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INHIBITORY POSTSYNAPTIC POTENTIALS IN NEONATAL RAT SYMPATHETIC PREGANGLIONIC NEURONES *IN VITRO*

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SUMMARY

1. Intracellular recordings were made from antidromically identified sympathetic preganglionic neurones (SPNs) in transverse sections of thoraco-lumbar spinal cord from neonatal (12–22 day) rats.

2. Two types of hyperpolarizing (inhibitory) postsynaptic potentials (IPSPs) were recorded in the SPNs. The first type, which we have termed unitary IPSPs, were small, discrete IPSPs that occurred spontaneously and also following chemical or electrical stimulation applied to the spinal cord slices. The second type of IPSP was a hyperpolarizing response evoked by either dorsal or ventral root stimulation.

3. Spontaneously occurring unitary IPSPs had an amplitude of 1 to 5 mV, and reversal potential of −60 to −75 mV; they were reversibly abolished by low Ca^{2+}, tetrodotoxin (TTX) or strychnine but not by bicuculline and picrotoxin.

4. Pressure application of *N*-methyl-D-aspartate (NMDA), an excitatory amino acid receptor agonist, induced the occurrence of unitary IPSPs in a proportion of SPNs; these were abolished by either strychnine or by the NMDA receptor antagonist D-2-amino-5-phosphonovalerate. Furthermore, electrical stimulation of dorsal rootlets elicited in several SPNs the discharge of strychnine-sensitive unitary IPSPs.

5. Electrical stimulation applied to dorsal or ventral rootlets elicited in nineteen and eight SPNs, respectively, an IPSP of larger amplitude (5 to 15 mV). The IPSP exhibited a reversal potential of −60 to −75 mV; it was changed to a depolarizing response in a low $[Cl^-]_o$ solution, but was not significantly affected in a low $[K^+]_o$. Strychnine but not bicuculline or picrotoxin reversibly blocked the IPSPs in nearly all the SPNs. Additionally, hexamethonium and *d*-tubocurarine antagonized the IPSPs evoked by ventral but not by dorsal root stimulations.

6. Our results suggest that unitary and evoked IPSPs recorded in SPNs are due primarily to an increase of Cl^- conductance by glycine or a glycine-like substance, released from interneurones, that can be activated by NMDA. Furthermore, IPSPs evoked by ventral root stimulation appear to represent a disynaptic event whereby nicotinic activation of a glycine-releasing interneurone results in a release of the inhibitory transmitter; this is then analogous to the Renshaw cell circuitry of the spinal motoneurones.

INTRODUCTION

Sympathetic preganglionic neurones (SPNs) constitute the final common pathway site in the spinal cord whereby descending and segmental information related to cardiovascular activity is received, processed and transmitted to the peripheral sympathetic ganglia (Barman, 1984; Schramm, 1986; Coote, 1988). Viewed in this context, the available information concerning the electrical behaviour and synaptic transmission which underlie the transfer of information from central and peripheral neurones to SPNs is limited.

An important aspect of this transmission concerns the mechanism of inhibition in the SPNs. The early report by Polosa (1967) that antidromic or orthodromic activation of SPNs is followed by a 'silent period' lasting several hundred milliseconds to minutes, implies the existence of intrinsic and/or extrinsic inhibitory mechanisms in these neurones. Yet, inhibitory postsynaptic potentials (IPSPs) were rarely observed in cat SPNs *in situ* (Coote & Westbury, 1979; McLachlan & Hirst, 1980; Dembowsky, Czachurski & Seller, 1985*b*).

In the present study we report frequent recordings of hyperpolarizing potentials in the SPNs contained in thin transverse sections of neonatal rat spinal cord. In addition, our data provide pharmacological evidence of a recurrent inhibitory pathway in a population of rat preganglionic neurones that may be involved in an intraspinal inhibitory mechanism resembling the Renshaw cell circuitry associated with motoneurones (Renshaw, 1941; Eccles, Fatt & Koketsu, 1954).

METHODS

Neonatal (12–22 day) Sprague–Dawley rats were used in this study. The procedures used in obtaining thin (500 μm) transverse thoraco-lumbar spinal cord slices have been described in detail before (Ma & Dun, 1986; Dun & Mo, 1988). 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 ± 1 °C) until used. The slices were transferred to the recording chamber and continuously superfused with a Krebs solution of the following composition (mM): NaCl, 117; KCl, 2·0; KH_2PO_4, 1·2; $CaCl_2$, 2·3; $MgSO_4$, 1·3; $NaHCO_3$, 26; 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 $34 \pm 0{\cdot}5$ °C. In the series of experiments in which low-Cl^- solution was used, $[Cl^-]_o$ was reduced to 4·3 mM by substituting NaCl with sodium isethionate. In the case of experiments involving low $[K^+]_o$, the concentration of KCl was decreased without adjustment of other ions. Magnesium-free solution was prepared by omitting Mg^{2+} ions from the Krebs solution.

Intracellular recordings were made from neurones situated in the lateral horn area of the thoraco-lumbar spinal cord slices by means of glass microelectrodes filled with 2 M-potassium acetate; their impedance varied between 60 and 120 MΩ. Electrical stimulation of the ventral and dorsal rootlets was accomplished via a concentric bipolar electrode positioned close to the respective rootlets. Signals were amplified via an Axoclamp II preamplifier and displayed on a Nicolet Digital Oscilloscope and on a Gould pen recorder. The drugs were dissolved in Krebs solution and applied to the slices by superfusion in known concentrations. *N*-Methyl-D-aspartate (NMDA, 1 mM) was applied to the SPNs from a closely positioned micropipette by a puff of nitrogen (Picospritzer, General Valve Co.) at 280 pK_a and duration of 5–50 ms. The results are expressed as mean ± S.D.

The following compounds, purchased from Sigma Co., were used: bicuculline, picrotoxin, *d*-tubocurarine chloride, hexamethonium bromide, tetrodotoxin, *N*-methyl-D-aspartate, strychnine hydrochloride, furosemide (frusemide), penicillin-G and D-2-amino-5-phosphonovalerate.

RESULTS

Stable recordings were obtained from ninety-two lateral horn neurones which were identified as sympathetic preganglionic neurones (SPNs) by the appearance of an antidromic spike following stimulation of ventral rootlets (see Ma & Dun, 1986; Dun

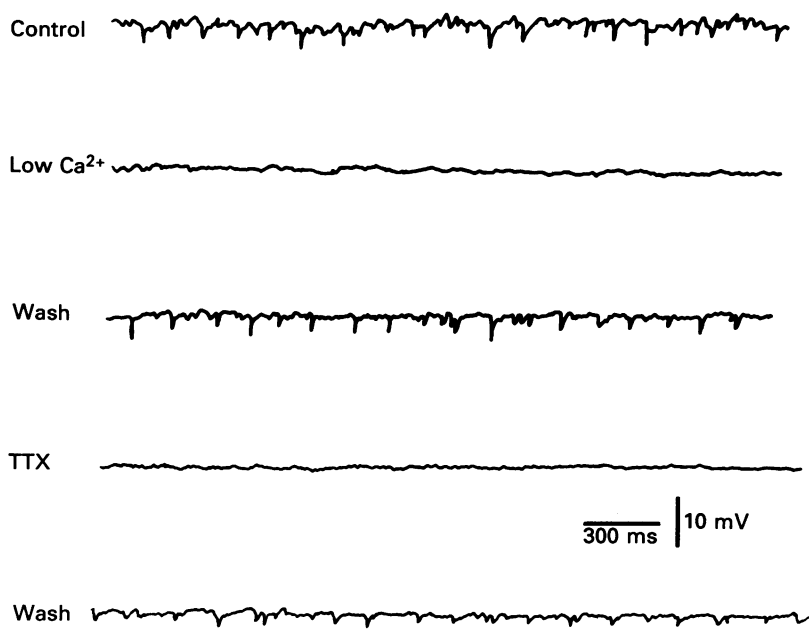

Fig. 1. High-frequency discharge of spontaneously occurring unitary IPSPs in a rat sympathetic preganglionic neurone. Small, discrete hyperpolarizing potentials occurred at a relatively high frequency in this otherwise quiescent neurone which had a resting membrane potential of −53 mV. Without causing a detectable change of resting membrane potential, low Ca^{2+} (0·25 mM) or TTX (0·1 μM) reversibly eliminated the unitary IPSPs. Recordings were made from the same neurone.

& Mo, 1988). These cells had a mean resting membrane potential, input resistance and time constant of -57 ± 7 mV, 114 ± 22 MΩ and 8 ± 3 ms respectively (Ma & Dun, 1986; Dun & Mo, 1988).

Two types of hyperpolarizing (inhibitory) potentials were recorded. The first type consisted of low-amplitude, discrete, hyperpolarizing potentials that occurred either spontaneously or following a stimulus applied to the slices. We will refer to these responses as unitary IPSPs, although at present we have no direct evidence that they result from activation of a single presynaptic fibre. The second type was a hyperpolarizing potential of larger amplitude, the IPSP evoked by stimulation of either dorsal or ventral roots (Figs 6 and 7).

Spontaneous unitary IPSPs

Spontaneously occurring unitary IPSPs, the frequency of which varied from less than 1 to over 20 s^{-1}, were detected in twenty-nine otherwise quiescent SPNs (Fig. 1).

The spontaneous unitary IPSPs appeared to occur at fairly regular intervals in a number of SPNs (see Figs 1 and 2). Superfusion of the slices with Krebs solution containing either low Ca^{2+} (0·25 mM, $n = 6$) or tetrodotoxin (TTX, 0·1 μM, $n = 4$) reversibly abolished the unitary IPSPs (Fig. 1). When recorded at the resting

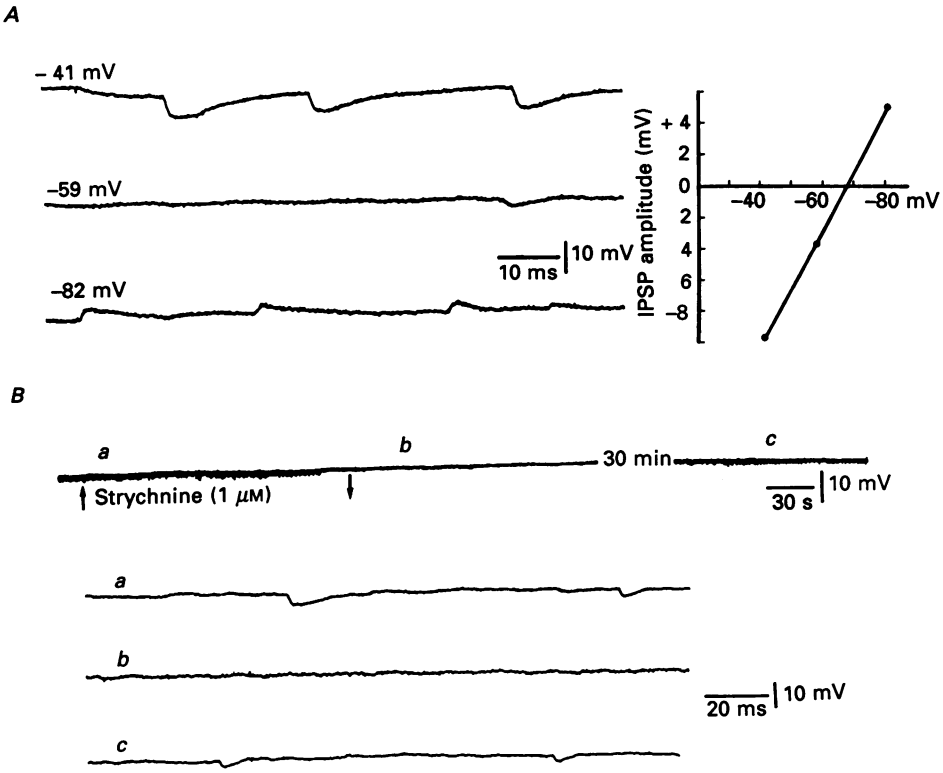

Fig. 2. Reversal and blockade of spontaneous unitary IPSPs by membrane hyperpolarization and by strychnine in two sympathetic preganglionic neurones. Cell *A*, membrane hyperpolarization reduced and ultimately reversed the unitary IPSPs. Graph to the right depicts the relationship between the amplitude of unitary IPSPs and membrane potential. The line intercepts at −67 mV which is taken as the reversal potential of unitary IPSPs in this neurone. The resting membrane potential of this neurone was −41 mV. Cell *B*, continuous, slow-speed chart recording of spontaneously occurring unitary IPSPs, interrupted by an interval of 30 min wash time (top trace). Arrows represent the start and withdrawal of strychnine (1 μM) superfusion. Lower three traces represent fast-speed chart recordings taken at points *a*, *b* and *c* of the top trace. Strychnine reversibly eliminated the hyperpolarizing potentials. This neurone had a resting membrane potential of −49 mV. Recordings in *A* and *B* were taken from two different SPNs.

membrane potential of between −50 and −60 mV, the amplitude and time-to-peak of unitary IPSPs ranged from 0·5 to 5 mV and 2 to 4 ms, respectively. Unitary IPSPs of peak amplitude greater than 0·5 mV were included in this study; membrane fluctuations below 0·5 mV which were near the noise level of the recording system were arbitrarily excluded. The decay of unitary IPSPs followed a single-exponential decay with a time constant (4–12 ms) similar to that of the cell membrane. The

amplitude of unitary IPSPs was decreased on hyperpolarization and increased on depolarization of the membrane by current injection. The unitary IPSP reversed at a membrane potential varying between −60 and −75 mV. In six SPNs, where the amplitude of unitary IPSPs was fairly constant at a given membrane potential, the

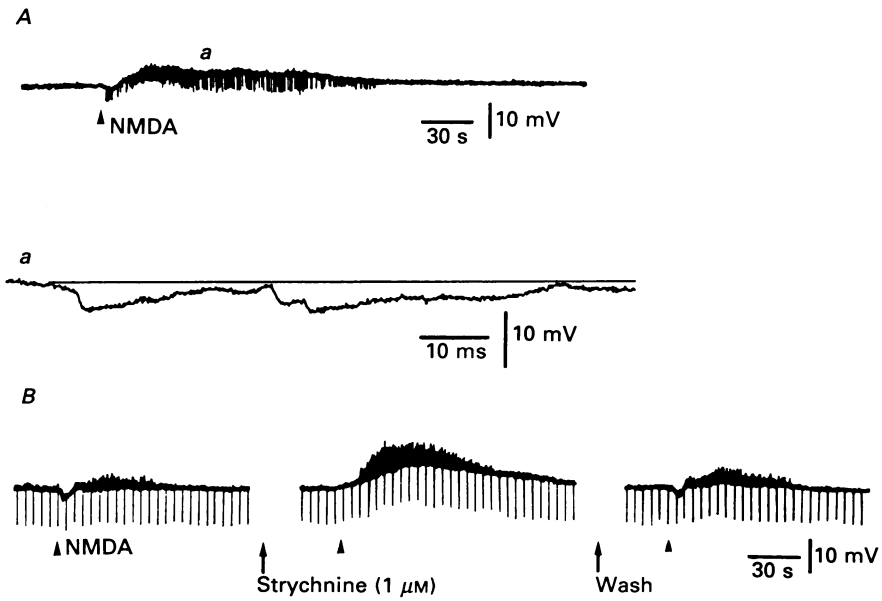

Fig. 3. NMDA-induced unitary IPSPs in two sympathetic preganglionic neurones. Cell *A*, pressure application of 1 mM-NMDA (arrow-head, 10 ms pulse duration) evoked a depolarization and a burst of unitary IPSPs. Several unitary IPSPs were clearly seen when recorded on an expanded time scale (lower trace of *A*). Cell *B*, NMDA delivered by pressure (5 ms pulse) elicited a hyperpolarization followed by unitary IPSPs superimposed on a small depolarizing response. In the presence of strychnine (1 μM), NMDA elicited a larger depolarization with intense cell discharge, instead of unitary IPSPs. The effect of strychnine was reversible after wash. Downward deflections represent hyperpolarizing electrotonic potentials induced by hyperpolarizing current pulses (not shown). Recordings in *A* and *B* were taken from two different preganglionic neurones which had resting membrane potentials of −52 and −55 mV, respectively.

reversal potential was determined from the straight line fitted to plots of the mean amplitude of five unitary IPSPs against the membrane potential (Fig. 2*A*); the extrapolated mean reversal potential was -68 ± 6 mV.

Strychnine (0·1–1 μM), a glycine antagonist (Curtis, Duggan & Johnston, 1971), effectively and reversibly abolished the unitary IPSPs in all five cells tested (Fig. 2*B*), whereas bicuculline (0·1–10 μM) and picrotoxin (1 and 10 μM), which antagonize the action of γ-aminobutyric acid (GABA), had no appreciable effect on the frequency or the amplitude of unitary IPSPs in any of the five cells.

NMDA-induced unitary IPSPs

During the course of our study of the effects of NMDA on the SPNs (Mo & Dun, 1987), it was noted that pressure application of NMDA (1 mM) evoked, in addition

to a depolarizing response, a barrage of unitary IPSPs in eighteen of the fifty-six SPNs tested (Fig. 3*A*). In five other neurones, pressure application of NMDA produced an initial hyperpolarization followed by unitary IPSPs superimposed on a depolarizing response (Fig. 3*B*). The NMDA-induced unitary IPSPs were similar to the spontaneous unitary IPSPs in terms of their amplitude and time course (compare Figs 1 and 3).

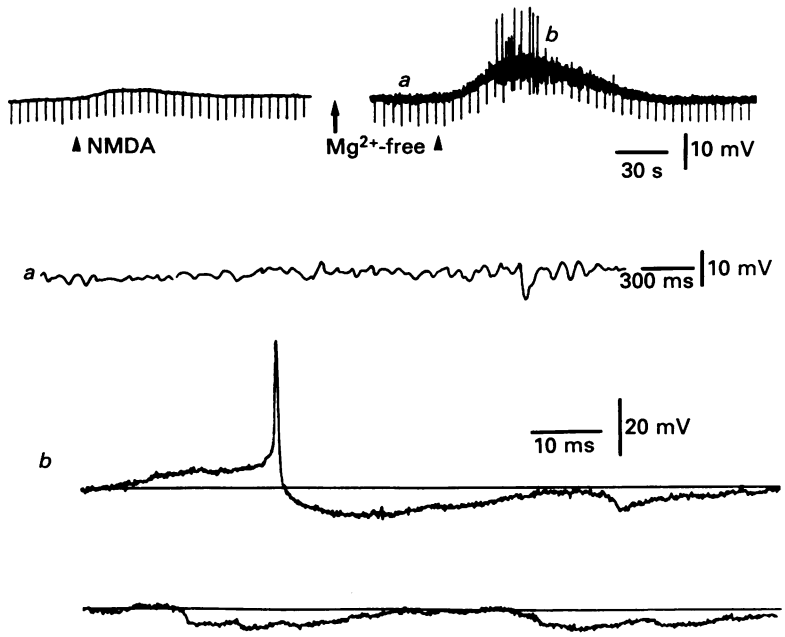

Fig. 4. Mg^{2+}-free solution caused the appearance of unitary IPSPs and enhanced the action of NMDA in a rat sympathetic preganglionic neurone. Pressure application of NMDA (arrow-head, 10 ms pulse) evoked a small depolarization in normal Krebs solution. After switching to a Mg^{2+}-free solution, spontaneously occurring unitary IPSPs were clearly evident even prior to NMDA application, as indicated by trace *a*. The same pulse of NMDA now evoked a much larger depolarization accompanied by spike discharges and unitary IPSPs, as shown in trace *b*. Note the peak amplitude of spike discharges (top trace) was attenuated by the limited frequency response of the pen recorder.

While the NMDA-induced unitary IPSPs and the initial hyperpolarizations were reversibly eliminated by superfusing the slices with low-Ca^{2+}(0·25 mM) solution, the depolarizing response persisted. More interestingly, the NMDA-induced depolarization was clearly enhanced in a low-Ca^{2+} solution in three of the five cells investigated. Similarly, strychnine abolished the initial hyperpolarization of the biphasic response as well as the unitary IPSPs induced by NMDA, but increased the depolarizing phase of the biphasic response (Fig. 3*B*). The increase of NMDA-induced depolarization by low Ca^{2+} or strychnine may have been due to the block of IPSPs which normally curtail the full expression of the excitatory action of NMDA.

Another interesting observation made in the course of this series of experiments

was that Mg^{2+}-free solution increased the amplitude and frequency of NMDA-induced unitary IPSPs in all eight SPNs investigated. In addition, Mg^{2+}-free solution caused the appearance of unitary IPSPs in five SPNs. An example of this phenomenon is shown in Fig. 4. Here, NMDA elicited a small depolarization with no sign of IPSPs in normal Krebs solution; after bathing the slice with a Mg^{2+}-free solution, unitary IPSPs could be detected clearly before and after NMDA application

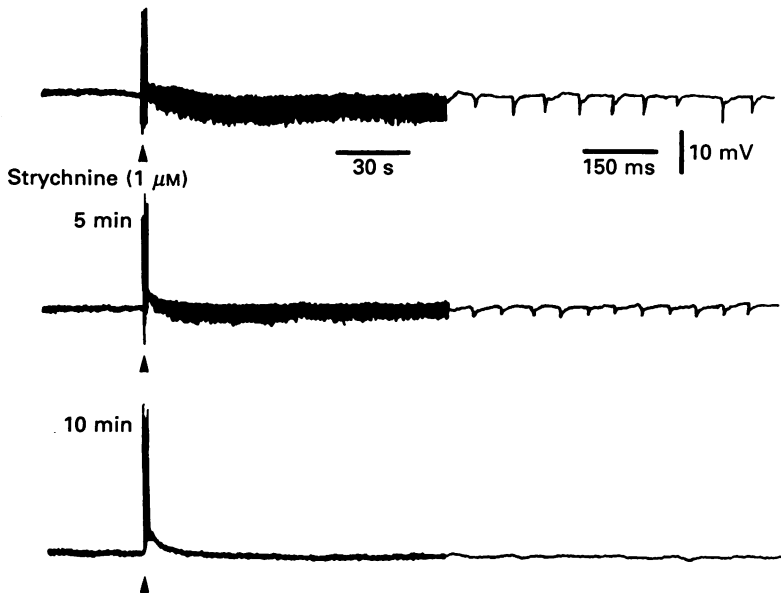

Fig. 5. Strychnine-sensitive unitary IPSPs evoked by repetitive dorsal root stimulation in a rat sympathetic preganglionic neurone. A train of stimuli (arrow-heads, 5 Hz, 3 s) evoked a burst of action potentials followed by intense discharge of unitary IPSPs lasting for more than 3 min (entire recording not shown). Note the chart speed was increased at the end of each trace to reveal individual unitary IPSPs. Strychnine (1 μM) depressed and blocked the unitary IPSPs following 5 and 10 min of superfusion, whereas the spike discharge was not affected. The resting membrane potential of this neurone was −56 mV.

as shown in trace *a*. NMDA now evoked a depolarization which was larger than that obtained in normal Krebs solution and a barrage of unitary IPSPs interspersed with spike discharges at the peak of depolarization (Fig. 4*b*). The depolarization as well as the unitary IPSPs induced by NMDA were reversibly eliminated by pre-treating the slice with a NMDA receptor antagonist D-2-amino-5-phosphonovalerate (APV, 1–5 μM) in the two cells tested (not shown).

Nerve-evoked unitary IPSPs

In addition to the spontaneously occurring and NMDA-induced unitary IPSPs, electrical stimulation of dorsal roots elicited the appearance of unitary IPSPs in seven of the forty-four SPNs tested; a representative experiment is shown in Fig. 5. Repetitive stimulation of dorsal roots (5 Hz, 3 s) elicited a burst of action potentials

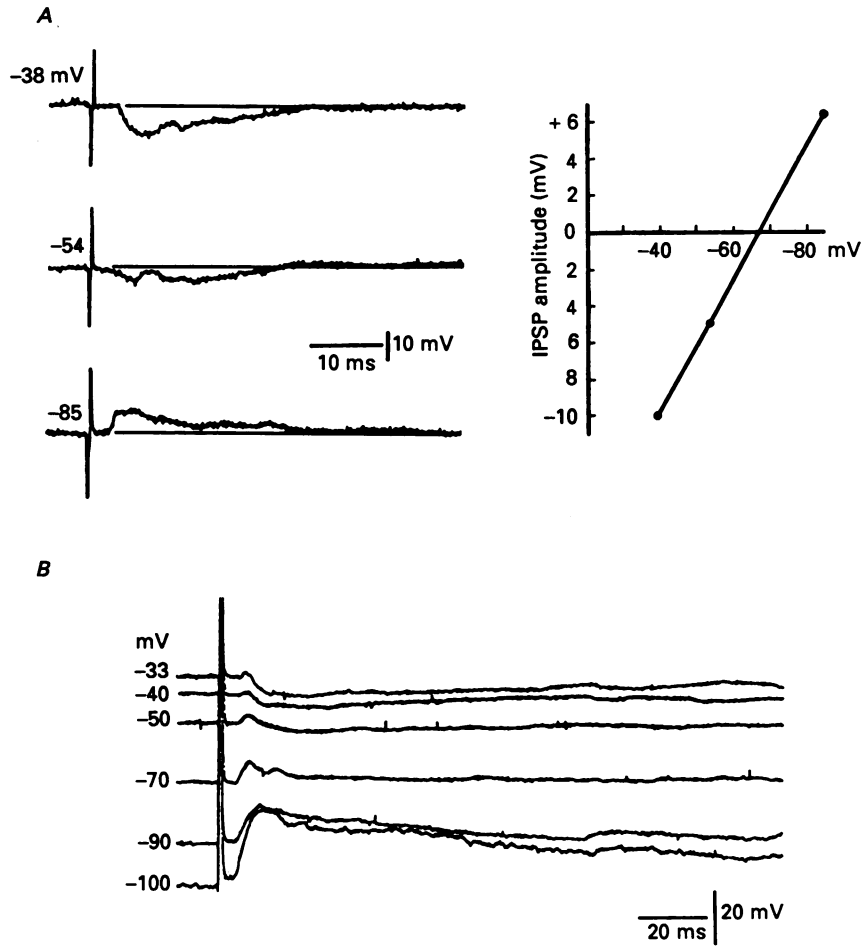

Fig. 6. IPSP and a mixed response consisting of EPSP and IPSP evoked by dorsal root stimulation in two rat sympathetic preganglionic neurones. Cell *A*, a single supramaximal stimulation (20 V, 0·5 ms) evoked an IPSP at the resting membrane potential of −54 mV. The IPSP was increased and decreased by depolarizing and hyperpolarizing the membrane, respectively; at −85 mV, the IPSP was reversed. Graph shows the relationship between the amplitude of evoked response and membrane potential; the line intercepts at −66 mV which is taken as the reversal potential of the evoked IPSP. Cell *B*, at the initial resting membrane potential of −50 mV, a single supramaximal stimulation (15 V, 0·5 ms) applied to dorsal rootlets evoked a biphasic response consisting of a small, short-lasting depolarization followed by a long-lasting hyperpolarization. Hyperpolarization increased and decreased, respectively, the depolarizing and hyperpolarizing response; an opposite effect was seen when the membrane was depolarized. At −70 mV, dorsal root stimulation elicited a depolarization only. Prolonged depolarization observed at the membrane potential of −90 and −100 mV probably represents the composite of the initial depolarizing and the inverted hyperpolarizing response.

followed by continuous discharge of unitary IPSPs for over 3 min in the cell illustrated. It is of interest to note that unitary IPSPs occurred at regular intervals, about one every 60 ms in this cell. Strychnine (1 μM) applied for 10 min eliminated the unitary IPSPs without affecting the action potential discharge.

IPSPs evoked by dorsal root stimulation

A single electrical stimulus (10–20 V, 0·5 ms) applied to the dorsal roots via a bipolar concentric electrode elicited in nineteen SPNs a hyperpolarizing potential, the IPSP (Fig. 6*A*), a biphasic response of EPSP and IPSP in six neurones (Fig. 6*B*), and in the remaining thirty-six SPNs an EPSP.

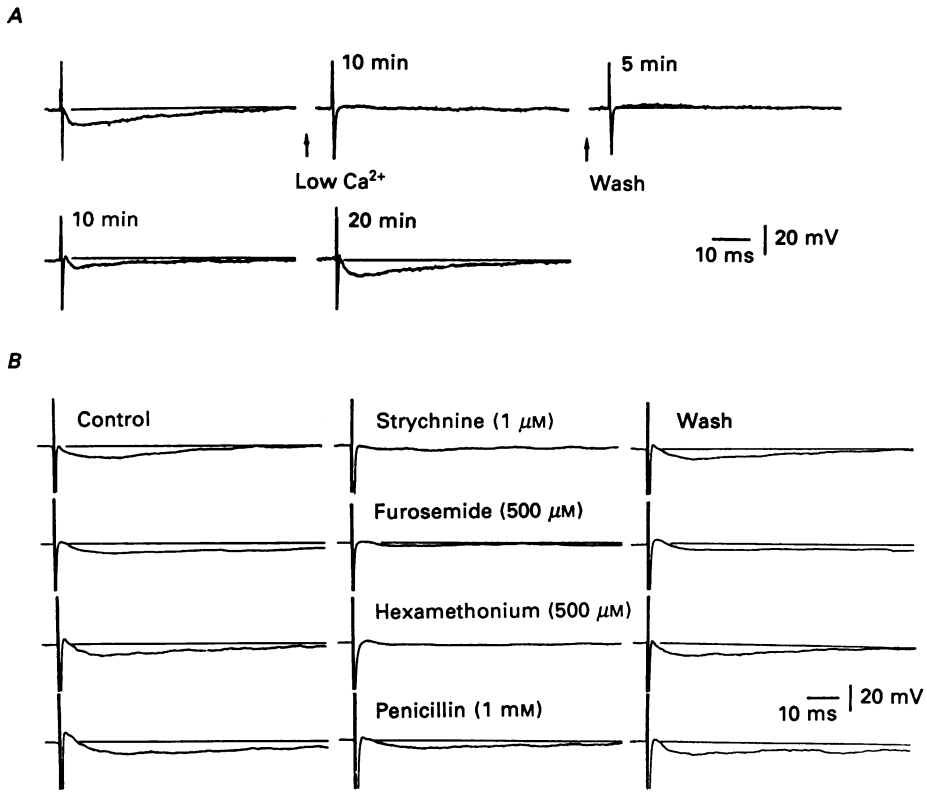

Fig. 7. Blockade of ventral root evoked IPSPs by low-Ca^{2+} solution and by various pharmacological antagonists in two sympathetic preganglionic neurones. Cell *A*, a single electrical stimulus (10 V, 0·5 ms) delivered to the ventral rootlets evoked an IPSP at the resting membrane potential of −54 mV. Superfusing the slice with a low-Ca^{2+} (0·25 mM) solution reversibly abolished the IPSP. Cell *B*, the IPSP evoked by ventral root stimulation was blocked by strychnine and hexamethonium, whereas furosemide and penicillin reduced the response by over 50 %. The depressant effects of these agents were all reversible upon wash. The resting membrane potential was −57 mV and stable throughout the entire recording period of nearly 3 h.

The IPSPs had a mean latency, amplitude, time-to-peak and decay time constant of 5·2 ± 2·7 ms, 8 ± 2 mV, 4·1 ± 1·6 ms and 17 ± 4·4 ms, respectively. Similar to the spontaneous unitary IPSPs, the amplitude of evoked IPSPs was reduced on hyperpolarization, and the mean reversal potential was −65 ± 4 mV ($n = 5$; Fig. 6*A*). The IPSPs evoked by dorsal root stimulation were also reversibly antagonized by strychnine ($n = 8$) in concentrations that were effective against the spontaneous

unitary IPSPs, whereas bicuculline and picrotoxin were without effect on the evoked IPSPs in these neurones. However, strychnine-resistant IPSPs were observed in two SPNs. In these two cases, bicuculline (0·5 μM) effectively blocked the IPSPs. Nicotinic antagonists, hexamethonium (0·1–2 mM) and *d*-tubocurarine (10–50 μM), had no appreciable effect on IPSPs evoked by dorsal root stimulations in any of the four cells examined.

The biphasic response observed in six neurones consisted of an initial depolarization followed by a hyperpolarization (Fig. 6*B*). Hyperpolarization of the cell by current injection had an opposite effect on these two responses: the initial depolarizing phase became larger, whereas the hyperpolarizing phase became smaller and was nullified at a membrane potential of about -70 mV. Further hyperpolarization revealed a large and long-lasting depolarization probably representing the composite response of the initial depolarization and the inverted response of IPSP.

IPSPs evoked by ventral root stimulation

Electrical stimulation of ventral roots elicited in eight neurones an IPSP with amplitude and time course similar to that of IPSPs evoked by dorsal root stimulation. The latency of IPSPs evoked by ventral root stimulation was $3{\cdot}0 \pm 1{\cdot}6$ ms, which was shorter than that evoked by dorsal root stimulation. The IPSPs evoked by ventral root stimulation were attenuated by hyperpolarization and the mean reversal potential was -67 ± 4 mV ($n = 3$).

Low-Ca^{2+} solution reversibly eliminated the IPSPs evoked by ventral root stimulation ($n = 3$, Fig. 7*A*), indicating that they were probably caused by the action of a synaptically released transmitter; strychnine but not bicuculline reversibly antagonized the IPSPs evoked by ventral root stimulation in three cells tested (Fig. 7*B*).

An additional feature of IPSPs evoked by ventral root stimulation was their sensitivity to the nicotinic antagonists hexamethonium and *d*-tubocurarine (not shown) as well as strychnine in the three neurones examined (Fig. 7*B*).

Ionic basis of IPSPs

When the perfusing Krebs solution was changed to one that contained low Cl^- (4·3 mM), the IPSP evoked by dorsal ($n = 3$) or ventral root ($n = 2$) stimulation was progressively attenuated and ultimately reversed to a depolarizing response after a few minutes of superfusion; the hyperpolarizing response was restored on returning to normal Krebs solution (Fig. 8). It is of interest to note that several unitary IPSPs were superimposed on the IPSP trace for the neurone illustrated in Fig. 8. The amplitude of unitary IPSPs became smaller and disappeared in a low-Cl^- solution as well. On the other hand, changing to a low-K^+ (1·9 mM) solution did not significantly alter the amplitude of the evoked IPSPs in any of the five cells tested (Fig. 8). Like the evoked IPSP, the unitary IPSPs were not changed appreciably by low-K^+ solution in the neurone shown in Fig. 8.

Moreover, pharmacological agents known to interfere with Cl^- transport or block Cl^- conductance such as furosemide (Brazy & Gunn, 1976; see also Nicoll, 1978) and penicillin (Hochner, Spira & Werman, 1976) were effective in attenuating the evoked

IPSPs. Thus, penicillin (1 mM; $n = 3$) and furosemide (500 μM; $n = 4$) reduced the IPSPs to about 45 and 30% of the control, respectively. It should be added that penicillin and furosemide when applied to the spinal cord slices did not cause any significant change in the resting membrane potential in any of the preganglionic neurones tested.

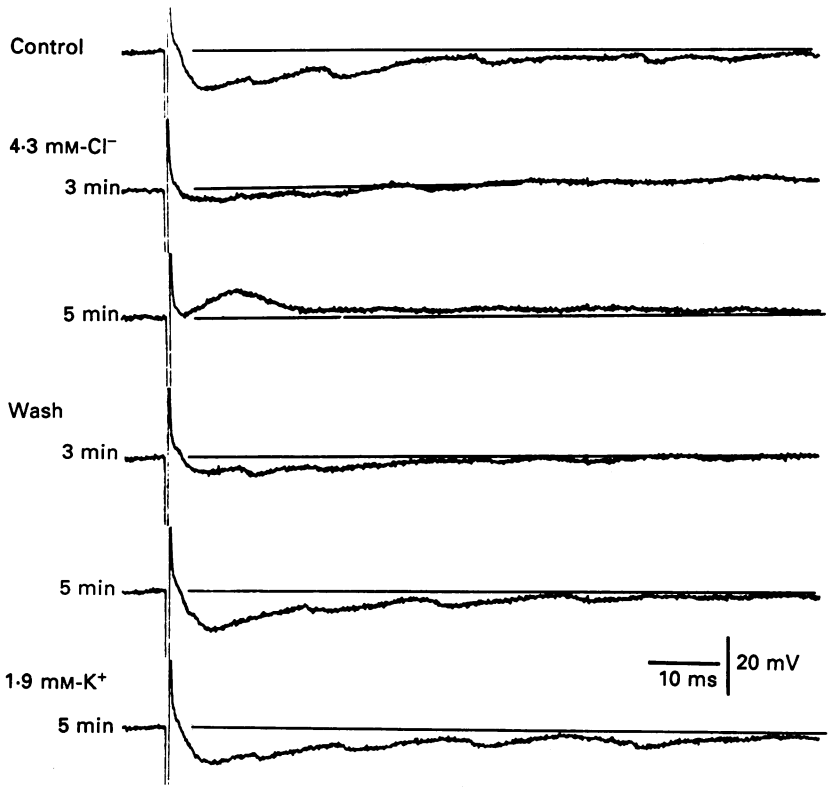

Fig. 8. Effects of low-Cl^- and low-K^+ solution on IPSP evoked by ventral root stimulation in a rat sympathetic preganglionic neurone. An IPSP was evoked by a single stimulus (15 V, 0·5 ms) applied to the ventral rootlets. Note the appearance of unitary IPSPs during the course of the evoked IPSP. Superfusing the slice with a low-Cl^- (4·3 mM) solution depressed and eventually reversed the IPSP; this effect was reversible. Superfusing the slice with a low-K^+ (1·9 mM) solution had no significant effect on the evoked IPSP. Numerals to the left of each trace represent the time in minutes after changing to perfusing solutions of different ionic composition as indicated.

DISCUSSION

Spontaneously occurring and evoked hyperpolarizing potentials were recorded, respectively, in twenty-nine and twenty-six neonatal rat SPNs *in vitro*. This is in contrast to the findings obtained *in situ* from the adult cat SPNs where inhibitory potentials were infrequently encountered (McLachlan & Hirst, 1980; Dembowsky *et al.* 1985*a*). Species and age differences notwithstanding, other factors such as cell diameter, input resistance and location of inhibitory synaptic inputs in relation to

the soma of SPNs might influence the size and consequently, the detection of unitary IPSPs. In this respect, the input resistance (10–40 MΩ) of cat SPNs *in situ* (McLachlan & Hirst, 1980; Dembowsky, Czachurski & Seller, 1986) was lower than that of neonatal rat SPNs reported here; this might partly explain the smaller size, and thus the less detectable, unitary IPSPs in the cat SPNs. Additionally, SPNs with an intact neural axis may receive continuous synaptic bombardments such that inhibitory potentials may be occluded by excitatory inputs (McLachlan & Hirst, 1980). Indeed, a mixed response of EPSP and IPSP was recorded in a few SPNs, indicating the convergence of excitatory and inhibitory inputs onto a single neurone. Finally, inhibitory interneurones which are relatively vulnerable to anoxia (Davidoff, Shank, Graham, Aprison & Werman, 1967) may be compromised in the case of the *in situ* experiments. Consequently, the IPSPs which are presumably generated by the interneurones may be more readily obtainable in well-oxygenated slice preparations than in *in situ* preparations.

Two types of hyperpolarizing potentials could be distinguished on the basis of their amplitude and time course. Those we termed unitary IPSPs had an amplitude ranging from less than 1 to about 5 mV and a time constant comparable to the membrane time constant of the SPNs. The amplitude of evoked IPSPs was larger and typically ranged from a few to over 10 mV. The finding that unitary IPSPs were reversibly eliminated by low Ca^{2+} as well as by TTX suggests that they were probably caused by activity-dependent release of inhibitory transmitters from interneurones which are presynaptic with respect to the SPNs. In this context, a similar suggestion was made with respect to unitary IPSPs recorded in the hippocampal pyramidal neurones (Algers & Nicoll, 1980). More recently, Miles & Wong (1984) showed by means of paired recordings that the occurrence of unitary IPSPs in hippocampal pyramidal neurones is correlated with the discharge of single inhibitory interneurones which impinge on the neurones in question. The present observation that spontaneous or evoked unitary IPSPs occur at regular intervals in preganglionic neurones (e.g. Figs 1 and 8) is consistent with the interpretation that their occurrence may be time-locked to the discharge of presynaptic inhibitory interneurones. While the removal of descending influence in the slice preparations could have caused the spontaneous discharge and/or rhythmicity of inhibitory interneurones, paired recordings similar to that performed in the hippocampal neurones (Miles & Wong, 1984) will be needed to characterize more precisely the initiation and pattern of discharge of inhibitory interneurones.

The IPSPs evoked by dorsal and ventral root stimulation probably represent population responses of unitary IPSPs, as the reversal potentials of the unitary IPSPs and evoked IPSPs were similar, and as strychnine blocked both types of responses. Hence, the transmitter involved in these two types of responses may be identical. If the unitary IPSP indeed represents the discharge of a single inhibitory interneurone, the evoked IPSP may constitute the synchronous discharge of inhibitory interneurones converging onto a single preganglionic neurone. In the spinal motoneurone the evoked IPSP has been estimated to be caused by the discharge of approximately seventy inhibitory neurones (Jankowska & Roberts, 1972); a somewhat smaller number of fifteen inhibitory interneurones is thought to synapse onto a single hippocampal pyramidal neurone (Miles & Wong, 1984). The

number of inhibitory interneurones synapsing onto a single preganglionic neurone remains to be determined. It is of interest to note that, in a few preganglionic neurones, repetitive dorsal root stimulation evoked prolonged (up to several minutes) discharge of unitary IPSPs. This sustained discharge of inhibitory interneurones may underlie the long-lasting 'silent period' reported in the cat SPNs (Polosa, 1967).

An interesting observation was made with respect to the activation of the interneurones by NMDA. Pressure application of NMDA induced in a population of SPNs unitary IPSPs and a hyperpolarization, in addition to a depolarizing response. The depolarizing response was likely to be a direct action of NMDA on the SPNs (see Mo & Dun, 1987). The hyperpolarizing responses on the other hand were abolished by low Ca^{2+} as well as by strychnine and the NMDA antagonist APV, suggesting they were probably an indirect effect of NMDA, acting on glycine-releasing interneurones. The observation that Mg^{2+}-free solution potentiated the action of NMDA on inhibitory interneurones as evidenced by increased frequency and amplitude of unitary IPSPs, suggests that the NMDA channel of spinal interneurones may also be gated by Mg^{2+} ions in a manner similar to that reported for other central neurones (Ault, Evans, Francis, Oakes & Watkins, 1980; MacDonald, Porietis & Wojtowicz, 1982; Mayer, Westbrook & Guthrie, 1984; Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984). Lastly, the finding that the unitary IPSPs induced by NMDA were abolished by APV, a selective NMDA receptor antagonist (McLennan, 1983; Fagg, Foster & Ganong, 1986), supports the contention that the receptor located on the inhibitory interneurones is of the NMDA subtype. Collectively, these findings raise the interesting possibility that inhibitory spinal interneurones may be activated through NMDA receptors.

What is the putative transmitter responsible for the inhibitory potentials in the preganglionic neurones? On the basis of their sensitivity to strychnine, the transmitter mediating these potentials in the majority of SPNs is likely to be glycine or a glycine-like substance. None the less, IPSPs evoked in two SPNs were found to be strychnine-insensitive and bicuculline-sensitive, suggesting that GABA may be the mediator of IPSP in a subpopulation of SPNs. With respect to the ionic mechanism underlying the unitary and evoked IPSPs, our results suggest that an increase of Cl^- conductance is involved. First, the reversal potential was more negative than the resting membrane potential. Second, superfusing the slice with a low-Cl^- solution readily changed the hyperpolarizing response into a depolarizing response, whereas the response was not significantly changed in a low-K^+ solution. Finally, agents such as penicillin and furosemide which affect Cl^- conductance or transport (Brazy & Gunn, 1976; Hochner *et al.* 1976) attenuated the hyperpolarizing responses. Thus, our results are in agreement with those obtained with respect to other central neurones in which glycine hyperpolarized the membrane by increasing primarily the Cl^- conductance (Coombs, Eccles & Fatt, 1955; Lux, Loracher & Neher, 1970; Llinas & Baker, 1972; Buhrle & Sonnhof, 1985).

The most interesting aspect of this study relates to the findings that the IPSPs could be evoked by ventral root stimulation and that these IPSPs could be blocked not only by strychnine but also by the nicotinic antagonists, hexamethonium and *d*-tubocurarine. This observation has a bearing on the much debated issue of whether

or not axon collaterals emanate from SPNs (see, for example, Schramm, 1986). Morphological evidence as to the existence of axon collaterals is inconsistent. Dembowsky, Czachurski & Seller (1985*a*) could not detect axonal collaterals in cat SPNs labelled intracellularly with horseradish peroxidase, although small protuberances with a very thin stalk and a bulbous head that might represent presynaptic boutons were observed. On the other hand, axonal collaterals were detected in pigeon SPNs labelled with the same agent (Cabot & Bogan, 1987). Our finding that the IPSP evoked by ventral root stimulation was abolished by strychnine and nicotinic antagonists would suggest a disynaptic arrangement whereby nicotinic activation of inhibitory interneurones by axon collaterals releases glycine or a glycine-like substance which in turn initiates the IPSP. Since the IPSPs evoked by dorsal root stimulation were not significantly affected by the nicotinic antagonists, the postulated cholinergic involvement must be unique for the ventral root pathway. While morphological evidence for the existence of axon collaterals in rat SPNs remains to be established, our results are compatible with the idea that a subpopulation of SPNs may have axon collaterals that terminate on intraspinal neurones as suggested in the pigeon (Cabot & Bogan, 1987). The synaptic pathway involved in the generation of IPSPs evoked by ventral root stimulation in a population of preganglionic neurones may thus be analogous to the Renshaw cell–motoneurone circuitry of the ventral horn (Renshaw, 1941; Eccles *et al.* 1954). Functionally, the IPSP evoked by ventral root stimulation may correspond to the 'silent period' following antidromic stimulation reported by Polosa (1967).

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