated fibres in the dorsal column to produce an inhibition of the responses of dorsal horn neurones to noxious stimuli. However, direct analysis of responses in SG neurones evoked by dorsal column stimulation has not been done, because of the difficulty in obtaining stable intracellular recordings from small SG neurones *in vivo*.

We have developed an *in vitro* adult rat spinal cord slice preparation to achieve stable intracellular recordings from SG neurones. Furthermore, we used a horizontal slice preparation, since this type of slice seemed suitable as a model for dorsal column stimulation-produced depression of nociceptive transmission. We examined the synaptic responses of SG neurones evoked by dorsal column and dorsal root stimulation, and the effects of dorsal column stimulation on the response evoked by dorsal root stimulation.

METHODS

Preparation of the slice

The methods for the preparation of the spinal cord slices used in this study were similar to those described elsewhere (Yoshimura & Jessell, 1989*a*), except that horizontal slices were used. Briefly, 7- to 9-week-old Sprague–Dawley rats (150–250 g) were deeply anaesthetized with urethane (1·5–2·0 g kg^{-1}, I.P.). After lumbosacral laminectomy, a 1·5–2·0 cm length of spinal cord, with attached ventral and dorsal roots, was excised and placed in pre-oxygenated cold (4–6 °C) Krebs solution. All ventral and dorsal roots, with the exception of the L5 or L6 dorsal root on one side, were cut and then the arachnoid membrane was removed. The spinal cord, with attached dorsal root, was placed in a shallow groove formed in an agar block. A horizontal slice with a thickness of about 500 μm was made using a vibratome while the spinal cord was immersed in cold Krebs solution. The slice was mounted on a nylon mesh in a recording chamber (volume, 1·0 ml). The slice was completely submerged and was superfused continuously with Krebs solution (25–40 ml min^{-1}) equilibrated with 95 % O_2–5 % CO_2 at 36 ± 1 °C. The composition of the Krebs solution was as follows (mM): 117 NaCl, 3·6 KCl, 2·5 $CaCl_2$, 1·2 $MgCl_2$, 1·2 NaH_2PO_4, 25 $NaHCO_3$ and 11 glucose.

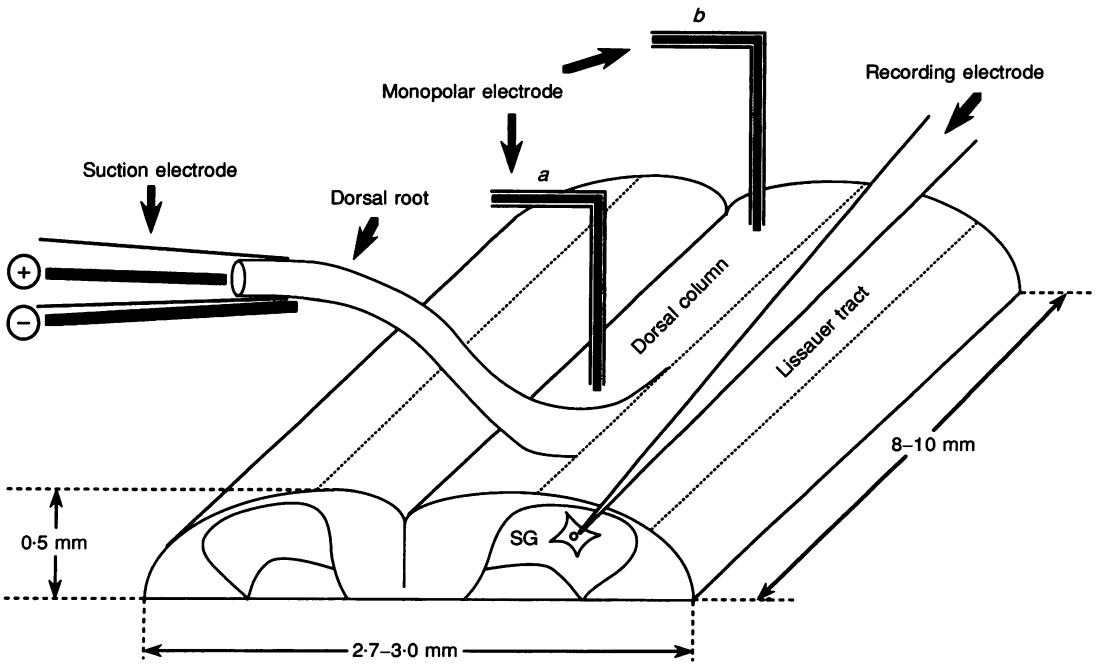

Figure 1. Schematic arrangement for intracellular recording and for stimulation of dorsal column and dorsal root

Microelectrodes were inserted into the substantia gelatinosa (SG) lateral to the dorsal root entry and through Lissauer's tract and lamina I. Dorsal column and dorsal root were stimulated by a silver wire monopolar electrode and suction electrode, respectively. In some experiments, the dorsal column was stimulated at two sites (*a* and *b*) separated by 3–5 mm.

Intracellular recording and stimulation

Intracellular recordings were performed from neurones located in the SG with microelectrodes, filled with 4 M potassium acetate, that had DC tip resistances of 120–200 MΩ. Signals were amplified with a high input impedance bridge amplifier (Axoclamp 2A, Axon Instruments, Foster City, CA, USA) and were monitored on a digital oscilloscope (VC-11, Nihon kohden, Tokyo, Japan). Values for the resting membrane potential were determined by rapid withdrawal of the microelectrode. Negative rectangular current pulses of 300–500 ms duration and 0·05 nA or less were injected thorough the recording electrode to measure neuronal input resistance.

A dorsal column was stimulated with a monopolar silver wire electrode which was insulated with Teflon, except at the tip, and had a diameter of 50 μm (Fig. 1). Using stimuli of relatively low intensity (5T, see below) and brief duration (0·1 ms), displacement of the stimulating electrode from the ipsilateral to the contralateral dorsal column resulted in the loss of synaptic responses, indicating that the spread of stimulating current was confined to a small region. Therefore, the synaptic responses evoked by the electrode located above the dorsal column were due to activation of fibres in the dorsal column but not due to activation of fibres or neurones in Lissauer's tract or in the SG. The electrode was positioned one to three segments rostral or caudal to the preserved dorsal root (3–8 mm from the recording electrode).

The dorsal root, which had a length of 5–12 mm, was orthodromically stimulated by means of a suction electrode (Fig. 1). Stimuli of short duration and low intensity (1–5 V, 0·1 ms) were used for myelinated fibres (Aβ and Aδ fibres) and relatively higher intensity and longer duration stimuli (6–12 V, 0·4 ms) for unmyelinated fibres (Yoshimura & Jessell, 1989*a*, 1990). The stimulus intensity was expressed relative to the threshold for Aβ fibres (1T = 1·0 V). The threshold for Aβ, Aδ and C fibres was measured by recording the compound action potential in the sciatic nerve–dorsal root preparation as described previously (Yoshimura & Jessell, 1990). In four rats a comparison was made of the thresholds for the main components of the compound action potential of the preparation when using a suction and a focal stimulation electrode applied to the dorsal root. No significant difference was found between the two with stimulus intensities of up to 20 V. The conduction velocity of fibres that were responsible for the monosynaptic response was calculated from the latency of evoked excitatory postsynaptic potentials (EPSPs) and the distance from the stimulating electrode to the recording site. More precise conduction velocity was determined in selected instances by placing two stimulating electrodes, separated by 3–5 mm, on the dorsal column and by recording the response latency intracellularly from SG neurones (Fig. 1). This method compensates for errors in calculation due to the decrement in conduction velocity of fibres within the dorsal column and avoids the need to estimate the length of fibres in the dorsal column. The conduction velocity calculated with this method was up to 20% faster than that calculated with single electrode stimulation.

Identification of SG neurones

Under a binocular microscope with transillumination, the superficial dorsal grey matter lateral to the dorsal root entry zone was discernible in the horizontal slice as a relatively translucent band under Lissauer's tract (Fig. 1). SG neurones were identified by their depth from the dorsal surface, and from their membrane properties which have been previously analysed in the transverse slice (Yoshimura & Jessell, 1989*b*). The recorded neurones were located at a depth of between 50 and 230 μm at the L4–L5 level of the spinal cord in adult rats; this depth was within the SG when measured in the transverse slice. In addition, the resting membrane potential, mean input resistance, time constant and spike duration of recorded neurones obtained in the horizontal slice were quite similar to those of SG neurones identified in transverse slices (North & Yoshimura, 1984; Yoshimura & Jessell, 1989*a*, *b*; Yoshimura & Jessell, 1990). Most of the neurones impaled in horizontal slices had input resistances of more than 200 MΩ. Neurones that had a low input resistance were located either in deeper layers more than 250 μm from the dorsal surface of the spinal cord, presumably in laminae III and IV, or in the most superficial layer of the dorsal horn, presumably in lamina I. Therefore, the neurones that showed a low input resistance were probably either non-SG neurones or neurones impaled improperly; neurones with an input resistance of less than 150 MΩ were not analysed further. Neurones located in deeper layers, more than 250 μm deep, could respond to low-intensity stimulation (1T, 0·1 ms) of the dorsal root and dorsal column. This stimulus intensity was insufficient to activate Aδ fibres (Yoshimura & Jessell, 1989*a*, 1990). Aβ fibres, therefore, seemed to be responsible for this low-intensity stimulation-evoked response.

Drug application

Drugs were applied by exchanging the perfusion solution with one containing a known drug concentration, without altering the perfusion rate or the temperature. The time required for the drug-containing solution to flow from the three-way tap to the recording chamber was about 5 s. Drugs used were 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM; Tocris Neuramin, Bristol, UK), DL-2-amino-5-phosphonovaleric acid (APV, 50 μM; Sigma, St Louis, MO, USA), strychnine (0·5–2 μM; Sigma) and bicuculline (10–20 μM; Sigma).

RESULTS

General characteristics of SG neurones

Intracellular recordings were obtained from 101 SG neurones located near the preserved dorsal root entry zone. Stable intracellular recordings could be obtained from slices maintained *in vitro* for more than 12 h and recordings were made from single SG neurones for up to 6 h. The resting membrane potential of SG neurones in the horizontal slice preparation was -67 ± 1 mV (mean ± S.E.M., $n = 49$), the input resistance was 266 ± 12 MΩ ($n = 44$) and the time constant was 29 ± 3 ms ($n = 18$). The input-resistance values obtained in the horizontal slices, in which the dendrites should be intact, was consistent with results from the transverse slice (North & Yoshimura, 1984; Yoshimura & Jessell, 1989*a*, *b*; Yoshimura & Jessell, 1990) but much higher than other comparable studies (Murase & Randić, 1983; King, Thompson, Urban & Woolf, 1988; Schneider & Perl, 1988). The high input-resistance value probably resulted from the fact that our recordings were confined to SG neurones and that we used adult animals.

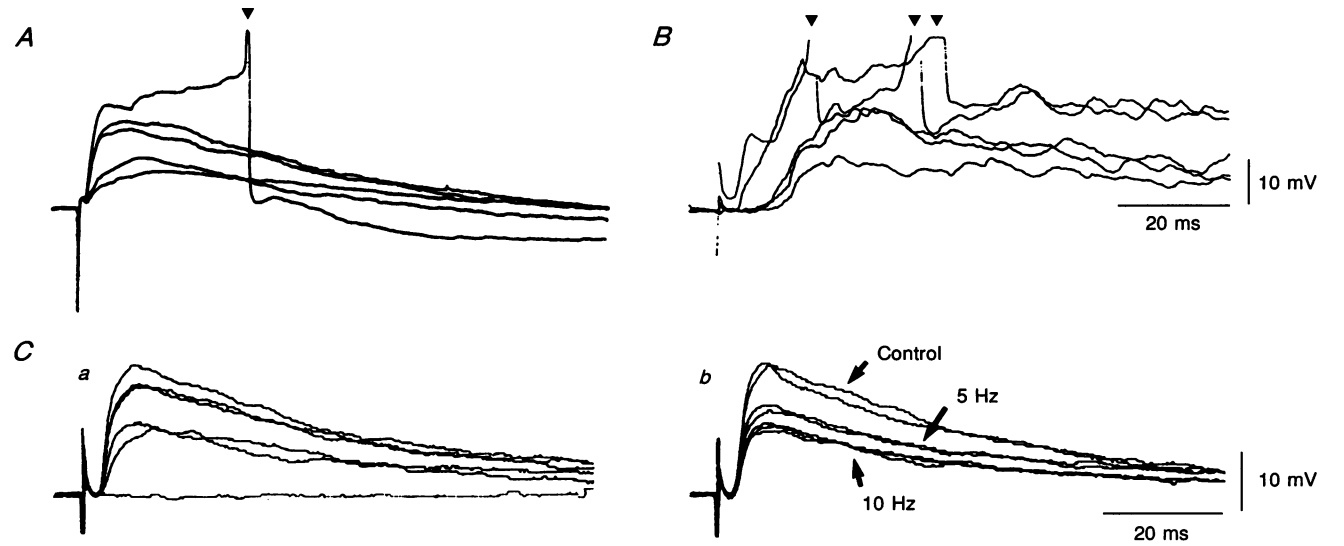

Figure 2. Fast monosynaptic and polysynaptic EPSPs recorded intracellularly from SG neurones in response to dorsal column stimulation

Records were obtained from three neurones (*A*, *B* and *C*). *A*, short and constant-latency EPSPs elicited by dorsal column stimulation at a membrane potential of −73 mV. The EPSPs increased in amplitude with increasing stimulus intensity. The responses were obtained with stimulus intensities of 2, 4, 6, 8 and 11*T*. *B*, long and variable-latency EPSPs evoked by dorsal column stimulation at a membrane potential of −74 mV. Note that the latency of EPSPs decreased with increasing stimulus intensity. *C*, monosynaptic EPSPs in an SG neurone evoked by dorsal column stimulation at a membrane potential of −69 mV. The latency of EPSPs remained constant when the stimulus intensity was increased gradually from subthreshold to supramaximal intensities (10*T*, 0·4 ms; *Ca*) and when the threshold stimuli were applied repetitively at high frequency (5 and 10 Hz; *Cb*). ▼, truncated spikes.

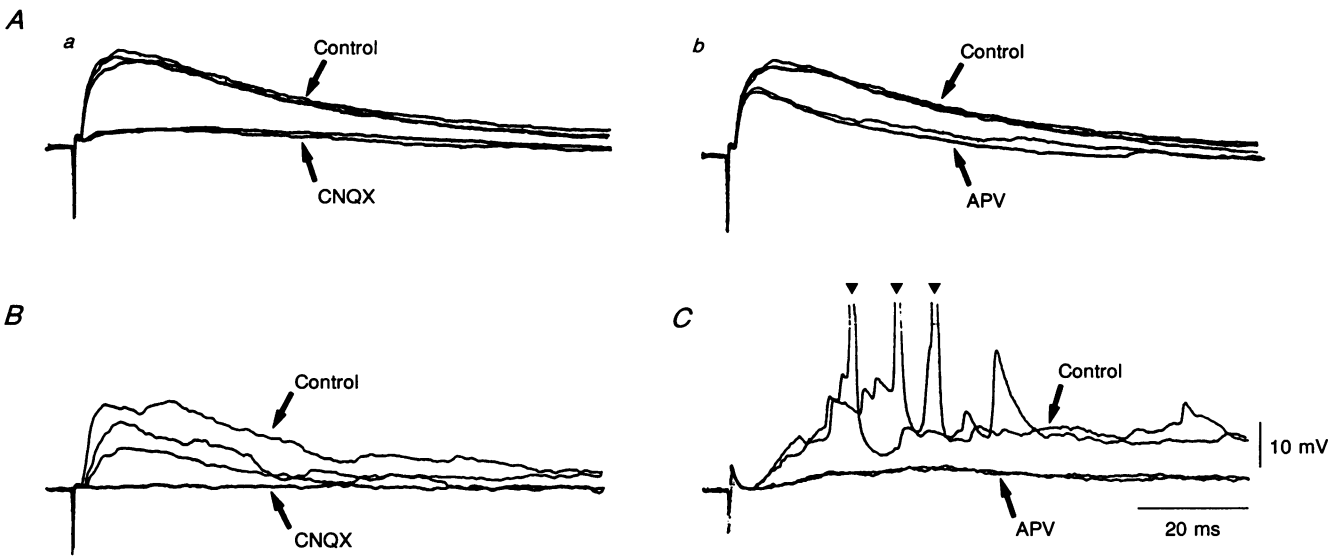

Figure 3. Inhibition of monosynaptic and polysynaptic EPSPs by the amino acid receptor antagonists CNQX and APV

Records shown were obtained from three neurones (*A*, *B* and *C*). *Aa*, the amplitude of the monosynaptic EPSP was significantly depressed by CNQX (10 μM). *Ab*, APV had a small effect on the peak amplitude of the EPSP but significantly depressed the falling phase of the EPSP. *B*, the polysynaptic EPSPs were abolished by CNQX. *C*, the polysynaptic EPSPs were depressed but not abolished by APV. Records before and during application of the antagonists are superimposed in each panel. Membrane potentials were −68 mV in *A*, −66 mV in *B*, and −68 mV in *C*. ▼, truncated spikes.

SG neurones in horizontal slices exhibited spontaneous EPSPs that rarely initiated action potentials similar to those reported in transverse slices (Yoshimura & Jessell, 1989*a*). All SG neurones tested ($n = 101$) received EPSPs from both dorsal column and dorsal root (Fig. 2). In addition to excitatory synaptic inputs, inhibitory postsynaptic potentials (IPSPs) were observed in 38 of the 101 SG neurones in response to dorsal column stimulation (Fig. 4). No antidromic spikes were observed in response to dorsal column stimulation.

Properties of the EPSPs evoked by dorsal column stimulation

In 83 of the 101 SG neurones recorded, dorsal column stimulation evoked short and constant-latency fast EPSPs that had amplitudes of 8–28 mV and durations of 40–100 ms (Fig. 2*A*). These short and constant-latency fast EPSPs were monosynaptic. The latency of the fast EPSPs remained constant both when the stimulus intensity was increased from threshold to supramaximal (Fig. 2*Ca*) and when threshold stimuli were applied repetitively at high frequency (5–10 Hz; Fig. 2*Cb*). In addition, the latency of EPSPs was short, ranging from 1·2 to 2·5 ms; these values were shorter than those of variable-latency EPSPs (Fig. 2*B*; see below). In the remaining 18 SG neurones, the post-stimulus latencies of the EPSPs were long and variable (Fig. 2*B*), suggesting polysynaptic inputs. In these neurones, the amplitudes and durations of EPSPs were usually augmented and the latency was shortened by an increase in stimulus intensity (Fig. 2*B*).

Measurements of conduction velocity and the stimulus intensity required to activate fibres in the dorsal column both indicated that the monosynaptic EPSPs were mediated by Aδ fibres. The values of conduction velocity were 1·5–3·5 m s^{-1} which are the slowest values within the range obtained *in vivo* for Aδ fibres (Woolf & Fitzgerald, 1983; Light & Perl, 1984). These values were somewhat slower than those of Aδ fibres in the dorsal root (2–7 m s^{-1}) (Yoshimura & Jessell, 1989*a*). This difference may result from the fact that the conduction velocity slows near the bifurcation point, where primary afferent fibres give off collaterals (Horch *et al.* 1976). In addition, monosynaptic EPSPs were detected after stimulation of the dorsal column with single pulses of between 1·9 and 5·0T and 0·1 ms in duration. This intensity was presumed to be sufficient to activate Aδ but not C fibres, since it evoked Aδ but not C fibre-mediated EPSPs when the dorsal root was stimulated with the same monopolar electrode ($n = 6$).

In five SG neurones, low-intensity stimulation (1·0–1·8T, 0·1 ms), which was insufficient to activate Aδ fibres, evoked the variable-latency EPSPs. These EPSPs may reflect polysynaptic inputs driven by the activation of Aβ fibres.

All SG neurones tested responded to stimulation of the dorsal column caudal as well as rostral to the recording site. Synaptic responses evoked by rostral and caudal dorsal column stimulation were essentially the same. Although we have not tested the synaptic responses evoked by stimulation of the dorsal column farther than three segments rostral or caudal to the recordings site, it is likely that the spread of many Aδ fibres is restricted to a few segments, since the

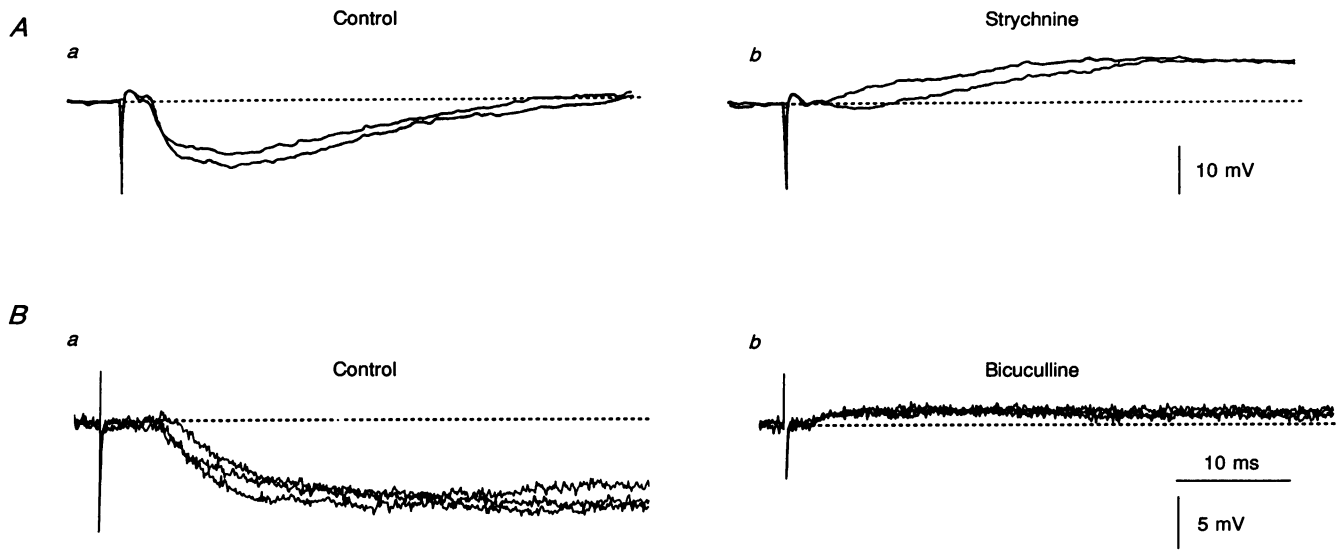

Figure 4. Effect of strychnine and bicuculline on fast and slow IPSPs
A, the fast IPSP with a duration of about 40 ms was blocked by strychnine (2 μM). During application of strychnine, an EPSP, presumably polysynaptic, was unmasked (*A b*). *B*, the slow IPSP with a duration of about 200 ms was blocked by bicuculline (20 μM; *B b*). During the application of bicuculline, an EPSP was unmasked (*B b*). Dotted lines indicate the control membrane potential. Membrane potentials were maintained at −52 mV in *A* and −54 mV in *B* by applying DC current through the recording electrodes.

synaptic responses decreased in amplitude with increasing distance of the stimulating electrode from the recording site. This notion is consistent with anatomical observations (Horch *et al.* 1976).

Effect of excitatory amino acid antagonists on the EPSPs evoked by dorsal column stimulation

We examined the sensitivity of monosynaptic and polysynaptic EPSPs, evoked by dorsal column stimulation, to the non-*N*-methyl-D-aspartic acid (non-NMDA) receptor antagonist CNQX and the NMDA receptor antagonist APV to test whether they were mediated by L-glutamate. CNQX and APV had no effect on the resting potential (102 ± 3 % relative to the resting potential, $n = 6$) and the input resistance (103 ± 1 % relative to control, $n = 4$) of SG neurones. The amplitude of monosynaptic EPSPs evoked by dorsal column stimulation was reduced by 74 ± 2 % ($n = 6$) in the presence of 10 μM CNQX (Fig. 3*A a*) and returned to control within 10–15 min of wash-out of CNQX. The half-decay time of monosynaptic EPSPs decreased by 30 % in the presence of APV (50 μM) and the amplitude of EPSPs was slightly reduced ($n = 5$; Fig. 3*A b*). These findings are consistent with other studies showing that the EPSP is mediated mainly by non-NMDA receptors and that NMDA receptors contribute primarily to the later components of EPSPs (Dale & Roberts, 1985; Forsythe & Westbrook, 1988; Yoshimura & Jessell, 1990).

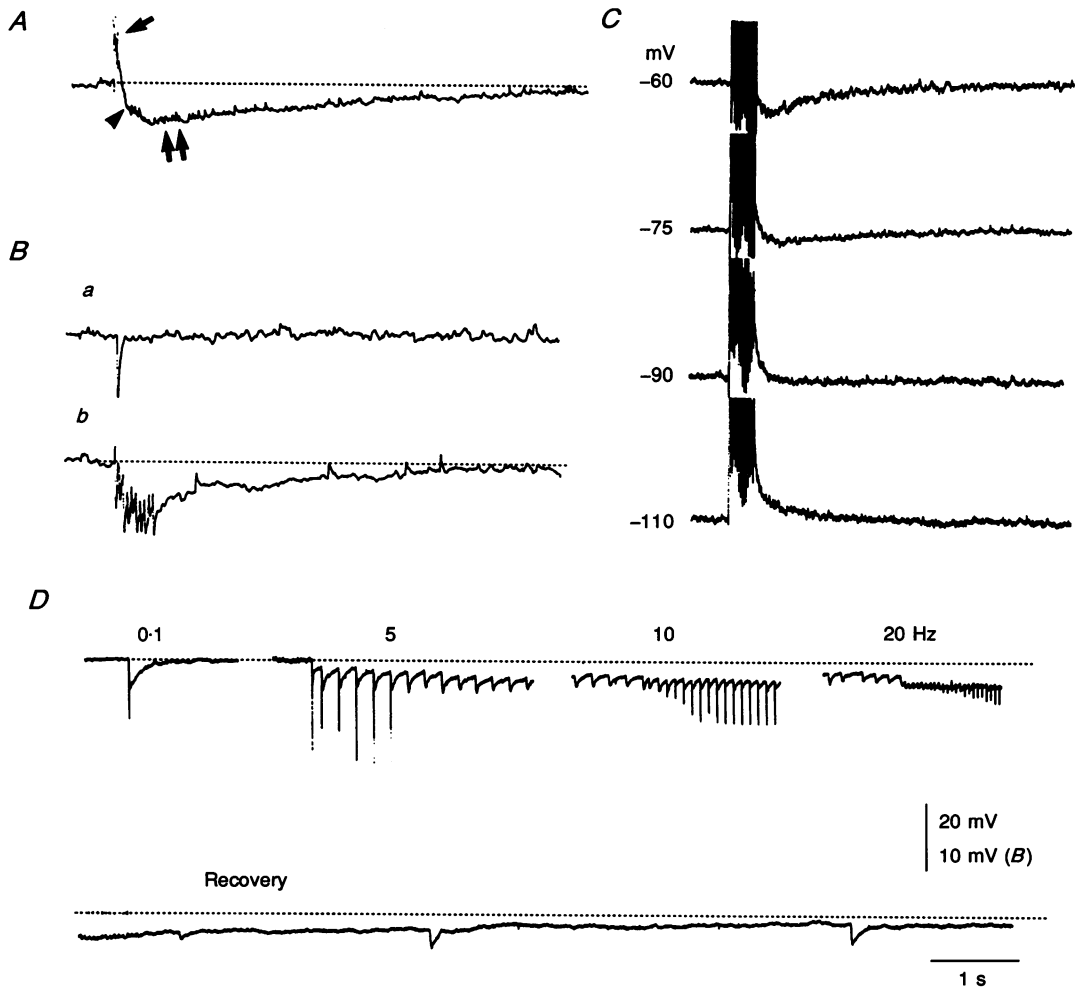

Figure 5. Long-lasting slow IPSPs evoked by dorsal column stimulation
Records shown were obtained from four neurones (*A*, *B*, *C* and *D*). *A*, the fast EPSP (arrow) was followed by a slow (arrowhead) and a long-lasting slow IPSP (double arrows) which had a duration of 5–15 s. *B*, single dorsal column stimulation sufficient to activate Aδ fibres evoked only the fast IPSP (*Ba*), but the long-lasting slow IPSP could be elicited by repetitive stimulation (20 Hz, 10 pulses) at the same intensity (*Bb*). *C*, the reversal potential of the long-lasting slow IPSP was near −90 mV. The dorsal column was stimulated repetitively with 15 pulses at 20 Hz. *D*, repetitive stimulation applied to the dorsal column at various frequencies resulted in prominent summation of the long-lasting slow IPSP. The long-lasting slow IPSP lasted for more than 20 s after cessation of the stimulation. Membrane potentials were maintained at −58 mV in *A*, −50 mV in *B* and −55 mV in *D*.

Next we examined the sensitivity of polysynaptic EPSPs to CNQX and APV. The polysynaptic EPSPs were almost completely blocked in the presence of 10 μM CNQX ($n = 5$; Fig. 3*B*). The polysynaptic EPSPs were also decreased by 50 μM APV ($n = 3$; Fig. 3*C*). These findings accord with the results obtained from transverse slices with dorsal root stimulation (Yoshimura & Jessell, 1990).

IPSPs evoked by the dorsal column stimulation

In 38 out of the 101 SG neurones (38 %), the stimulation of dorsal column Aδ fibres evoked IPSPs that were occasionally preceded by EPSPs. Based on the time course, three types of IPSP (fast, slow and long-lasting slow IPSP) could be distinguished. The fast and slow IPSPs were evoked by single shock stimulation. The fast IPSP was observed in 16 of the SG neurones (42 %) that received inhibitory inputs and had a duration of 52 ± 7 ms (range, 40–70 ms; Fig. 4*A a*). A slow IPSP was observed in 22 SG neurones (58 %) and had a much slower time course (mean duration, 385 ± 51 ms; range, 100–500 ms; Fig. 4*B a*). Both IPSPs reversed polarity at membrane potentials near −70 mV (not shown), suggesting that these IPSPs were mediated by an increase in chloride conductance.

Several lines of evidence indicate that glycine and GABA are the main inhibitory transmitters in the spinal cord (Willis & Coggeshall, 1991). To elucidate the transmitter mediating the IPSPs observed in SG neurones in response to dorsal column stimulation, we examined the effect of glycine and $GABA_A$ receptor antagonists. The glycine receptor antagonist strychnine (0·5–2 μM) reversibly depressed the fast IPSPs (Fig. 4*A b*). This concentration of strychnine had no appreciable effect on the slow IPSPs. In contrast, the $GABA_A$ receptor antagonist bicuculline

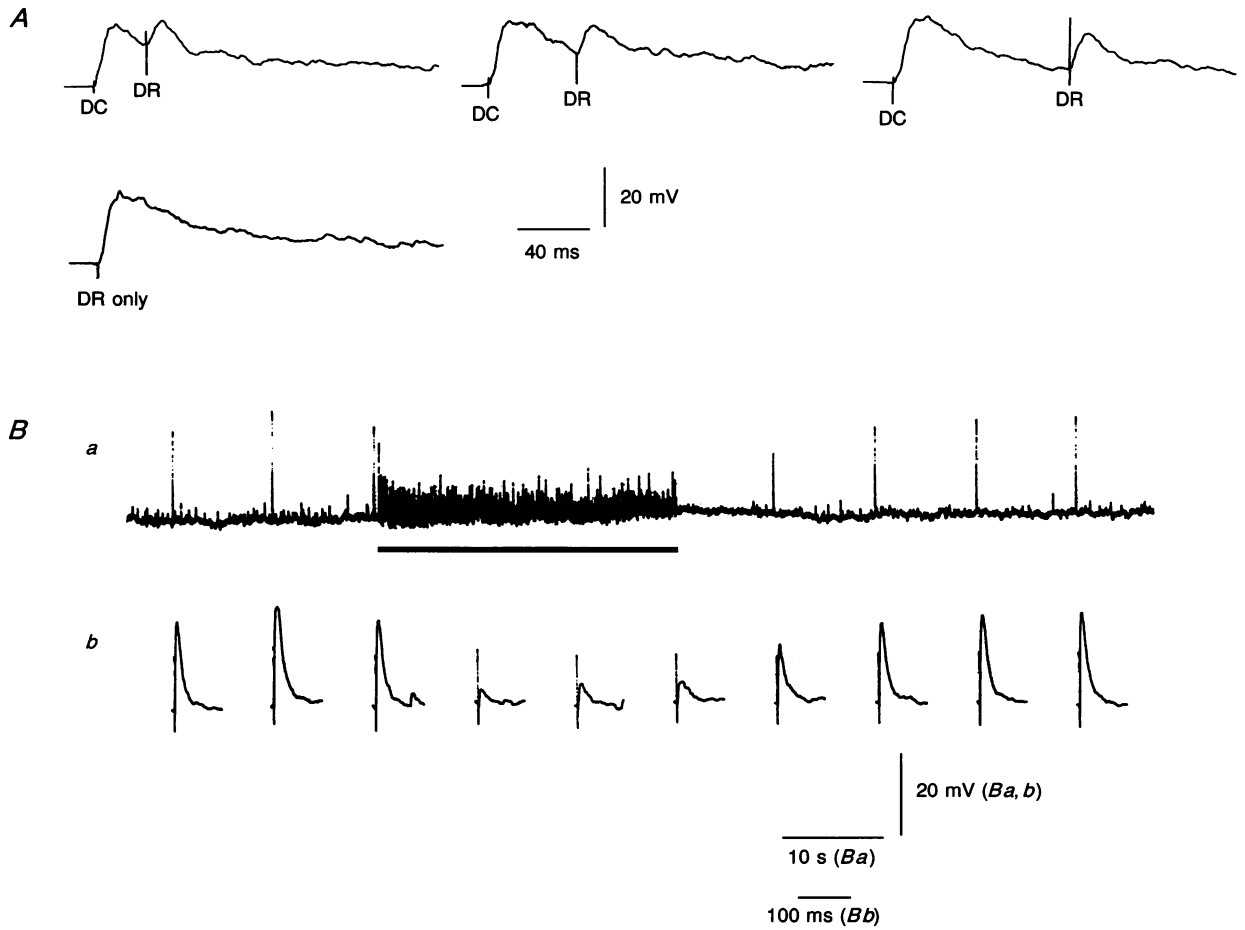

Figure 6. Effect of dorsal column stimulation on the monosynaptic EPSPs evoked by dorsal root stimulation

A, the monosynaptic EPSP evoked by dorsal root stimulation was depressed by preceding dorsal column stimulation. The depression lasted more than 500 ms. DR, dorsal root stimulation; DC, dorsal column stimulation. *B*, during repetitive dorsal column stimulation at 10 Hz (indicated by the bar), the monosynaptic EPSP evoked by dorsal root stimulation was effectively depressed. This effect lasted for about 30 s after the end of stimulation. Primary afferent Aδ fibres in the dorsal root were stimulated every 10 s (*B b*). Membrane potential was −67 mV.

(10–20 μM) depressed the slow IPSPs (Fig. 4*Bb*). These observations suggest that the fast and slow IPSPs were mediated by glycine and by GABA, respectively, with the latter interacting with $GABA_A$ receptors.

Using these antagonists, it was shown that in ten of the thirty-eight SG neurones, single shock stimulation of the dorsal column evoked a sequence of fast–slow IPSPs. In twenty of the thirty-eight SG neurones (53 %) that exhibited the fast and/or slow IPSPs, a long-lasting slow IPSP was elicited by repetitive dorsal column stimulation (Fig. 5*A* and *B*). The long-lasting slow IPSPs showed prominent summation (Fig. 5*B*). With a single stimulus, the long-lasting slow IPSP was detectable in only four SG neurones. The long-lasting slow IPSP increased in amplitude and duration with an increasing number of pulses (Fig. 5*Bb*). The long-lasting slow IPSP recorded in SG neurones had a duration of less than 1 s with a single stimulus, but was prolonged up to 30 s by brief repetitive stimulation (Fig. 5*D*). The long-lasting slow IPSP reversed its polarity at a membrane potential near −90 mV (Fig. 5*C*) which was consistent with the potassium equilibrium potential (Yoshimura & North, 1983; North & Yoshimura, 1984). The transmitter for the long-lasting slow IPSP could not be identified. The long-lasting slow IPSP was insensitive to both strychnine and bicuculline, being rather augmented in amplitude and duration in the solution containing those antagonists. Further study is required to elucidate the pharmacological properties of long-lasting slow IPSPs detected in SG neurones.

Effect of dorsal column stimulation on the EPSPs evoked by dorsal root stimulation

The effect of dorsal column stimulation on Aδ and C afferent fibre-evoked responses was studied in thirty-five SG neurones. The monosynaptic EPSPs evoked by dorsal root stimulation were depressed in amplitude by preceding stimulation of the dorsal column at an intensity sufficient to activate Aδ fibres ($n = 7$; Fig. 6*A*). The stimulation of Aβ fibres in the dorsal column did not produce changes in the amplitude of dorsal root-evoked monosynaptic EPSPs. This depression was observed (for more than 500 ms) even after the dorsal column-evoked EPSPs had decayed to the control membrane potential, suggesting the involvement of a presynaptic mechanism. When the dorsal column was stimulated repetitively at 10 Hz for 30 s, the depression of dorsal root-evoked EPSPs lasted about 30 s after cessation of the stimulation ($n = 10$; Fig. 6*B*). The dorsal root-evoked EPSPs were depressed in amplitude by more than 70 % during the stimulation (Fig. 6*Bb*).

In eighteen SG neurones, repetitive (10 Hz) dorsal column stimulation caused a steady membrane hyperpolarization due to the summation of IPSPs (Fig. 7*A*). During the repetitive stimulation, the C fibre-mediated excitation of SG neurones was effectively depressed (Fig. 7*B*). The

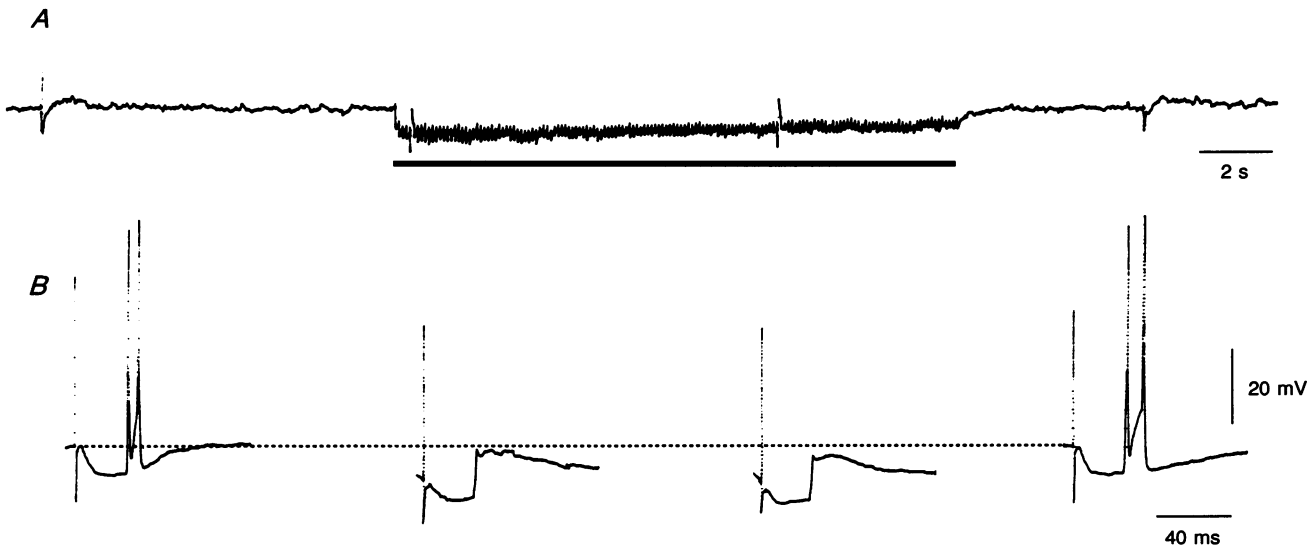

Figure 7. Blockade of spike firing by repetitive dorsal column stimulation
A, repetitive dorsal column stimulation at 10 Hz (indicated by the bar), at an intensity sufficient for Aδ fibre activation, produced a persistent membrane hyperpolarization due to summation of IPSPs. Primary afferent Aδ and C fibres were stimulated (0·1 Hz) before, during and after dorsal column stimulation. *B*, high-intensity stimulation of the dorsal root evoked initial fast IPSPs which were followed by monosynaptic C fibre-mediated EPSPs. The EPSPs reached threshold and initiated spikes in the absence of dorsal column stimulation (left trace). The C fibre-mediated EPSP became subthreshold during repetitive dorsal column stimulation (2nd and 3rd traces). The membrane potential recovered to resting level within 1 s of the cessation of dorsal column stimulation. The C fibre-mediated EPSP again reached threshold and evoked action potentials (5 s after cessation of dorsal column stimulation). Dotted line indicates the control membrane potential (−55 mV).

membrane hyperpolarization and depression of excitability persisted only for several hundred milliseconds after the end of repetitive dorsal column stimulation, so that whilst it may be due to the involvement of fast or slow IPSPs, it is not due to the slow IPSP.

Comparison of synaptic responses evoked by dorsal column and dorsal root stimulation

In all eighty-eight SG neurones tested, the responses to dorsal column stimulation had configurations and durations similar to the responses to dorsal root Aδ fibre stimulation. As shown in Fig. 8, when a neurone exhibited a fast EPSP (Fig. 8*A*) or an IPSP (Fig. 8*B*, *D* and *E*) or a combination of EPSP and IPSP (Fig. 8*C*) in response to dorsal column stimulation (upper traces), similar responses were evoked by dorsal root Aδ fibre stimulation (lower traces). However, one notable difference between the responses evoked by dorsal column and dorsal root stimulation was the occurrence of C fibre-mediated responses when the dorsal root was stimulated but not when the dorsal column was stimulated. Examples are shown in Fig. 8*A*, *D* and *E*. In these neurones, when the intensity of dorsal root stimulation was increased to a strength sufficient to activate C fibres (more than 6*T*, 0·4 ms, $n = 19$), monosynaptic (Fig. 8*A* and *D*) or polysynaptic (Fig. 8*E*) C fibre-evoked EPSPs were superimposed on the Aδ fibre-evoked responses. No C fibre-

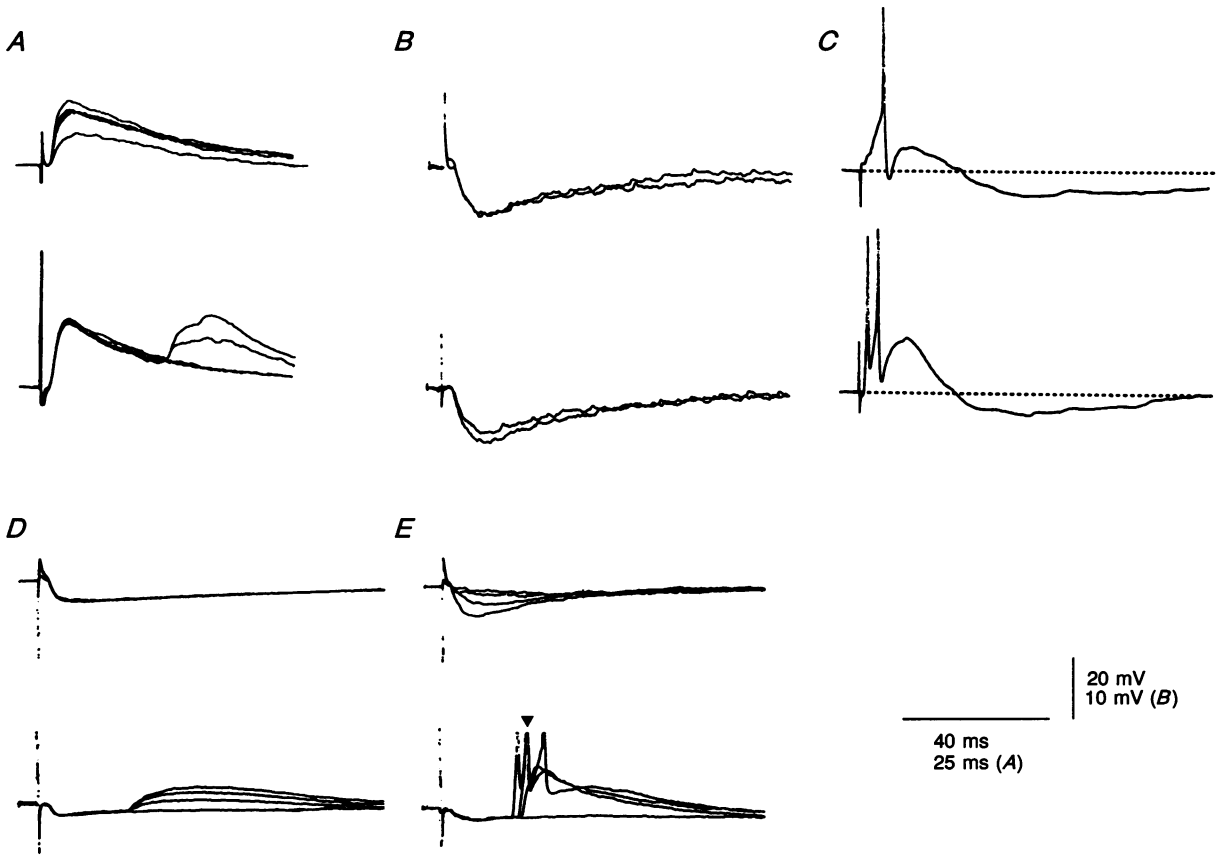

Figure 8. Comparison of the responses evoked by dorsal column (upper traces) and dorsal root (lower traces) stimulation

Records were obtained from five SG neurones (*A*, *B*, *C*, *D* and *E*). Synaptic responses evoked from the dorsal column and dorsal root are quite similar except that there are C fibre-mediated EPSPs with dorsal root stimulation (*A*, *D* and *E*). *A*, Aδ fibre-mediated monosynaptic EPSPs were elicited by dorsal column stimulation (upper trace). Stimulation of the dorsal root elicited a similar, short-latency, monosynaptic EPSP. This short-latency EPSP was followed by a long and constant-latency EPSP when the dorsal root was stimulated with higher intensity. Such long-latency C fibre-mediated EPSPs were never observed with dorsal column stimulation. *B*, fast IPSPs were evoked by both dorsal column and dorsal root stimulation. *C*, the fast EPSP was followed by an IPSP in both records. *D*, IPSPs were evoked by dorsal column stimulation. With high-intensity stimulation of the dorsal root, the IPSPs were followed by a long-latency C fibre-mediated monosynaptic EPSP. *E*, as in *D*, the stimulation of dorsal column and dorsal root evoked IPSPs. The dorsal root stimulation also evoked long but variable-latency, presumably C fibre-mediated, polysynaptic EPSPs. ▼ in record *E*, truncated spikes. Membrane potentials were −71 mV in *A*, −55 mV in *B*, −58 mV in *C*, −58 mV in *D*, and −58 mV in *E*.

evoked responses were detected in these neurones with dorsal column stimulation, even when supramaximal stimulation (20T, 0·4 ms) was used ($n = 88$; Fig. 8E).

These observations raise the possibility that the Aδ fibres in the dorsal column are collaterals of primary afferent Aδ fibres. To elucidate this possibility, maximal intensity stimulation was applied to both dorsal column and dorsal root (Fig. 9A and B). When the dorsal root was stimulated simultaneously with the dorsal column, no summation or occlusion of the synaptic responses occurred (Fig. 9C). In five SG neurones tested, the amplitude of EPSPs evoked by the simultaneous stimulation of dorsal column and dorsal root was 105 ± 5 % of that evoked by the dorsal root alone. These observations are consistent with the idea that the synaptic responses evoked from the dorsal column and the dorsal root in SG neurones are mediated largely by the same Aδ fibres.

DISCUSSION

The electrical stimulation of a dorsal column of the spinal cord is one method for the management of intractable pain. However, it is not clear how pain relief is achieved by this procedure. The central role of the SG in the integration of nociceptive information has been well established (Wall, 1978; Cervero & Iggo, 1980) and it is conceivable that the mechanism responsible for the dorsal column stimulation-produced analgesia resides in the SG. In the present study we have analysed the synaptic responses of SG neurones to dorsal column stimulation using the *in vitro* adult rat spinal cord slice preparation.

Horizontal slice preparation

The horizontal slice used in the present study provides certain advantages over the transverse slice. In the horizontal slice, the dorsal column can be stimulated in a manner similar to clinical therapy. Moreover, both primary afferent fibres and longitudinally oriented dendrites are preserved in the horizontal slice whereas they are severed in preparation of the transverse slice. In fact, all SG neurones impaled received mono- or polysynaptic input from both dorsal root and dorsal column in the horizontal slice, whereas some SG neurones in the transverse slice did not respond to dorsal root stimulation (Yoshimura & Jessell, 1989a). However, the horizontal slice preparation has obvious disadvantages in the identification of the recorded neurones. This difficulty was overcome by monitoring both the depth of recorded neurones from the dorsal surface and the membrane properties which have been previously analysed in the transverse slice (North & Yoshimura, 1984; Yoshimura & Jessell, 1989a, b; Yoshimura & Jessell, 1990) and which can be used for identifying SG neurones. In the adult rat, the configuration of the dorsal grey matter and the depth of the SG from the dorsal surface are not significantly different from preparation to preparation. The horizontal slice preparation from newborn animals has also been reported (Murase & Randić, 1983; Schneider & Perl, 1988).

The circuit associated with the responses to dorsal column and dorsal root stimulation

In the present study we described SG neurones receiving abundant monosynaptic inputs from the dorsal column. Based on threshold stimulus intensity and on conduction

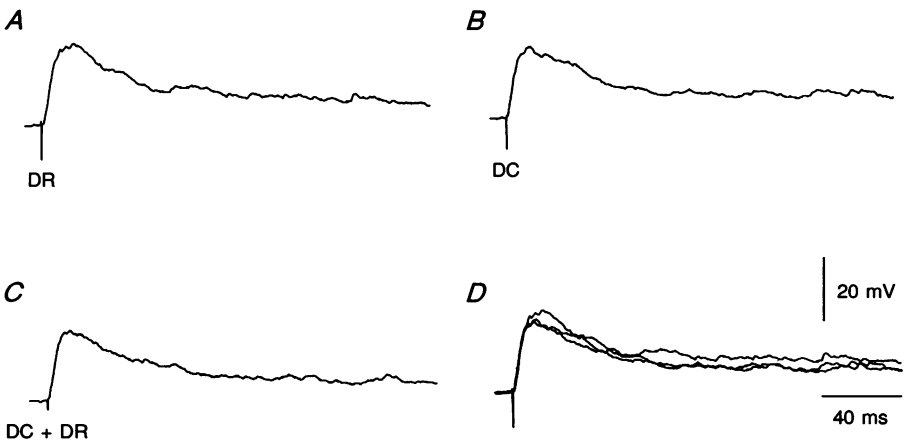

Figure 9. No summation was observed in the synaptic responses evoked by dorsal column and dorsal root stimulation

All records were obtained from the same neurone. *A* and *B*, stimulation of the dorsal root (DR) and dorsal column (DC) evoked Aδ fibre-mediated monosynaptic EPSPs. *C*, when the dorsal root and dorsal column were stimulated simultaneously, the amplitude of the EPSP was almost the same as that of EPSPs evoked by either dorsal root or dorsal column alone. This is shown in *D* where the EPSPs evoked by dorsal root (*A*) and by dorsal root and dorsal column together (*C*) are superimposed. Membrane potential was −69 mV.

velocity, Aδ fibres in the dorsal column were probably responsible for the monosynaptic and polysynaptic responses observed in SG neurones. It has been thought that large myelinated fibres are predominant in the dorsal column, while the fine myelinated afferent fibres enter the dorsolateral fasciculus of Lissauer's tract or the dorsal part of the lateral funiculus (Chung & Coggeshall, 1983; Chung, Sharma & Coggeshall, 1985), and that only some of them join the dorsal column (Horch *et al.* 1976; Light & Perl, 1979). The present intracellular recordings suggest that there is more Aδ fibre input to the dorsal column from the primary afferents than was previously thought.

In contrast with the evidence of abundant large myelinated fibres in the dorsal column (Brown, 1973), Aβ fibre-mediated synaptic responses were observed in only a few SG neurones and these responses appeared in the present study to be polysynaptic. Monosynaptic EPSPs mediated by Aβ fibres were, however, detected in this study in some neurones located in deeper laminae, presumably laminae III and IV. The rarity of Aβ fibre-mediated responses in the SG in the present study is consistent with previous results obtained in the transverse slice preparation (Yoshimura & Jessell, 1989*a*). This is probably due to impairment of the large afferent fibres in the slice preparation, because of their long trajectory in the spinal cord.

Some unmyelinated fibres have been shown to enter the dorsal column (Chung & Coggeshall, 1985; Briner, Carlton, Coggeshall & Chung, 1988). However, we could not detect any C fibre-mediated response of SG neurones to dorsal column stimulation. There are several possible reasons for this result. Firstly, the C fibres in the dorsal column may be too scarce to detect the response. Secondly, the C fibres in the dorsal column do not terminate on SG neurones. Thirdly, the C fibres may be more sensitive to damage by mechanical manipulation during preparation of the slice, although this is not likely, since the C fibre responses to dorsal root stimulation were well preserved. It is also unlikely that the stimulus intensity used for the dorsal column stimulation was insufficient for the activation of C fibres, since we tested it with intensities that were supramaximal for dorsal root C fibre activation (up to 40 T, which was more than 6 times the dorsal root C fibre threshold).

Possible mechanism for the dorsal column stimulation-induced depression of the EPSP evoked in SG neurones by dorsal root stimulation

We showed that the monosynaptic EPSPs of SG neurones resulting from primary afferent Aδ fibre stimulation were depressed by preceding dorsal column stimulation. The depression lasted longer than the duration of the dorsal column-evoked EPSPs. It lasted for up to 30 s after the cessation of brief repetitive stimulation (250 ms at 20 Hz), suggesting that presynaptic inhibition might be involved in this depression. A component of presynaptic inhibition can be detected with extracellular recording in *in vivo* experiments as negative dorsal root potentials, which are mediated by $GABA_A$ receptors located on primary afferent terminals (Barber, Vaughn, Saito, McLaughlin & Roberts, 1978; Todd & Lochhead, 1990). The depression of EPSPs lasted for up to 500 ms with a single stimulus, which is somewhat longer than $GABA_A$ receptor-mediated presynaptic inhibition (Eccles, Schmidt & Willis, 1963). In addition, the $GABA_A$ receptor antagonist bicuculline did not have any appreciable effect on the depression. It is therefore probable that some other transmitters, such as opiate peptides, noradrenaline, serotonin, or GABA interacting with $GABA_B$ receptors, participate in the generation of the presumed presynaptic inhibitory action (Price, Wilkin, Turnbull & Bowery, 1984; Carstens, Gilly, Schreiber & Zimmermann, 1987; Hori, Endo & Takahashi, 1992).

We also described three distinct types of IPSP in SG neurones evoked by dorsal column stimulation. Glycine and GABA are probably transmitters for the fast and slow IPSPs, respectively. Although the present study suggests that the long-lasting slow IPSP is produced by the opening of potassium channels, the transmitter implicated in the mediation of long-lasting slow IPSPs is uncertain. Enkephalin and noradrenaline have been shown to produce a membrane hyperpolarization in many SG neurones by increasing K^+ conductance (Yoshimura & North, 1983; North & Yoshimura, 1984). However, the antagonists for these transmitters did not antagonize the long-lasting slow IPSPs. These three types of IPSP may be involved in the mechanism underlying the dorsal column stimulation-produced depression of nociceptive transmission; in particular, the long-lasting slow IPSPs might have a prominent inhibitory effect on transmission. As shown in Fig. 7, the excitation of SG neurones by primary afferent C fibre stimulation was effectively depressed during the IPSPs evoked by dorsal column stimulation. Primary afferent C fibres are thought to convey diffuse and slow pain. Therefore, the depression of the C fibre response by dorsal column stimulation is consistent with clinical observations showing that dorsal column stimulation is more effective in patients with chronic pain due to peripheral nerve injury (Nielson, Adams & Hosobuchi, 1975) and less effective for a pin-prick pain.

Thus, the synaptic transmission between primary afferents and SG neurones seems to be depressed both presynaptically and postsynaptically following the stimulation of Aδ fibres in the dorsal column. However, the same presynaptic and postsynaptic inhibitory effects were also observed when the dorsal root instead of the dorsal column was stimulated as conditioning before the dorsal root stimulation. This result is consistent with the fact that Aδ fibres in the dorsal column are mostly the collaterals of dorsal root Aδ fibres, as shown in the present study. If one

assumes that Aδ fibres convey pain sensation, then stimulation of Aδ fibres in the dorsal column itself should produce pain. This assumption may be incorrect; Aδ fibres in the dorsal column could convey only information unrelated to pain, while the primary afferent Aδ fibres that are responsible for pain sensation preferentially enter Lissauer's tract but not the dorsal column. This possibility is supported by the physiological observation that Aδ fibres from high-threshold mechanoreceptors travel just medial to Lissauer's tract, while Aδ hair afferents run in the dorsal column (Light & Perl, 1979). However, the synaptic responses evoked from the dorsal column and dorsal root Aδ fibres are not significantly different in amplitude, suggesting that the majority of primary afferent Aδ fibres also enter the dorsal column. Alternatively, the Aδ fibres in the dorsal column are not responsible for analgesia, but simply transmit nociceptive information to neurones of the superficial dorsal horn several segments rostral and caudal to the dorsal root entry.

In conclusion, the results obtained in the present study provide information concerning the pathway of primary afferent Aδ and C fibres in the spinal dorsal horn and dorsal column, and their connectivity with dorsal horn SG neurones. This may contribute to an understanding of mechanisms underlying nociceptive transmission and its modulation in the SG of the spinal cord by dorsal column stimulation.

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