Pergamon

0306-4522(95)00187-5

Neuroscience Vol. 68, No. 4, pp. 1207–1218, 1995
Elsevier Science Ltd
IBRO
Printed in Great Britain
0306-4522/95 $9.50 + 0.00

THE EFFECTS OF EXTRASYNAPTIC SUBSTANCE P ON NOCICEPTIVE NEURONS IN LAMINAE I AND II IN RAT LUMBAR SPINAL DORSAL HORN

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Abstract—Inflammation of the skin induces release and extrasynaptic spread of neuropeptides such as substance P mainly in spinal laminae I and II and causes changes in discharge properties of nociceptive neurons in spinal dorsal horn. To evaluate the role of extrasynaptic substance P we have superfused the spinal cord at recording segment with artificial cerebrospinal fluid or with substance P. A total of 102 multireceptive neurons responding to both noxious and innocuous skin stimulation were recorded in laminae I or II of lumbar spinal dorsal horn in pentobarbital anaesthetized rats. During superfusion with substance P (10 or 100 μM) significant increases of background activities (from 2.2 ± 0.6 to 8.4 ± 3.2 imp./s, mean $\pm$ S.E.M.), enlargement of cutaneous receptive fields (from 359.9 ± 60.4 to 465.5 ± 77.3 mm^2) and enhanced responses to mechanical (from 89.1 ± 22.7 to 147.0 ± 27.5 imp./5 s) but not thermal noxious skin stimuli were observed in the 22 neurons tested. Noxious heat-evoked responses and C-fibre-evoked responses were changed in both directions. In 50 other neurons, the coefficients of dispersion of interspike intervals, which is an indicator of burst-like discharges, were significantly reduced (from 60.4 ± 5.5 to 52.7 ± 5.3) after application of substance P. Substance P induced oscillations in background activities in 13 of 40 non-rhythmic neurons and depressed oscillations in 2 of 11 neurons. Cross-correlations of discharges of pairs simultaneously recorded neurons were flat ($n = 4$), or had a central peak ($n = 19$) or a central trough ($n = 2$) and were not changed qualitatively by extrasynaptic substance P.

Thus, extrasynaptic substance P can modify not only discharge rates but also discharge patterns in the spinal dorsal horn.

Converging evidence suggests that substance P (SP) may function as a neurotransmitter or neuromodulator in spinal nociception. Iontophoretic application of SP selectively excites nociceptive spinal dorsal horn neurons which are activated by noxious chemical,[32,34] thermal,[17] or mechanical[33] stimuli. In a slice preparation of the rat spinal dorsal horn, bath application of SP resulted in a slow depolarization in most neurons.[30] This may be caused by enhancement of persistent inward Ca^{2+}-sensitive currents and inhibition of M-current, a species of voltage-dependent K^+ currents.[31]

Intrathecal administration of SP induces caudally-directed scratching and biting,[2,23] which has been interpreted as indications for pain sensation. The physiological meaning of this behaviour is, however, controversial.[40]

In the mammalian spinal cord SP is concentrated in the dorsal root cell bodies of unmyelinated and small myelinated neurons.[21,22] SP receptors are concentrated in superficial dorsal horn.[6] Inflammation of skin and strong, long-lasting noxious stimuli[17] may induce release of SP, which spreads extrasynaptically throughout the superficial and in some cases also throughout the deep dorsal horn.[13] This extrasynaptic spread of chemical signals has been termed "volume transmission"[1] but its function remains unclear. Of course, volume transmission cannot mediate the fast, punctual synaptic transmission, but may be relevant for long-term changes in the spinal neuronal network following inflammation of peripheral tissues.[35] For example, inflammation may change the discharge properties of nociceptive spinal dorsal neurons[19,35] and may enlarge cutaneous receptive fields.[12,35] These central changes may contribute to the hyperalgesia during inflammation of peripheral tissues.

In spite of accumulated knowledge about direct effects of SP on single neurons, virtually nothing is known about the effects of extrasynaptic SP on the function of intact neuronal network of the spinal dorsal horn. Plastic changes of network properties such as altered synaptic strength in afferent pathways may, for example, result in considerable modifications of size and the location of cutaneous receptive fields which cannot be explained solely by

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Abbreviations: ACSF, artificial cerebrospinal fluid; AS, autospectral density; CC, cross-correlation histogram; CD, coefficient of dispersion; ISIH, interspike interval histogram; PETH, peristimulus time histogram; SP, substance P.

excitability changes of the neurons under study. It is suggested that sensory information may be encoded not only by mean discharge rates but also by discharge patterns.[8,29] Synchronization of discharges of convergent neurons is a highly effective way to enhance the degree of spatial summation at postsynaptic membrane[37] and may play a role for assembly coding of information.[39] In order to evaluate the role of extrasynaptic SP, we have determined the effects of controlled superfusion of the rat lumbar spinal cord dorsum with SP on discharge rates and discharge patterns. In the present study all data were obtained from neurons located in laminae I or II because release and spread of SP induced by different noxious stimuli is most prominent in the superficial dorsal horn,[13,14,17] where specific receptors for SP are also concentrated.[6,20] Some of the results were published in preliminary form.[37]

EXPERIMENTAL PROCEDURES

Experiments were performed on 34 male Sprague–Dawley rats (250–400 g body weight) under deep pentobarbital anaesthesia induced by 60 mg/kg pentobarbital sodium given intraperitoneally. Rats were obtained from the Zentralinstitut für Versuchstierzucht (Hannover, Germany). The trachea was cannulated to allow mechanical ventilation with room air, if necessary. A catheter was inserted into one external jugular vein for continuous i.v. infusion of a glucose-tyrode solution containing 15 mg/ml of pentobarbital sodium to maintain a deep level of anaesthesia (verified by stable mean arterial blood pressure and a constant heart rate during noxious skin stimulation). One carotid artery was cannulated to continuously monitor mean arterial blood pressure which ranged from 80 to 100 mmHg. Colorectal temperature was kept constant around 37–38°C by means of a feedback controlled heating blanket. A laminectomy was performed to expose the lumbar enlargement of spinal cord and dura mater was incised longitudinally. A specially synthesized silicone rubber was used to form a small well on the cord dorsum at the recording segments to allow controlled superfusion of the spinal cord with artificial cerebrospinal fluid or drugs (see Ref. 4 for details). The left sural nerve was dissected free for bipolar electrical stimulation with platinum hook-electrodes. The left hind paw was fixed pad upwards with paraffin wax in a holder to allow noxious radiant heating of the glabrous skin. All exposed nervous tissues were covered with warm paraffin oil, except for those spinal segments which were superfused.

Extracellular recordings were made in laminae I or II with glass microelectrodes containing a carbon fibre (impedance 10–20 MΩ). Data were stored in a PC/AT computer and action potentials were discriminated with the principal component method based on the shape of wave form, which was implemented by Brainwave systems. Cutaneous mechanoreceptive fields of recorded neurons were measured with von Frey filaments (6.4 g) and noxious mechanical stimuli were applied with von Frey filaments exerting 37.8 g. The sural nerve was stimulated electrically (20 V, 0.5 ms) 5 times in 40 s intervals to excite both A- and C-fibres. Periodic histograms were calculated off-line to determine the number of action potentials evoked by afferent Aδ or C-fibres. Based on an approximate distance of 12 cm from the stimulating electrodes to recording electrodes, latencies of discharges less than 4 ms (conduction velocity ≥30 m/s), between 4 ms and 30 ms (conduction velocity 4–30 m/s) and more than 80 ms (<1.5 m/s) after stimulation were considered to be evoked by Aβ-, Aδ- and C-fibres, respectively (Fig. 1A and G). Aβ-evoked discharges overlapped with field potentials and were not analysed. Noxious radiant skin heating (50°C, 10 s) was given three times at 2 min intervals. Heat-evoked responses were calculated as total number of impulses in 20 s subtracting background activity beginning with the onset of heat stimulation. Background activities were analysed with peristimulus time histogram (PETH, bin width 1 s), interspike interval histogram (ISIH, bin width 1 ms), cross-correlation histogram (CC, bin width 1 ms), autospectral density (AS) and coefficient of dispersion (CD = variance/mean) of ISI, see Fig. 1 for an example.

Centred signed rank statistics was used for statistical comparison, $P < 0.05$ was considered significant.

RESULTS

A total of 102 multireceptive neurons were recorded in laminae I or II (corresponding to a mean depth of 242.5 ± 12.4 μm) (mean ± S.E.M.) from the dorsal cord surface. The mean discharge rate was 7.9 ± 1.2 imp./s (mean ± S.E.M.). Most (72/100) neurons responded to noxious skin heating (50°C, 10 s). Fifty six of the 83 neurons tested responded with a long (≥80 ms) latency to electrical stimulation of the sural nerve at strength supramaximal for the activation of C-fibres. All neurons responded either to C-fibre stimuli or to noxious skin heating or to both stimuli (Fig. 1A). All neurons had cutaneous receptive fields on the ipsilateral hind paw or leg. Receptive fields are difficult to measure precisely for neurons with high level of background activity, so in only 79 neurons receptive fields were determined quantitatively and the mean size was 357 ± 58.1 mm².

The effects of extrasynaptic substance P on discharge rates, receptive fields and evoked discharges

The effects of extrasynaptic SP on the level of background activity, responses to Aδ and C-fibre stimulation, responses to noxious skin heating and the size of cutaneous receptive fields and responses to noxious mechanical stimuli were quantitatively assessed in 22 neurons. To evaluate the time course of modulation, the parameters were determined before and in 5, 20, 40 and 60 min after the onset of SP superfusion. Because some of the neurons were lost during superfusion the number of neurons encountered decreased with time of superfusion.

Twenty min after the onset of SP superfusion the mean discharge rate of background activities was significantly increased from 2.2 ± 0.6 to 8.4 ± 3.2 imp./s (mean ± S.E.M., $n = 18$, $P < 0.01$) (Figs 2B and 3A). From 18 neurons, ten displayed strong increase of discharge rates, two neurons had slightly reduced rates and six neurons did not change discharge rates. The change of discharge rates in these 18 neurons ranged from −2 to +40 imp./s. Forty to 60 min after the onset of SP superfusion the mean level of background activity was no longer different from the control.

Five min and 20 min after the onset of SP superfusion the mean size of cutaneous receptive fields was significantly enlarged from 359.9 ± 60.4 mm² to 465.5 ± 77.3 mm² ($n = 22$) and from 389.4 ± 68.0 mm²

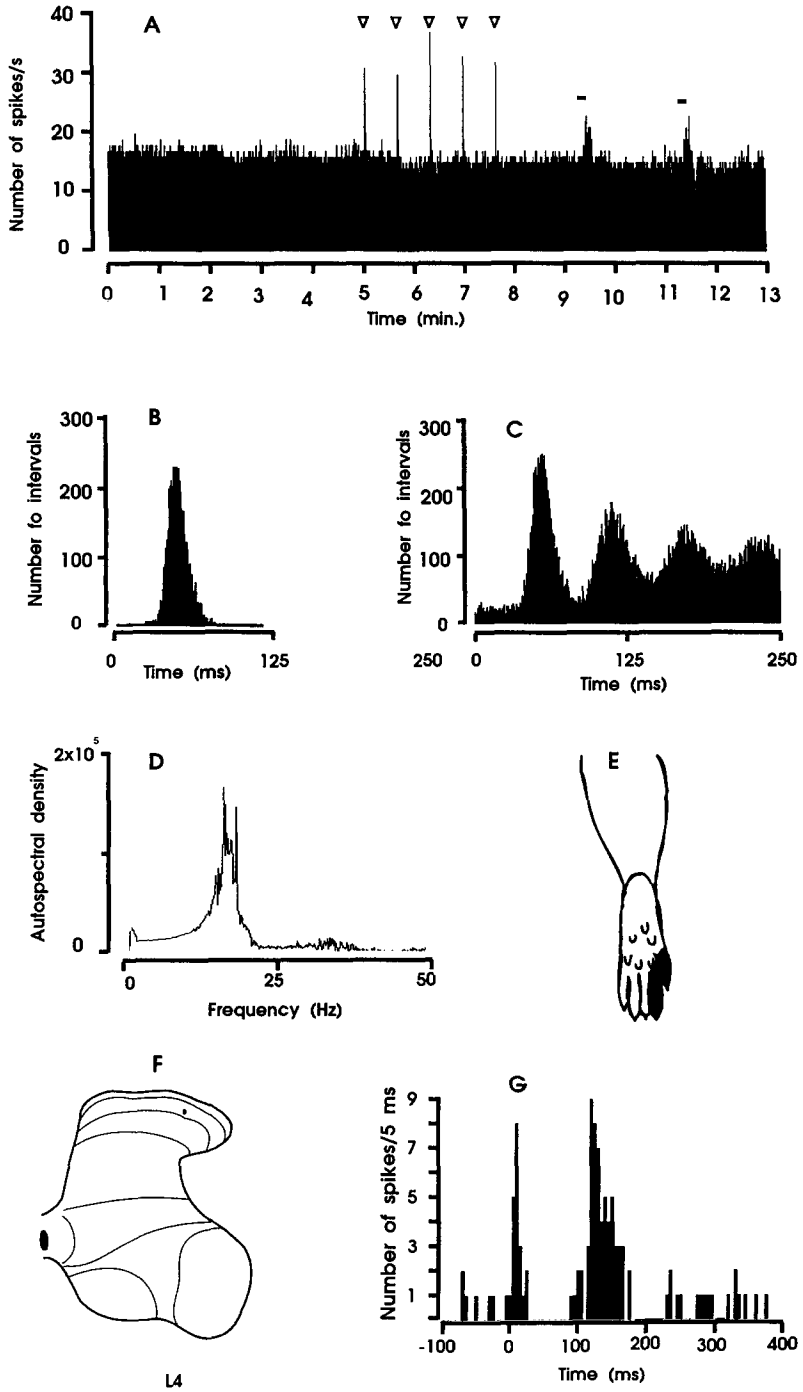

Fig. 1. Properties of one multireceptive neuron recorded in lamina II are shown. (A) Peristimulus time histogram (bin width 1 s) of background and evoked activity. ▽: indicate single pulse of electrical stimulation (20 V, 0.5 ms). Black horizontal bars indicate period of noxious radiant heating of the glabrous skin at the ipsilateral hind paw. Interspike interval histogram (B) (bin width 1 ms), auto-correlation histogram (C) (bin width 1 ms) and autospectrum (D) were calculated from the background activity in the recording period from 0 to 5 min as shown in A. (E) Cutaneous mechanoreceptive field is indicated as determined by responses to probing with a von Frey filament (6.4 g). The histologically verified recording site is shown in F. (G) The sum response to five consecutive electrical nerve stimuli (indicated by open triangles in A) is shown by a periodic histogram. Note that A-fibre and C-fibre evoked discharges can be clearly distinguished.

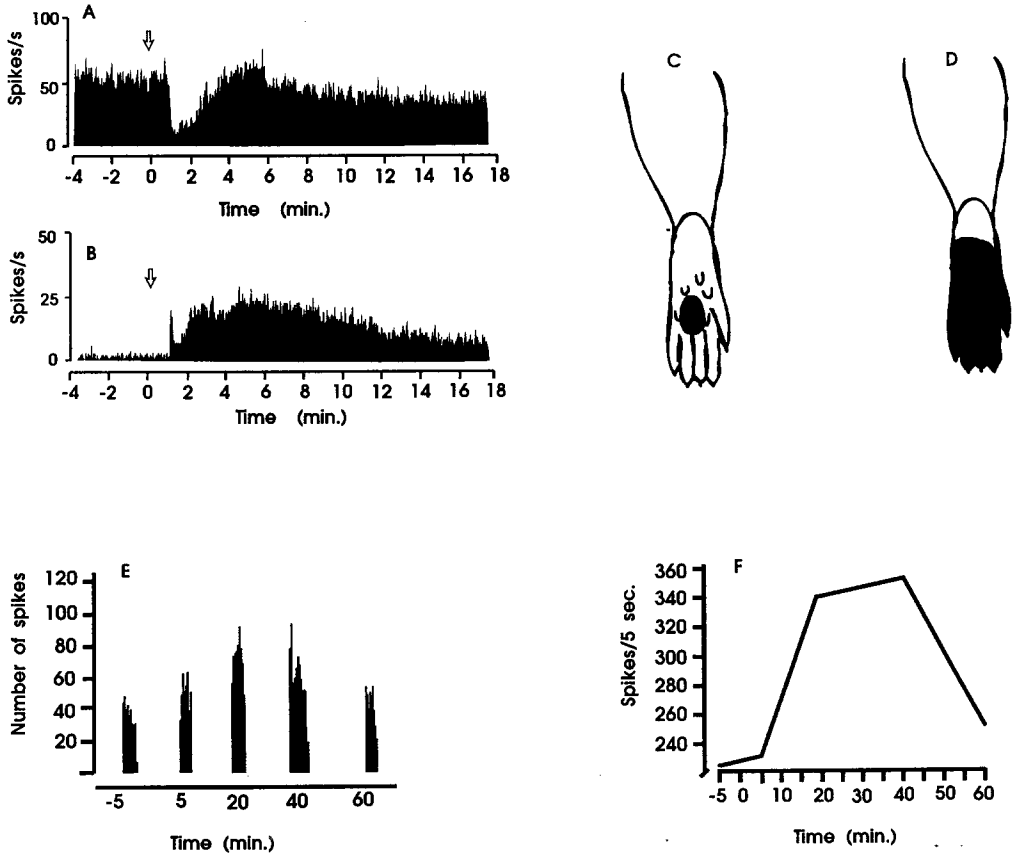

Fig. 2. Examples of the effects of SP on background activity and responses to mechanical skin stimuli are shown. (A and B) Peristimulus time histograms (bin width 1 s) of discharges of two multireceptive neurons, which were recorded simultaneously in lamina II. Downward arrows (time zero) indicate onset of SP superfusion (100 μM, 30 min). Note that the effects of SP on background activity were different in this pair of neurons. (C) Cutaneous mechanoreceptive field of the neuron shown in B before SP. (D) Receptive field of the same neuron 20 min after the onset of SP superfusion. The size of the cutaneous receptive field of the neuron shown in A was not changed during SP superfusion. (E) Peristimulus time histogram (bin width 1 s) from another neuron without background activity but with responses to mechanical noxious skin stimuli (von Frey filament 37.8 g). Twenty to forty minutes after the onset (time 0) of continuous SP superfusion the response was maximally enhanced. At each time point three tests were made and representative responses are shown. (F) Mean responses of the same neuron to mechanical stimuli are plotted against time after the onset of SP superfusion.

to $543.0 \pm 84.6\,mm^2$ ($n = 18$), respectively ($P < 0.01$ in both cases) (Figs 2C, D and 3B). At 20 min the size of cutaneous receptive fields of 11 neurons enlarged by up to 400 mm^2 and in one neuron it contracted by 150 mm^2 and in six neurons receptive fields remained unchanged.

With a long latency of 40 min SP enhanced mean responses to noxious mechanical skin stimulations (von Frey filament, 37.8 g) from 89.1 ± 22.7 imp./5 s to 147.0 ± 27.5 imp./5 s ($P < 0.05$) (Figs 2E, 2F and 3C).

Responses to noxious skin heating were tested before and 5, 20, 40 and 60 min after the onset of SP superfusion. Before SP mean response to noxious skin heating was 220.3 ± 150.7 imp./20 s. During SP superfusion mean response was not significantly changed. This is because SP changed heat-evoked responses of different neurons in both directions (Fig. 4A and 4B). For example, 40 min after the onset of SP superfusion the responses in two neurons were decreased, in five neurons they were unchanged, in five neurons they increased. The change of heat-evoked responses ranged from -70 to $+320$ imp./20 s

Responses to supramaximal electrical nerve stimulation recruiting A and C-fibres were also measured in 18 neurons before and during SP superfusion (at 5, 20, 40 and 60 min). Before SP mean Aδ- and C-fibre-evoked discharges were 2.2 ± 0.4 imp./stim. and 20.1 ± 5.5 imp./stim., respectively. During SP superfusion responses were changed in both directions, thus, no significant change of mean response was observed.

The effects of substance P are antagonized by RP 67580

In 11 neurons recorded in superficial dorsal horn of four rats the effects of SP plus NK1-receptor

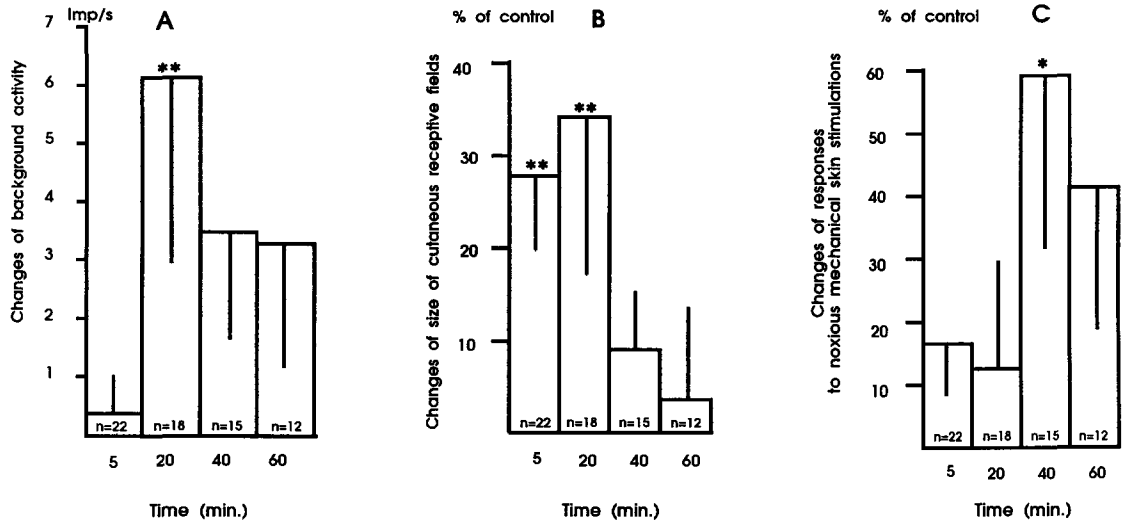

Fig. 3. Summary of the effects of SP superfusion on background activity and responses to mechanical skin stimuli. In A, mean changes in discharge rates are plotted versus time after the onset of superfusion. In B mean changes in size of cutaneous mechanical receptive fields and in C mean changes in responses to noxious mechanical skin stimulations (von Frey filament 37.8 g) are shown. The number of neurons studied are indicated at the bottom of each bar. **: $P < 0.01$; *: $P < 0.05$, vertical lines indicate S.E.M.

antagonist RP 67580 on discharge rates, size of cutaneous mechanoreceptive fields and response to mechanical noxious skin stimulation were determined. After control values have been assessed the spinal cord was superfused with the NK1-receptor antagonist RP 67580 (1 μM) for 10 min followed by superfusions with SP (100 μM) plus RP 67580 (1 μM). Discharge rates, sizes of cutaneous receptive fields and the

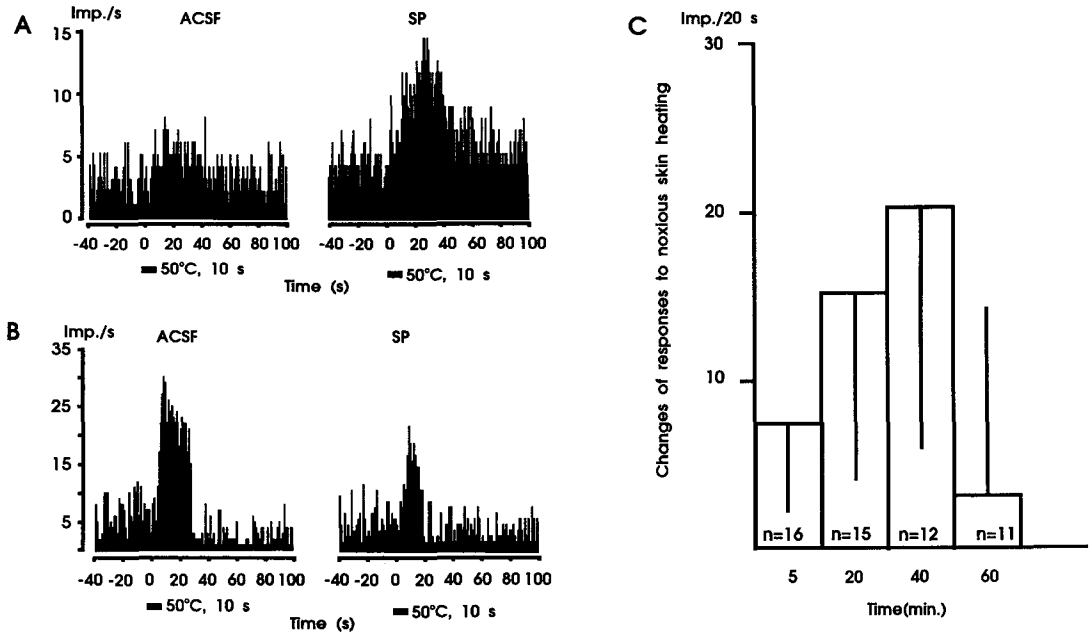

Fig. 4. Differential effects of SP on responses to noxious skin heating are shown. (A) Peristimulus time histograms (bin width 1 s) of discharges of a multireceptive neuron recorded in lamina II during superfusion with artificial cerebrospinal fluid (ACSF) and in 20 min after the onset of SP superfusion show that responses to noxious radiant heating can be enhanced by SP. (B) Peristimulus time histograms (bin width 1 s) of another multireceptive neuron recorded in lamina I before (ACSF) and 20 min after onset of SP superfusion exhibit that responses to noxious radiant heating can also be decreased by SP. (C) Mean response to noxious skin heating (and S.E.M.) are plotted versus time after the onset of SP superfusion. The number of neurons tested are indicated at the bottom of each bar. No significant change of mean responses was observed.

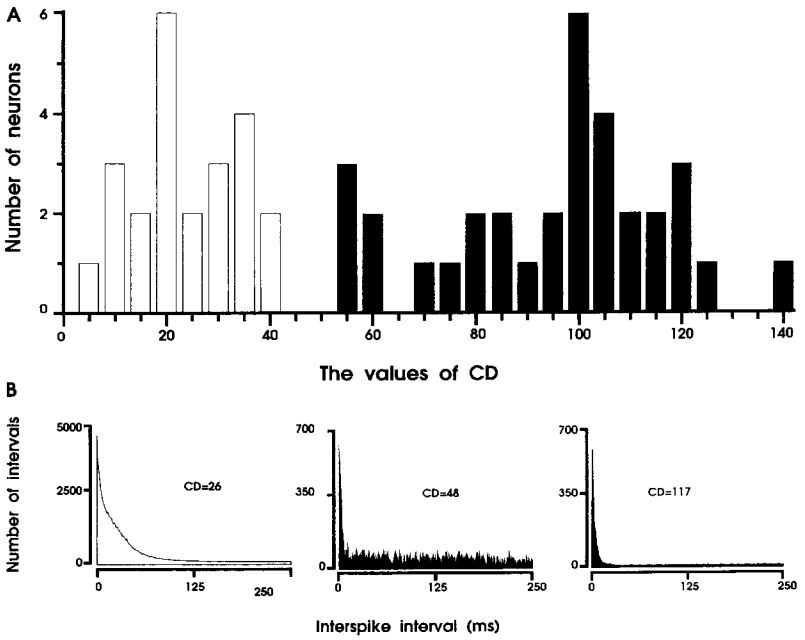

Fig. 5. A shows the distribution of coefficients of dispersion (CD) calculated for short interspike intervals from 1 to 250 ms. Three distinct groups are apparent. B illustrates interspike interval histograms (bin width 1 ms) of background activities of representative neurons from the three different groups. Note that there is close correlation between the CD values and the patterns of interspike interval histograms.

responses to mechanical noxious stimulations were measured at 5, 20, 40, and 60 min after beginning of superfusion with SP plus antagonist. During superfusion with artificial cerebrospinal fluid (ACSF) the mean size of receptive fields of 11 neurons was $282.0 \pm 49.4\,mm^2$, mean discharge rate was 1.9 ± 0.5 imp./s and mean responses to mechanical noxious skin stimulation was 53.6 ± 45.9 imp./5 s. In the presence of RP 67580 SP failed to induce changes in any of the parameters at any of time points tested.

Extrasynaptic substance P affects discharge patterns of nociceptive spinal dorsal horn neurons

To evaluate the impact of SP on discharge patterns, stationary periods of background activity of at least 1000, typically about 5000 action potentials were analysed in 54 neurons. In 50 neurons, discharges before and during SP superfusion were analysed by ISIH, CD of interspike intervals and AS.

The CD is considered to be a sensitive parameter to detect burst-like discharges.[8] When all interspike intervals, including also very long intervals of more than 250 ms were considered, mean CD of interspike intervals was not different before and during SP superfusion. However, when only the interspike intervals which ranged from 1 to 250 ms were calculated, a significant difference of CDs before and during SP superfusion was observed. In our neuron sample, the distribution of CD values calculated for intervals ranging from 1 to 250 ms showed at least three clearly distinguishable groups (Fig. 5A). The first group which consisted of 21 neurons, had CD values which ranged from 5 to 40. The CD of the second group (5 neurons) ranged from 55 to 60 and 28 neurons of the third group had CD values which ranged from 70 to 140.

In 50 neurons the values of CD of short interspike intervals were determined before and during superfusion with SP. SP decreased the values of CDs in 26 neurons, in 17 neurons the CDs were unchanged and in seven neurons they were increased. The mean change of CD values of all 50 neurons tested were significantly decreased from 67.7 ± 5.4 to 56.2 ± 5.2 ($P < 0.05$) (Fig. 6A and Fig. 7).

In the same neurons, AS of background activity was performed before and during SP superfusion. Before SP, 11 neurons showed rhythmic discharges, 40 neurons were non-rhythmic. During SP, 13 of 40 (33%) non-rhythmic neurons became rhythmic (Fig. 8A) and 2 of 11 (18%) rhythmic neurons lost rhythmicity.

Substance P antagonist RP 67580 blocks the effect of substance P on discharge patterns

In 15 neurons the effects of superfusion with RP 67580 (1 μM) on the CD of short intervals were tested. During superfusion the mean value of CDs was not significantly changed.

In these 15 neurons AS was also performed. Before superfusion with RP 67580, nine neurons were rhythmic, six were non-rhythmic and during superfusion five of nine (55.5%) rhythmic neurons lost

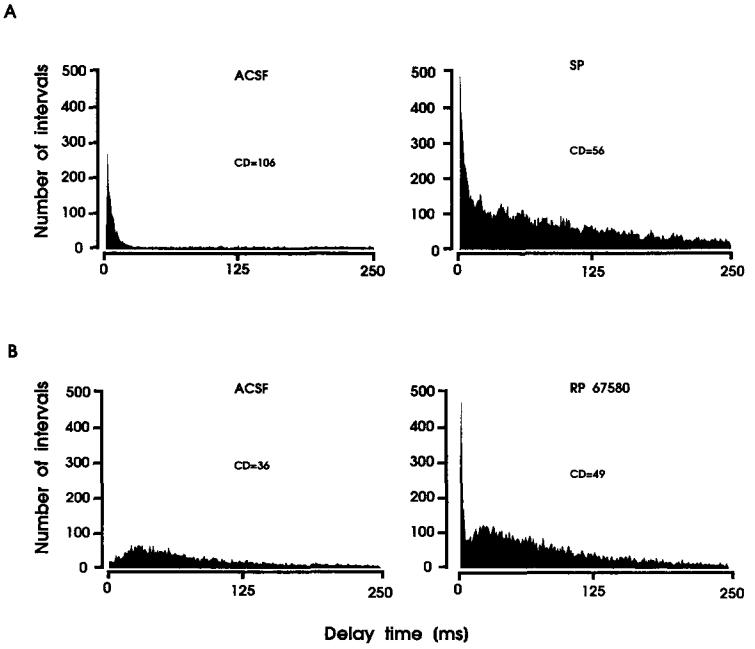

Fig. 6. (A) Interspike interval histograms of background activity (bin width 1 ms) from a multireceptive neuron recorded in lamina II during superfusion of spinal cord with artificial cerebrospinal fluid (ACSF) or with SP (100 μM) are shown. The discharge rate of this neuron was increased from 1.3 imp./s to 8.4 imp./s during SP superfusion and the value of coefficient of dispersion decreased from 106 to 56. (B) Shows interspike interval histograms of background activity (bin width 1 ms) from another multireceptive neuron recorded in lamina II before ACSF and during superfusion with the SP antagonist RP 67580 (1 μM). The value of coefficient of dispersion increased from 36 to 49 and the discharge rate was decreased from 16 imp./s to 12 imp./s during SP superfusion.

rhythmicity (Fig. 8B shows an example), two of six (33.3%) non-rhythmic neurons retained rhythmicity.

The effect of substance P on temporal correlation of discharges

In 25 pairs of simultaneously recorded nociceptive dorsal horn neurons cross-correlation histograms (CC) were calculated before and during SP. Before SP, four pairs showed non-correlated discharges as revealed by flat cross-correlograms, 19 pairs had central peaks, i.e. discharges were synchronized and CCs of two pairs had a central trough. During SP superfusion the majority of CCs (23/25) were not changed qualitatively, but two CCs with previously central peaks became flat (Figs 9 and 10).

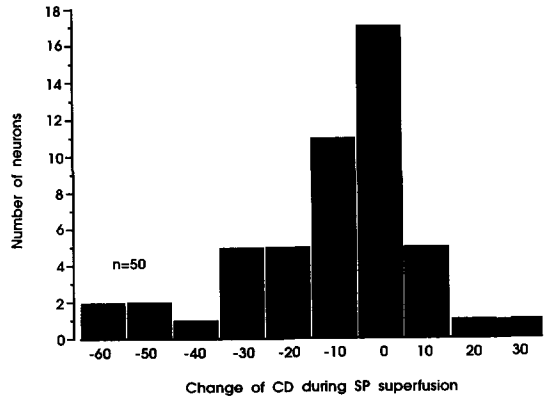

Fig. 7. SP superfusion decreases the values of coefficients of dispersion (CD) of background activity of most spinal multireceptive neurons recorded in laminae I or II. The figure illustrates the distribution of changes of the CD values calculated for interspike intervals from 1 to 250 ms during SP superfusion (100 μM) as compared with CD values obtained before SP. Note that the mean CD was significantly reduced.

DISCUSSION

The present study provides evidence that extrasynaptic SP modifies discharge properties of neurons in laminae I and II, which cannot be explained solely by direct effects of SP on neurons under study but which may involve modifications of the functional spinal neuronal network. SP superfusions not only affected excitability of multireceptive neurons as revealed by changes in discharge rates but also the temporal patterns of discharges. The level of background activities, the size of cutaneous receptive fields and responses to noxious mechanical but not thermal stimulation were significantly increased. Autospectral histograms showed that SP induced harmonic oscillations of background activities. Temporal correlation of discharges of few of the simultaneously recorded pairs of neurons were changed qualitatively, as revealed by cross-correlation analysis. This indicates that extrasynaptic SP mimicks some, but not all

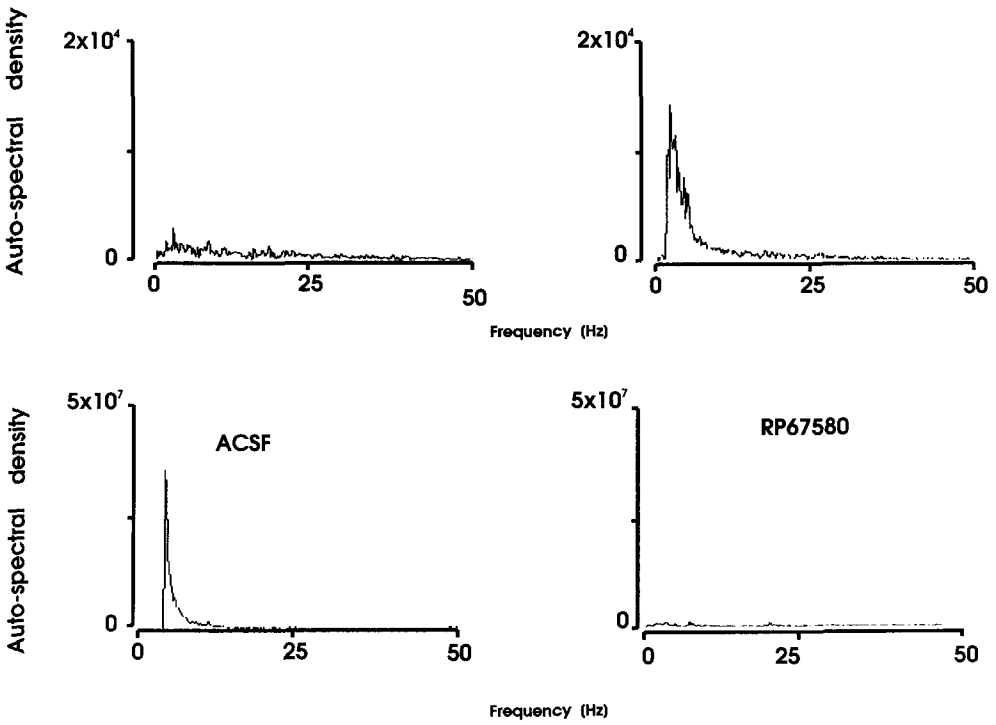

Fig. 8. SP induces harmonic oscillations in some multireceptive neurons in the superficial dorsal horn, as revealed by spectral analysis of the autocorrelation function (autospectral density) of background activity. (A) Autospectral density of a multireceptive neuron recorded in lamina I before ACSF and during SP (100 μM) superfusion. SP induced a significant broad peak centred around 2.0 Hz. (B) During superfusion with SP antagonist RP 67580 (1 μM) a significant peak around 1.4 Hz was completely depressed in another multireceptive neuron recorded in lamina I.

of the plastic changes, which can be induced in the spinal cord by skin inflammation.[37]

Controlled superfusion and 'volume transmission'

There are at least two types of chemical communication in central nervous system: (1) conventional fast synaptic transmission leading to punctate high frequency information transfer along neuronal chain, and (2) 'volume transmission' referring to chemical signals in the medium surrounding the neuronal network.[1] The activity of neurons in intact neuronal networks may be controlled by both types of signalling. Abundant evidence demonstrated that intense and prolonged noxious stimulation, e.g. following trauma or inflammation of peripheral tissues, induces the release of neuropeptides including SP in spinal dorsal horn, which can be detected extrasynaptically in the interstitial space and even in the cerebrospinal fluid.[7,13,15–17,27,38] Such noxious stimulations also cause some forms of long-term plastic changes in the spinal dorsal horn.[12] Thus, under some pathophysiological conditions 'volume transmission' of neuropeptides may play a role for neuronal plasticity. To study the effects of volume transmission of SP, controlled superfusions of the lumbar spinal cord were performed. We have shown recently that following superfusion of rat cord dorsum for 30 min with a single dose of [^{125}I]neurokinin A, significant radioactivity is detected up to a depth of 1.5 mm below the dorsal surface of the cord. The highest peptide concentration was located in laminae I and II.[4] This distribution is very similar to that found after spinal release of SP.[17] Clearly iontophoretic application is a suitable means to reveal the effects of neuropeptides on single neurons, while controlled superfusion of the cord dorsum with peptides may more closely mimic the extrasynaptic spread of neuropeptides following intense noxious stimulation and may reflect the effects of neuropeptides on the neuronal network in spinal dorsal horn.

Multiple effects of extrasynaptic substance P on discharge rates of spinal dorsal horn neurons

Some previous studies showed that iontophoretic application of SP excites selectively nociceptive spinal dorsal horn neurons.[17,32,34] In other studies inhibitory effects were also observed.[11,42] The present work has shown that mean discharge rates of nociceptive neurons in the superficial dorsal horn were significantly increased by extrasynaptic SP. The effect of SP on different neurons recorded simultaneously at the same site may be qualitatively different. In contrast, brief, acute noxious skin stimuli led to excitation of all nociceptive neurons encountered. These differential effects of SP on nociceptive neurons suggest that an extrasynaptic spread of SP probably plays no

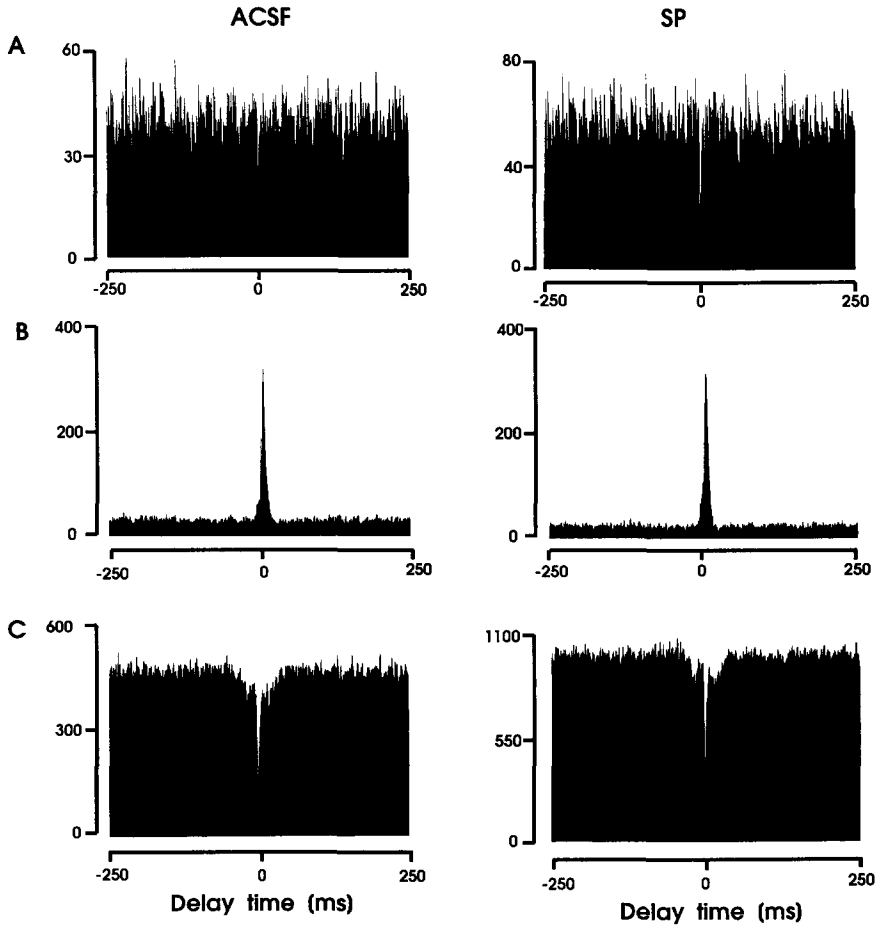

Fig. 9. Cross-correlograms (CC) (bin width 1 ms) of the background activities of three different pairs of neurons which were simultaneously recorded during superfusion with artificial cerebrospinal fluid (ACSF) and SP (100 μM) are shown. The CCs of the neuronal pair in A are flat, indicating that discharges were not correlated in time. The CCs shown in B display a high and narrow central peak which indicates synchronous discharges, possibly due to a common input with the same polarity. The CCs in C display a central trough which is suggestive of common input with oppositive polarity. During SP superfusion the patterns of cross-correlation were stable.

major role for the encoding of acute noxious stimuli. However, inflammation of the skin, like SP superfusion, produced both excitatory and inhibitory effects on simultaneously recorded nociceptive spinal dorsal horn neurons[36] further supporting the notion that spinal nociception may be fundamentally different under acute vs pathophysiological conditions. This has also been concluded from analysis of non-linear dynamics of discharges of nociceptive neurons: brief noxious thermal stimuli increased the number of degrees of freedom while inflammation of skin reduced complexity of discharge patterns.[37] We currently investigated whether extrasynaptic SP also reduces the number of degrees of freedom of the discharges of nociceptive neurons in superficial dorsal horn.

The mechanisms for the differential effects of SP on discharge rates of nociceptive spinal dorsal horn neurons are presently not known. Possibly, not all neurons in superficial dorsal horn express the same receptors and/or the same density of receptors for SP. Extrasynaptic SP may also have indirect effects via the neuronal network, e.g. by activating inhibitory interneurons.

Extrasynaptic substance P preferentially enhances the responses to mechanical stimulation

Up to date three distinct types of tackykinin receptors have been identified in mammalian tissues, which are named NK-1, NK-2 and NK-3, respectively. SP preferentially binds to NK-1 receptors, but the selectivity is not absolute.[41] It is reported that iontophoresis of SP in lamina II may cause a selective reduction of responses to innocuous brushing of skin in multireceptive spinocervical tract neurons recorded in laminae IV/V, while selective agonist for NK-2 receptor induces a specific facilitation of responses to noxious thermal skin stimuli.[18] Here we also report a differential effect of SP on mechanical versus thermal skin stimuli: extrasynaptic SP facilitated responses to

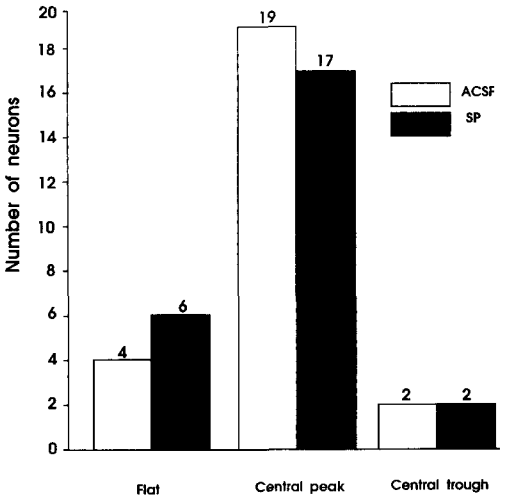

Fig. 10. Summary of the effect of SP on cross-correlations of 25 simultaneously recorded pairs of multireceptive neurons. During SP superfusion CCs changed their patterns qualitatively from central peaks to flat in two pairs of neurons. No qualitative changes were observed in the remaining 23 pairs of neurons.

mechanical stimulation, e.g., increased the size of cutaneous mechanoreceptive fields and the responses to noxious skin pressure. This suggests that NK-1 receptor may be involved in selective modulation of mechanical input. This is in line with the fact that noxious mechanical, but not noxious thermal stimulation of skin may induce release and synaptic spread of SP in the spinal dorsal horn.[25] However, this finding is not in agreement with the results from Duggan and his co-workers, which demonstrated that noxious heating of the skin releases immunoreactive substance P in the superficial dorsal horn of the cat.[17]

The observation that iontophoretic application of SP remote from the recording site may depress brush evoked responses is difficult to reconcile with the present data, showing a significant increase in the size of mechanoreceptive fields during controlled superfusion of the cord. Of course, it cannot not be excluded that SP acting in the superficial dorsal horn may have qualitatively different effects on low threshold mechanical responses of neurons in the superficial (present study) versus deep dorsal horn.[18] It is, however, more appealing to assume that these differences are due to the fact that local SP concentration may differ considerably along extended dendritic trees following iontophoretic application, while a more homogeneous SP distribution results during SP superfusion.[4]

Extrasynaptic substance P attenuates degree of burst-like discharges and induces harmonic oscillations

In the central nervous system many neurons may discharge action potential in clusters (bursts) which may be due to two different mechanisms: (1) high frequency spikes as a manifestation of intrinsic membrane properties and independent of synaptic input, (2) synaptically driven bursts. Most neurons may produce clusters of spikes in response to repetitive, high frequency synaptic input.[9] The present work revealed that 67% of the neurons recorded in laminae I and II exhibited pronounced burst-like discharges (i.e. the values of CDs were higher than 55). Because in this work recordings were performed in intact spinal cord, cellular and network mechanism cannot directly be distinguished. Since low-threshold Ca^{2+} current may be responsible for intrinsic bursts[28] and SP may enhance low and high threshold Ca^{2+} currents in rat spinal dorsal horn neurons, SP might induce intrinsic bursts. However, the present data have shown that during SP superfusions the values of CD were significantly decreased. This suggests that in intact spinal cord substance P does not induce intrinsic bursts but rather depresses burst-like activity, which is probably synaptically driven. This conclusion is supported by the observation that changes in the CD were not correlated with changes in mean discharge rates during SP superfusion. Intrinsic bursts are considered to be voltage-dependent and mean discharge rates may monotonically by related to the level of the membrane potential.

Extrasynaptic SP induced harmonic oscillations of background activity in some of the multireceptive neurons recorded in the present study. Recent work from our group indicates that rhythmicity may be generated within the local neuronal network of the spinal dorsal horn.[36] The mechanism of generating oscillations may involve negative feedback connections and coupling of excitatory and inhibitory membrane conductances within single neurons.[39] In thalamocortical relay neurons oscillations may block transmission of sensory information. Switching to transfer mode was characterized by non-rhythmic discharges.[10] In spinal dorsal horn neurons inflammation of skin induced, but acute noxious skin heating, depressed oscillations.[37] These results are consistent with the hypothesis that oscillations may be involved in gating transmission of sensory information. To further elucidate the role of rhythmicity in processing of sensory information in spinal dorsal horn neurons additional experiments will be necessary. Oscillations may also be important for the development of neuronal plasticity or long-term potentiation. It has been suggested that regular 2nd messenger pulses such as c-AMP and Ca^{2+} are more efficient than stochastic pulses to induce maximal response of target cells.[26] The fact that extrasynaptic SP and skin inflammation induce both rhythmic oscillations and the expression of immediate-early genes such as c-fos and jun B[3] is in line with this hypothesis.

Synchronization of discharges of convergent neurons is a highly effective way to enhance the degree of spatial summation at the postsynaptic membrane and may be involved in assembly coding of sensory information.[39] Thus, qualitative changes in the pattern of cross-correlograms may reflect changes in the

functional connection between neurons in spinal dorsal horn. Inflammation of skin induced synchronizations of discharges in spinal dorsal horn neurons.[37] Here we tested whether extrasynaptic SP which may selectively enhance release of glutamate in the spinal dorsal horn[24] may also synchronize discharges of neurons in superficial dorsal horn, e.g., by opening glutaminergic pathways between the neurons under study. However, SP superfusion failed to qualitatively change the patterns of CCs of most pairs of neurons and never produced synchronizations. Apparently other neuromodulators must be involved in synchronizing discharges of spinal dorsal horn neurons following inflammation.

CONCLUSIONS

In the intact spinal cord, extrasynaptic SP changes not only the excitability and discharge rates but also the discharge patterns of nociceptive neurons in the superficial dorsal horn.

Extrasynaptic SP can induce some of the changes which are produced by skin inflammation, such as enhancement of discharge rates, enlargement of cutaneous receptive fields, increased responses to mechanical skin stimulation and induction of harmonic oscillation. However, some changes which are induced by skin inflammation such as increased response to noxious skin heating and qualitative changes in the cross-correlation are not induced by SP superfusion. Thus, other spinal neuromodulators must also be involved.

Acknowledgements—We wish to express our thanks to Dr C. Garret for providing us with a free sample of substance P antagonist RP 67580. This work was supported by a grant from the Deutsche Forschungsgemeinschaft to J.S.

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(*Accepted* 21 *April* 1995)