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IN VITRO EFFECTS OF SUBSTANCE P ON NEONATAL RAT SYMPATHETIC PREGANGLIONIC NEURONES

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SUMMARY

1. Intracellular recordings were made from antidromically identified sympathetic preganglionic neurones (SPNs) in thin transverse neonatal rat thoracolumbar spinal cord slices.

2. Applied either by pressure ejection or superfusion, substance P (SP) caused a slow, monophasic depolarization in 60% of sympathetic preganglionic neurones; a biphasic response consisting of an initial hyperpolarization followed by a depolarization was observed in a few neurones. In addition, SP induced the occurrence of repetitive inhibitory postsynaptic potentials (IPSPs) in about 20% SPNs.

3. Low-Ca^{2+} or tetrodotoxin (TTX)-containing Krebs solution abolished the hyperpolarizing phase of the biphasic response and the small IPSPs, thereby augmenting the depolarizing response of SP.

4. SP-induced depolarizations were often associated with a moderate increase in membrane resistance. Generally, the response was made smaller on hyperpolarization and reversed at the membrane potential between -90 and -100 mV. These findings suggest that a reduction of membrane K^+ conductance may underlie the depolarizing action of SP.

5. Subthreshold fast, excitatory postsynaptic potentials (EPSPs) evoked by stimulation of dorsal rootlets were consistently augmented during SP-induced depolarization, leading to cell discharge.

6. Focal stimulations elicited, in addition to a fast EPSP, a slow EPSP in about 40% of SPNs. The slow EPSP was often associated with an increased membrane resistance and became smaller on hyperpolarization.

7. In 15% of SPNs that generated a slow EPSP, the latter was reversibly abolished during SP-induced depolarization; the blockade persisted when the membrane potential was restored to the resting level by hyperpolarizing current.

8. It is concluded that SP is excitatory to SPNs and that its synaptic release may initiate a slow EPSP which serves to augment impulse transmission through SPNs. Further, it appears that inhibitory interneurones may also be sensitive to SP and their activation may provide a negative feed-back mechanism which can limit excessive excitation of SPNs by the peptide.

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INTRODUCTION

Immunohistochemical studies have revealed an uneven distribution of substance P (SP) or a SP-like peptide in nerve fibres in the mammalian spinal cord (Cuello & Kanazawa, 1978; Ljungdahl, Hökfelt & Nilsson, 1978; Cuello, Priestly & Matthews, 1982). The origin and postulated physiological role of SP-immunoreactive fibres appear to differ depending on the region of spinal cord. Thus, SP-immunoreactive fibres in the dorsal horn arise mainly from dorsal root ganglion cells and are likely to subserve sensory transmission (Hökfelt, Kellerth, Nilsson & Pernow, 1975; Otsuka, Konishi & Takahashi, 1975). On the other hand, the majority of SP-positive fibres in the intermediolateral cell column (IML) appear to have their origin in the ventral medulla (Helke, Neil, Massari & Loewy, 1982). There is pharmacological evidence that activation of descending SP-pathways from the ventral medulla to the IML increases vasomotor activities. For example, stimulation of neurones in the ventral medulla by local injection of kainic acid caused an increase in blood pressure and heart rate which could be reversed by the SP antagonist (D-Pro^2, D-Trp^{7,9})-SP (Loewy & Sawyer, 1982). Likewise, intrathecal administration of a stable SP analogue (pGlu^5, MePhe^8, MeGly^9)-SP$_{(5-11)}$ resulted in a heightening of vasomotor activities which was attenuated by the SP antagonist (D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11})-SP (Keeler, Charlton & Helke, 1985). Lastly, SP applied iontophoretically to *in vivo* cat sympathetic preganglionic neurones (SPNs) accelerated the firing rate of these neurones (Gilbey, McKenna & Schramn, 1983; Backman & Henry, 1984).

This study was undertaken to determine the effects of substance P applied directly to the membrane of SPNs and to compare these results with the action of synaptically mediated events evoked by local stimulation. A preliminary communication appeared previously (Mo & Dun, 1986).

METHODS

Neonatal (12–20 days) Sprague–Dawley rats were used in this study. The procedures used in obtaining thin transverse thoracolumbar spinal cord slices have been described (Ma & Dun, 1985, 1986). Neonatal rats were anaesthetized with ether. Following a laminectomy, a 1–1·5 cm segment of the thoracolumbar spinal cord was removed and quickly immersed in oxygenated Krebs solution which was kept at room temperature. The pia mater was carefully removed under a dissecting microscope and the spinal segment was subdivided into several blocks using a sharp razor blade. One block was affixed with cyanoacrylic glue to the bottom of a Plexiglas block fastened to the cutting chamber of an Oxford Vibratome filled with oxygenated Krebs solution. Several 500 μm slices of spinal cord with their corresponding dorsal and ventral rootlets were cut from each block of the spinal segment. Under favourable conditions slices with ventral and dorsal rootlets of 2–3 mm in length could be isolated. The first one or two slices were generally discarded; the remaining slices were incubated in Krebs solution at room temperature (21 $\pm$ 1 °C). One slice was transferred to the recording chamber and continuously superfused with a Krebs solution of the following composition (mM): NaCl, 117; KCl, 3·1; KH_2PO_4, 1·2; $CaCl_2$, 2·4; $MgSO_4$, 1·3; $NaHCO_3$, 26; and glucose, 10; the solution was saturated with 95% O_2 and 5% CO_2, and the temperature of the solution reaching the slice was maintained at 34 $\pm$ 0·5 °C.

Intracellular recordings were made from neurones situated in the lateral horn area of the thoracolumbar spinal cord slices by means of glass microelectrodes filled with 2 M-potassium acetate, their impedances varying between 60 and 100 MΩ. Electrical stimulation of the ventral and dorsal rootlets was accomplished via a concentric bipolar electrode positioned close to the

respective rootlets. In a few experiments, the stimulating electrode was placed close to the recording SPNs (focal stimulation). Signals were amplified via a WPI 707A preamplifier and displayed on a Nicolet Digital Oscilloscope and on a Gould pen recorder. SP was administered to the lateral horn neurones by either superfusion in known concentrations (0·1–10 μM) or by pressure ejection (Picospritzer II, General Valve Co.). In the latter case, the peptide was ejected from a SP-containing micropipette (0·1 mM, 280 pK_a, pulse duration: 100–500 ms) to the vicinity of impaled SPNs. The results are expressed as mean ± S.D. SP and other peptides were purchased from Peninsula Laboratories.

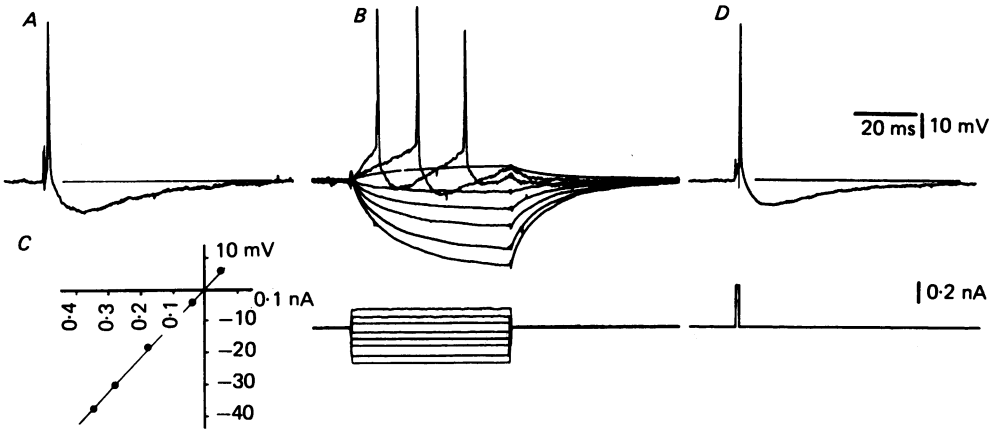

Fig. 1. Antidromic and direct action potentials evoked in a rat sympathetic preganglionic neurone (SPN). *A*, antidromic spike induced by stimulation of ventral rootlets. The conduction velocity was calculated to be 1·1 m/s at 33 °C bath temperature. *B*, current–voltage relations of this neurone. *C*, plot of steady-state current–voltage relations for subthreshold range of membrane potential. *D*, action potential evoked by short depolarizing current pulse.

RESULTS

Stable recordings were obtained from fifty-seven lateral horn neurones that could be activated antidromically by the stimulation of ventral rootlets. These neurones were classified as sympathetic preganglionic neurones (SPNs) on the basis of their conduction velocities (Fig. 1; see also Mo & Dun, 1986). Indeed, axons of adult rat SPNs are composed of mainly C fibres which conduct at a rate of about 1 m/s (Perri, Sacchi & Casella, 1970; Gilbey, Peterson & Coote, 1982). In our experiments, the conduction velocity of antidromic spikes evoked from lateral horn neurones ranged from 0·8 to 1·7 m/s, with a mean of 1·2 ± 0·3 m/s (mean ± S.D.) when measured at 34 ± 0·5 °C bath temperature. On the basis of conduction velocities, SPNs can be readily distinguished from rat motoneurones as their axonal conduction velocity in a slice preparation is above 10 m/s for γ- and 30 m/s for α-motoneurones (Jiang & Dun, 1986; Z. G. Jiang & N. J. Dun, unpublished). SPNs exhibited mean resting potential, input resistance and time constant of −58 ± 7 mV, 110 ± 34 MΩ and 8·9 ± 2·7 ms, respectively. The antidromic spike evoked from SPNs was short lasting (< 3 ms) and followed by a well-defined after-hyperpolarization with a mean amplitude and half-decay time of 8 mV and 36 ms, respectively (Fig. 1).

Effects of substance P

Initial experiments indicated that SP applied either by superfusion (0·1–10 μM) or pressure ejection produced similar responses in SPNs. Comparable responses were

obtained in five SPNs to which SP was administered by both methods. However, more rapid onset and offset of the SP response was obtained with the pressure-ejection method. The results reported below were obtained by pressure ejections, unless otherwise stated. Generally, the responsiveness of the neurone to SP of

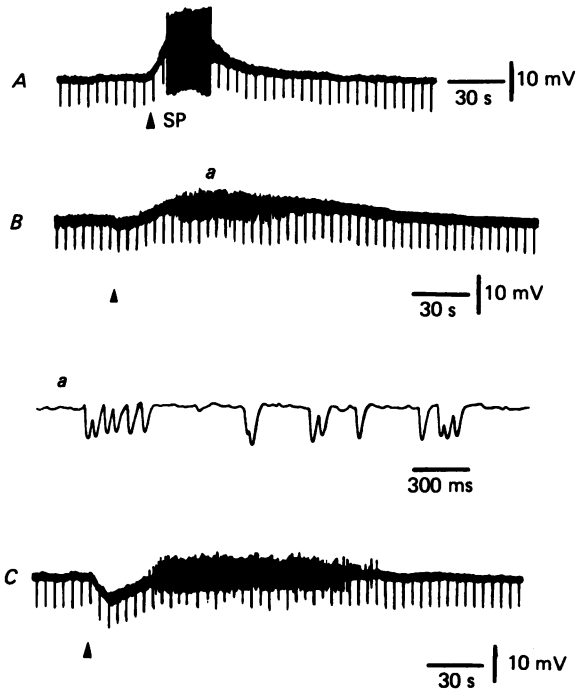

Fig. 2. Three types of response to SP applied by pressure ejection recorded from three SPNs. Arrow-heads indicate the ejection of SP. Downward deflections represent hyperpolarizing electrotonic potentials induced by hyperpolarizing current pulses the duration and intensity of which varied in different cells. *A*, fast-rising depolarization induced by 100 ms ejection pulse of SP was accompanied by intense cell discharge. *B*, slow-rise depolarization produced by 150 ms ejection pulse of SP was accompanied by inhibitory postsynaptic potentials (IPSPs) which appeared as thickening of the trace. When recorded at a fast speed taken at the time *a*, individual IPSPs could clearly be seen. *C*, a biphasic response consisting of a hyperpolarization followed by a low-amplitude depolarization which displayed intense discharge of IPSPs. The pulse duration used to eject SP in this experiment was 100 ms. The apparent input resistance as reflected by the amplitude of hyperpolarizing electrotonic potentials shows a small decrease and increase in recordings *A* and *B*, respectively. Because of the intense discharge of IPSPs in trace *C*, input resistance change cannot be assessed accurately. Recordings in *A*, *B* and *C* were made from three different SPNs.

varying pulse durations was first tested on a trial and error basis until the pulse duration (usually between 100 and 500 ms) that produced an optimal response was determined; thereafter this duration was used throughout a given experiment. The initial series of experiments also demonstrated that the depolarization induced by pressure application of SP was abolished during bath application of SP ($n = 3$), indicating that desensitization of SP receptors to exogenously applied SP developed readily in the SPNs.

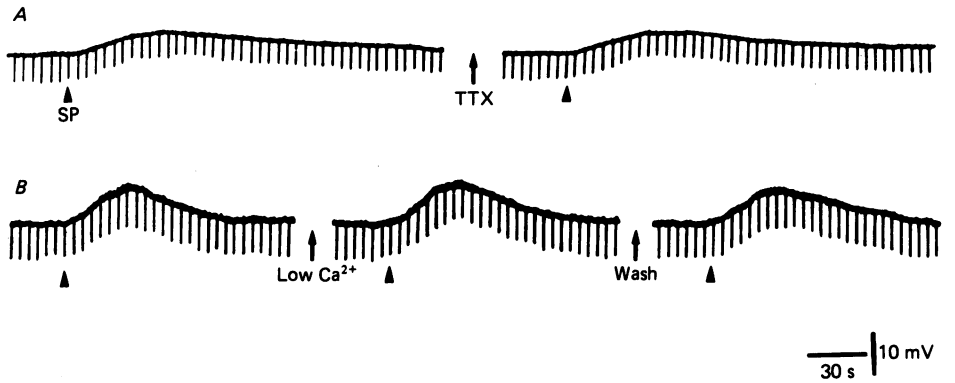

Fig. 3. SP-induced depolarizations remained relatively unaltered before and after TTX (0·1 μM) or low-Ca^{2+} (0·25 mM) superfusion in two rat SPNs. SP was applied onto the neurones as indicated by arrow-heads. The ejection pulse duration used in experiments *A* and *B* was 200 and 300 ms, respectively. Note the absence of IPSPs in recordings *A* and *B*. Superfusion of TTX and low-Ca^{2+} Krebs solution lasted 10 and 15 min, respectively. Downward deflections are hyperpolarizing electrotonic potentials which show relatively minor changes during SP-induced depolarizations in these two cells.

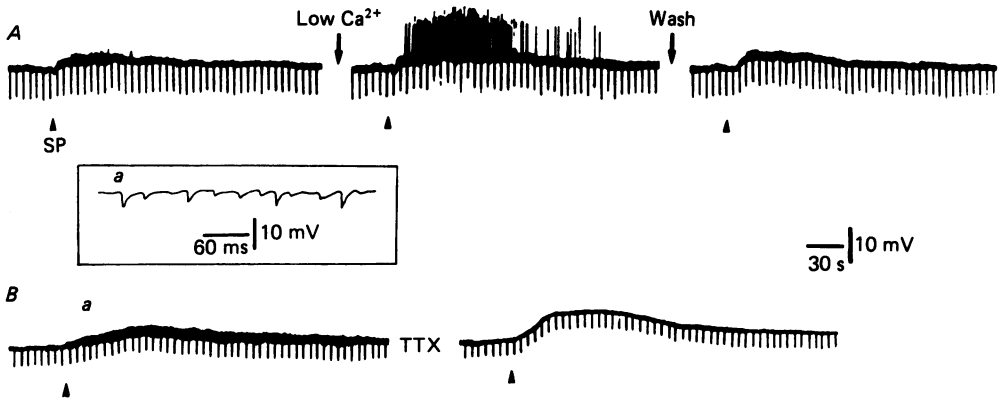

Fig. 4. Effects of low Ca^{2+} and TTX on IPSPs induced by SP. *A*, SP (150 ms pulse duration) evoked a low-amplitude depolarization accompanied by IPSPs evidenced as thickening of the trace. In a low-Ca^{2+} (0·25 mM) solution, SP now evoked a larger depolarization with bursts of discharge and no sign of IPSPs. After washing, SP caused a small response and the reappearance of IPSPs. *B*, SP applied by pressure ejection (300 ms pulse duration) elicited a slowly rising depolarization and a thickening of the trace which became most intense at the peak of depolarization. Recording of individual IPSPs taken at time *a* is shown in inset. In the presence of TTX (0·1 μM), SP evoked a depolarization substantially larger than that evoked in normal Krebs solution. Further, the trace is clean with no signs of IPSPs.

Initial analysis of the results indicated that SP produced two types of response in SPNs: a monophasic, slow depolarization ($n = 35$, 60% of neurones tested) and a biphasic response consisting of an initial hyperpolarization followed by a depolarization ($n = 7$, 14%). The remaining neurones ($n = 15$) were insensitive to SP whether applied by pressure ejections or by superfusion. Additionally, small inhibitory postsynaptic potentials (IPSPs) which appeared as a thickening of the baseline when recorded at a slow chart speed were detected in nine SPNs following

SP application. At a fast chart speed, individual IPSPs were clearly discernible (Figs 2 and 4). Examples of each type of response are illustrated in Fig. 2. In the case of the monophasic response, the slow depolarization developed after a latency of 2–4 s (mean = 2·8 ± 0·8, $n = 14$), and it varied from a few millivolts to over 20 mV; the mean was 7·2 ± 2·4 mV when recorded at the membrane potential of −50 to −60 mV. The duration of SP-induced depolarization ranged from 60 to 150 s, with a mean of 89·4 ± 28·6 s. Spike discharges were frequently seen during the rising or plateau phase of SP-induced depolarizations (Figs 2, 4, 5 and 6). With respect to the small IPSPs that appeared in a portion of SPNs, their amplitude varied from less than 1 to 5 mV and the frequency ranged from 5 to over 10 per second.

The biphasic response to SP was observed in seven SPNs. The initial hyperpolarization had an amplitude of 3–7 mV and duration of 10–30 s (Fig. 2). Interestingly, bursts of IPSPs could be detected during the depolarizing phase of the biphasic response in three of these neurones (Fig. 2).

Effects of low Ca^{2+} and tetrodotoxin (TTX)

Superfusing the slices with low-Ca^{2+} (0·25 mM, $n = 4$) or TTX (0·1 μM, $n = 3$)-containing Krebs solution did not cause any significant change of the amplitude or duration of SP-induced monophasic depolarizations (Fig. 3). On the other hand, in the case of neurones in which SP produced either a biphasic response or IPSPs, low Ca^{2+} or TTX eliminated the hyperpolarizing phase of the biphasic response and IPSPs and, more significantly, augmented the depolarizing response to SP (Fig. 4). The increase of SP-induced depolarization in low-Ca^{2+} or TTX solution can be explained by the removal of inhibitory influence of IPSPs which curtailed the full expression of the depolarizing action of SP.

Effects of strychnine

In an earlier study, we reported the recordings in the SPNs of strychnine-sensitive spontaneous IPSPs and evoked IPSPs (Mo & Dun, 1987*a*). Consistent with the results of this earlier study, strychnine (0·1 μM), a glycine antagonist (Curtis, Duggan & Johnston, 1971), reversibly abolished the SP-induced IPSPs in all three SPNs tested. Likewise, strychnine eliminated the hyperpolarizing phase of the biphasic response in the two cells examined. Moreover, as it eliminated the IPSPs and the hyperpolarizing phase of the biphasic response, strychnine enhanced the depolarizing responses to SP in the neurones tested.

Reduction of K^+ conductance

The membrane input resistance as monitored by the amplitude of hyperpolarizing electrotonic potentials showed either no change or a slight increase or decrease during SP-induced depolarization of the SPNs. The slow time course of the depolarization made it possible to clamp manually the membrane potential during the response (Fig. 5). Under these conditions, a clear increase in membrane resistance was observed in thirteen of the sixteen SPNs tested; this increase ranged from 8 to 45%, with a mean of 24%. In the remaining three SPNs, the membrane resistance showed no measurable change.

The amplitude of the SP-induced depolarization was inversely related to

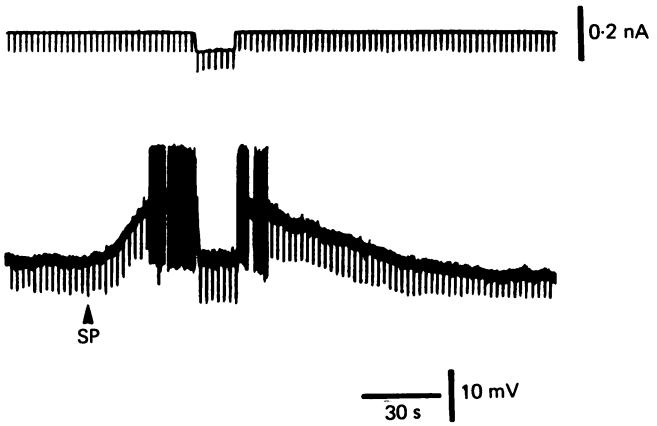

Fig. 5. SP-induced depolarization and increased input resistance of a rat SPN. Arrowhead shows time of ejection of SP (100 ms ejection duration) onto the neurone. Lower trace, voltage recording; downward deflections of trace are electrotonic potentials. Upper trace, current record. Pressure ejection of SP caused a slow depolarization accompanied by intense discharge. At the peak of response, the membrane potential was returned momentarily to the resting level by passage of hyperpolarizing current. Under these conditions, there was a 30% increase of membrane input resistance.

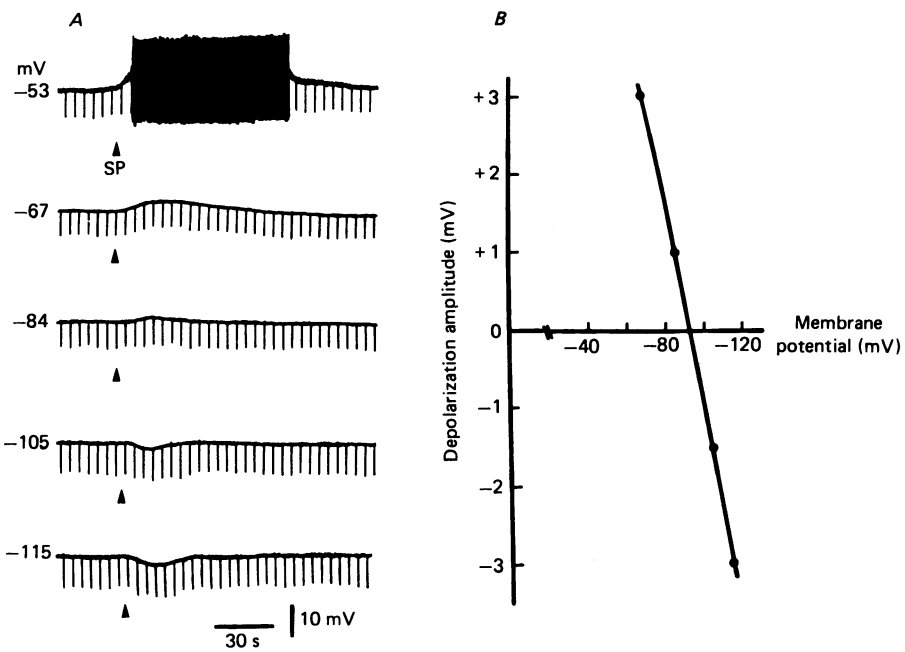

Fig. 6. Reversal of SP-induced depolarization by membrane hyperpolarization. *A* and *B*, effect of altered membrane potential (as indicated to the left of traces) on the responses and graph of depolarization amplitude against membrane potential. Membrane hyperpolarization reduced the amplitude of SP depolarization; at the membrane potentials of −105 and −115 mV, SP caused a hyperpolarization instead of a depolarization. The reversal potential of −92 mV is indicated in the graph.

membrane potential in nine of the ten SPNs studied. The response was made smaller on membrane hyperpolarization, and it was reversed at the membrane potential of −90 to −100 mV. A representative experiment is shown in Fig. 6. It should be noted that while in three neurones the response became smaller on membrane hyperpolarization, a clear reversal was not seen. Membrane hyperpolarization increased the amplitude of SP-induced depolarization in one cell.

Pharmacological antagonists

Effects of several SP antagonists were studied in an effort to determine the pharmacological profile of SP response evoked in SPNs. Neither (D-Pro^4, D-Trp^{7,9})-SP nor (D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11})-SP (Loewy & Sawyer, 1982; Akagi, Konishi, Otsuka & Yanagisawa, 1985) used in concentrations of up to 10 μM caused any measurable depression of SP-induced depolarization in any of the twelve SPNs tested. Negative results were also obtained with another antagonist, (D-pGlu^1, D-Phe^2, D-Trp^{3,6})-luteinizing hormone-releasing hormone which was reported to antagonize the excitatory action of SP on the rat SPNs (Takano, Sawyer, Sanders & Loewy, 1985). Superfusion of this compound in the concentrations of 1, 10 and 50 μM for 10–15 min did not cause any significant decrease of the amplitude of SP-induced depolarization in any of the five cells investigated.

Facilitation of fast excitatory potentials (EPSPs)

Electrical stimulations of dorsal rootlets by a concentric bipolar electrode evoked a fast-rising EPSP in the SPNs; when the fast EPSP reached the threshold an action potential resulted. Subthreshold fast EPSPs evoked from SPNs were consistently facilitated during SP-induced depolarization leading to spike discharges in four of the five cells; a representative recording is shown in Fig. 7. A facilitation of lesser magnitude could still be demonstrated when the membrane potential was held at the resting level by passage of hyperpolarizing current (Fig. 7).

Physiological correlate: slow EPSP

In about 40% of SPNs sampled, single supramaximal focal stimulus (0·1 ms, 10–20 V) elicited a fast EPSP that was followed by a slow depolarizing potential, the slow EPSP. The slow EPSP displayed a latency of 300–500 ms, rise time of 1–3 s, and duration of 5–20 s. The amplitude of slow EPSP varied from 3–5 mV after a single stimulus, to over 15 mV following a train of stimuli (10–30 Hz, Fig. 8*A*). The slow EPSP was most often associated with an increase of membrane resistance when the potential change was nullified by hyperpolarizing current (Fig. 8*B*).

The possibility that SP was the transmitter or one of the transmitters mediating the slow EPSP was investigated. Application of SP caused a depolarization in six of the eight SPNs that generated a slow EPSP. More interestingly, the slow EPSP but not the fast EPSP in two of the SP-sensitive neurones was eliminated by the application of SP. Figure 8*C* shows a neurone that was capable of generating the slow EPSP prior to SP application; however, it failed to initiate a slow EPSP following the SP-induced depolarization, even when the membrane potential was momentarily restored to the resting level by passage of hyperpolarizing current. The slow EPSP

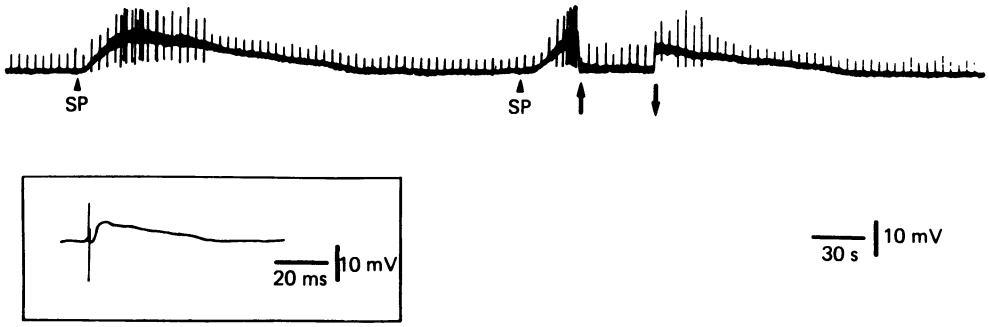

Fig. 7. Potentiation of fast EPSPs by SP in a SPN. Upward deflections are subthreshold fast EPSPs evoked by dorsal rootlet stimulations (a fast EPSP is shown in inset). SP (150 ms pulse) caused a depolarization during which the subthreshold fast EPSPs reached the threshold and gave rise to action potentials. An enhancement of the amplitude of fast EPSPs could still be demonstrated when the membrane potential at the peak of SP depolarization was momentarily restored to the resting level (between two arrows). Although the fast EPSPs under these conditions failed to reach the threshold, the amplitude was clearly increased.

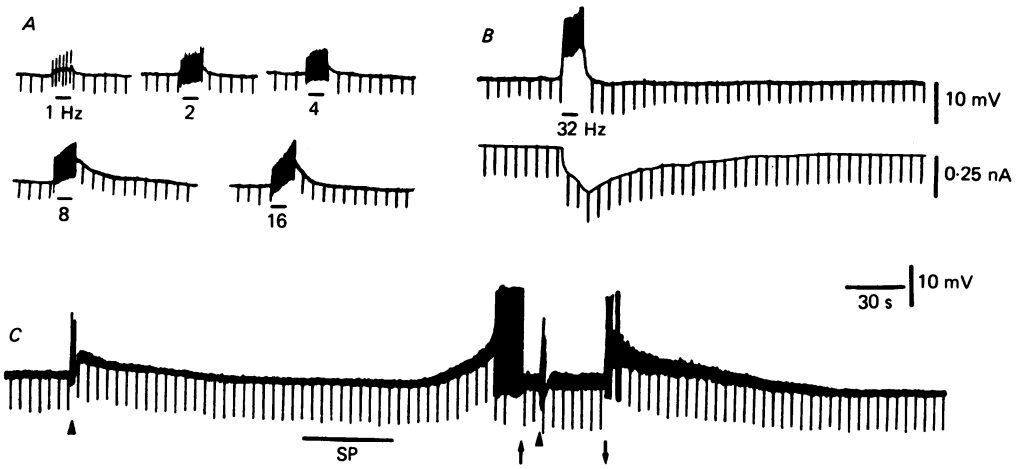

Fig. 8. Fast and slow EPSPs evoked in two SPNs. *A*, focal stimulations at a low frequency of 1 Hz evoked a fast EPSP (vertical line) followed by a small slow depolarization (slow EPSP). The amplitude of slow EPSP clearly became larger during higher frequencies of stimulation (2–16 Hz for 5 s). *B*, an increase of input resistance (60%) can be detected when the membrane was manually clamped at the resting level. *C*, repetitive focal stimulations (10 Hz, 2 s, arrow-heads) evoked a burst of fast EPSPs followed by a slow EPSP. SP (1 μM) was applied to the slice by superfusion for a period indicated by the bar, causing a slow depolarization and cell discharge (spikes were truncated). At the peak of depolarization, membrane potential was momentarily restored to the resting level (indicated by two arrows) during which time repetitive stimulation elicited only fast EPSPs that were not followed by a slow EPSP. Recordings *A*, *B* and *C* were taken from two different SPNs.

could again be evoked a few minutes after the SP-induced depolarization had subsided. It should be mentioned that SP depolarized four additional SPNs which generated a slow EPSP, but failed to block it.

DISCUSSION

The principal observation made in this study is that the undecapeptide SP depolarized and excited antidromically identified SPNs and probably inhibitory interneurones. With respect to the SPNs, the most consistent effect of SP was a slow depolarization and induction of cell discharges. About one-third of SPNs were insensitive to SP whether applied by pressure ejections or superfusion. This may imply that SP receptors are localized on a subpopulation of SPNs.

Hyperpolarizations and IPSPs were observed in a number of SPNs following SP applications. The finding that the hyperpolarization and IPSPs were abolished in low-Ca^{2+} or TTX-containing solution as well as by strychnine suggests that these responses were synaptic in nature and not due to a direct action of SP on the SPNs. Hence, SP ejected from the pipette probably activated an interneurone(s) in the immediate vicinity of the recorded SPN, causing a release of glycine or a glycine-like substance which in turn initiated the hyperpolarizing response and/or the IPSPs. This interpretation is supported by our previous finding that glycine or a glycine-like substance is the mediator of spontaneous IPSPs and IPSPs evoked in the SPNs (Mo & Dun, 1987*a*). The finding that glycine-containing interneurones in the intermediolateral cell column may be endowed with SP receptors is interesting and may have a functional significance. In this connection, the depolarizing response to SP was enhanced following the blockade of the hyperpolarizing phase of the biphasic response and of the IPSPs, indicating that the hyperpolarizing responses effectively attenuated the responsiveness of the SPNs to the peptide. As a corollary, activation of SP receptors on the inhibitory interneurone may provide a braking mechanism on the SPN whereby excessive stimulation by the peptide can be timely and effectively tuned down. It remains to be shown whether an analogous phenomenon can be induced by synaptically released SP.

It is pertinent to mention that the characteristics of SP-induced depolarizations in SPNs, namely, a considerable delay in onset, the slow rise and slow decay of the response, and the induction of cell discharge, are similar to the characteristics described for the SP response of other central and peripheral neurones that have been studied (Katayama & North, 1978; Dun & Minota, 1981; Nowak & MacDonald, 1982; Murase & Randić, 1984; Stanfield, Nakajima & Yamaguchi, 1985).

The SP-induced depolarization of SPNs was associated with a decrease in membrane resistance, it was reduced by hyperpolarization, and it was reversed at the membrane potential of -90 and -100 mV; all these findings suggest that a reduction of membrane K^+ conductance constitutes the primary ionic mechanism underlying the depolarizing action of SP. A similar ionic mechanism has been postulated for the depolarizing action of SP on guinea-pig myenteric neurones (Katayama & North, 1978; Nowak & MacDonald, 1983). However, in one case the response was increased on membrane hyperpolarization, and while in a few SPNs the response was decreased by hyperpolarization, it was not reversed. These findings

suggest that other ionic species, e.g. Na^+ and/or Ca^{2+}, in addition to K^+, may have been affected, as has been suggested for the SP response of the guinea-pig inferior mesenteric ganglion cells (Dun & Minota, 1981; Brown & Griffith, 1984) and rat dorsal horn cells (Murase & Randić, 1984; Murase, Ryu & Randić, 1986). On the other hand, a reduction of a voltage-sensitive K^+ current, the M-current, and of an inwardly rectifying K^+ current has been shown to underlie the depolarizing action of SP on bull-frog sympathetic neurones and rat cultured magnocellular neurones, respectively (Adams, Brown & Jones, 1983; Stanfield *et al.* 1985).

Several reputed SP antagonists tested here failed to block the action of SP on the SPNs. Takano *et al.* (1985) suggested that since (D-ρGlu^1, D-Phe^2, D-Trp^{3,6})-LH-RH blocked the [^{3}H]SP bindings in the rat intermediolateral cell column, the SP receptor on rat SPNs is of the substance P-P subtype. In our study, this compound in concentrations of up to 50 μM exerted little or no measurable depression of the SP-induced depolarizations. One possible explanation for the discrepancy is that neonatal rats were used in our study, while adult rats were used in the study of Takano *et al.* (1985). The pharmacological profile of SP receptors in the spinal cord may undergo developmental changes; thus, their sensitivity to antagonist may vary with age.

Is there any evidence for SP-mediated synaptic transmission in the SPNs? Two types of excitatory potential, fast and slow, were recorded in the SPNs. Our early studies showed that the fast EPSP evoked in the SPNs was reversibly suppressed by D-2-amino-5-phosphonvalerate and by ketamine (Mo & Dun, 1987*b*). Since these compounds antagonize the action of excitatory amino acids on *N*-methyl-D-aspartate receptors (McLennan, 1983; Dingledine, 1986), it was inferred that the transmitter mediating the fast EPSP in the SPNs is an excitatory amino acid (Mo & Dun, 1987*b*). The slow EPSP on the other hand exhibits characteristics that are similar to those of the depolarization evoked by SP. More importantly, the slow EPSP in two SPNs was reversibly abolished by exogenously applied SP. This finding together with the observation of marked tachyphylaxis of SP receptors to exogenously applied SP suggests that the blockade of slow EPSP in the presence of SP may be due to desensitization of the receptors mediating the slow EPSP. Alternatively, the blockade may be caused by the occlusion of the same ionic channels, i.e. K^+, inactivated by SP and by the endogenous transmitter, not necessarily SP, mediating the slow EPSP. For example, the blockade of the depolarization or current induced by bethanechol, a muscarinic agonist, in the presence of luteinizing hormone-releasing hormone is attributed to the occlusion of the same ganglionic K^+ channels inactivated by these two pharmacologically distinct compounds (Kuffler & Sejnowski, 1983). The development of specific antagonists to SP receptors will be needed to answer the question whether SP blocks the slow EPSP by the mechanism of desensitization or occlusion.

Our electrophysiological finding together with the presence of SP-immunoreactive fibres in the intermediolateral cell column (Helke *et al.* 1982) and of [^{3}H]SP bindings in this region (Maurin, Buck, Wamsley, Burks & Yamamura, 1984; Charlton & Helke, 1985) raises the possibility that synaptically released SP or a related peptide may initiate a slow EPSP in a population of SPNs. In this regard, a similar role of SP has been postulated in guinea-pig myenteric (Katayama & North, 1978) and

inferior mesenteric (Dun & Jiang, 1982) ganglion cells and rat dorsal horn neurones (Urban & Randić, 1984). It should be noted however that while effective in causing a slow depolarization, SP failed to block the slow EPSP in the majority of SPNs, suggesting the involvement of other transmitters, in addition to SP, in the generation of slow EPSP in the rat SPNs.

The most consistent and apparent effect of SP on synaptic transmission of the SPNs was a facilitation of the fast EPSPs. Two possible mechanism may be involved. First, the SP-induced depolarization brings the cell closer to the threshold level; second, the increased membrane resistance that was often associated with the SP-induced depolarization should amplify the current flow produced by the fast EPSPs, resulting in a greater voltage deflection. Furthermore, this increase in membrane resistance should increase the space constant of the soma–dendritic membrane, and thus the likelihood of spatial summation of fast EPSPs, while the long (seconds to minutes) time course of SP-induced depolarization or of the slow EPSP should maximize the likelihood of their temporal summation. The physiological consequence of the synaptic release of SP at the SPNs would then be a facilitation of their discharge, leading to an increase in vasomotor activity.

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