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ELECTROPHYSIOLOGICAL PROPERTIES OF RAT SPINAL DORSAL HORN NEURONES *IN VITRO*: CALCIUM-DEPENDENT ACTION POTENTIALS

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SUMMARY

1. The electrophysiological properties of dorsal horn neurones have been investigated in the immature rat *in vitro* spinal cord slice preparation.

2. Intracellular recordings from dorsal horn neurones show that direct or orthodromic stimulation generates action potentials followed by a brief after-hyperpolarization. Synaptic potentials were elicited by the activation of primary afferent fibres in the dorsal root.

3. Input resistance for dorsal horn neurones ranged from 48 to 267 MΩ, and the membrane time constant was in the range of 4–19 ms.

4. In response to strong depolarizing currents dorsal horn neurones perfused with TTX and TEA frequently exhibit a slow regenerative depolarizing potential followed by a slow after-hyperpolarization. The depolarizing potential probably results from an influx of Ca. It is blocked by low concentration Ca, Co or Mn, and enhanced by high levels of extracellular Ca.

5. There is, in addition, a low-threshold Ca-dependent response which is activated at membrane potentials more negative than −65 mV and has a maximum rate of rise at the polarization level of about −80 mV.

6. The addition of Ba or TEA to the perfusing medium provided support for the Ca-dependence of the low- and high-threshold responses, and the lack of fast inactivation of the high-threshold Ca potential.

INTRODUCTION

Intracellular studies of predominantly large mammalian spinal dorsal horn neurones in intact animals (Frank & Fuortes, 1956; Haapanen, Kolmodin & Skoglund, 1958; Hunt & Kuno, 1959; Eccles, Eccles & Lundberg, 1960; Hongo, Jankowska & Lundberg, 1966) and spinal neurones in cell culture (Ransom, Neale, Henkart, Bullock & Nelson, 1977; Heyer, Macdonald, Bergey & Nelson, 1981) have provided important information about their electrophysiological properties and functional organization. However, the neurones of the superficial parts of the spinal dorsal horn have long resisted electrophysiological analysis due to the relatively small size of the cells and the technical difficulties in obtaining stable intracellular recordings *in vivo*

(Cervero, Molony & Iggo, 1977; Yaksh, Wall & Merrill, 1977; Hayashi, Price, Dubner & Ruda, 1978; Kumazawa & Perl, 1978; Bennett, Hayashi, Abdelmoumene & Dubner, 1979). We have therefore developed an *in vitro* rat spinal cord slice preparation in order to achieve stable recordings and more thoroughly analyse the biophysical properties of the nerve cells in the superficial laminae of the spinal dorsal horn. Our initial studies have demonstrated the viability of this preparation (Miletić & Randić, 1980; Miletić & Randić, 1981) and that satisfactory intracellular recordings could be maintained for several hours enabling us to analyse the membrane actions of several neuropeptides known to be concentrated in the superficial parts of the spinal dorsal horn (Murase & Randić, 1981; Murase, Nedeljkov & Randić, 1982).

In this paper we will describe the ionic nature of action potentials of neurones in the superficial parts of the spinal dorsal horn, as recorded in the immature rat slice preparation *in vitro*, and the effects of modifying the ionic composition of the bathing solution and of using agents known to block specific voltage-dependent ionic conductances. We will provide additional evidence in support of the present concept that action potentials in mammalian spinal neurones are generated by voltage-dependent conductance increases to Na and Ca ions (Heyer *et al.* 1981). Two distinct types of Ca spikes are probably present in immature rat dorsal horn cells. A preliminary report has appeared (Murase & Randić, 1982).

METHODS

Preparation

Experiments were performed on 9–18-day-old Sprague–Dawley rats. The animals were anaesthetized with ether and cooled by immersing the thorax and abdomen in an ice–water slurry. During the period of cooling, close attention was paid to respiration. In the majority of animals respiration continued for at least 10 min following the onset of cooling, when it became shallow and regular, by which time the skin temperature had fallen to 20–22 °C. The dissection was then started and a laminectomy performed to expose the lower-thoracic and lumbosacral spinal cord together with dorsal roots. Following laminectomy, about 1–1·5 cm long segment of lumbosacral spinal cord, with attached dorsal rootlets, was quickly excised and immersed into aerated (95% O_2 and 5% CO_2) Ringer solution at approximately 24 °C. The composition of the solution was (mM): NaCl, 124; KCl, 5; KH_2PO_4, 1·2; $CaCl_2$, 2·4; $MgSO_4$ 1·3; $NaHCO_3$, 26; glucose, 10; pH 7·4. After the removal of the pia mater on the lateral aspects of the spinal cord, the spinal segment was cut manually into 5 mm blocks, and one of the blocks fixed with cyanoacrylic glue (Borden, Inc.) to the bottom of a Plexiglass cutting chamber of an Oxford Vibratome. The bath of the Vibratome was filled with the aerated Krebs solution, maintained at 24 °C. The blade of the Vibratome was positioned 300 μm below the dorsal surface of the spinal cord, and the spinal segment sectioned to yield one horizontal 300 μm thick dorsal horn slice. The duration of the entire procedure from the removal of the spinal cord until the slice was made rarely exceeded 5 min. The slices were incubated in Ringer solution at 35 °C for about an hour. After incubation, a slice was transferred to the recording chamber where it was continuously perfused with oxygenated modified Ringer solution (NaCl, 127 mM; KCl, 1·9 mM; KH_2PO_4, 1·2 mM; $CaCl_2$, 2·4 mM; $MgSO_4$, 1·3 mM; $NaHCO_3$, 26 mM; glucose, 10 mM) at 33 ± 1 °C at a flow rate of about 2 ml/min. The recording chamber had a capacity of 0·5 ml. Variations in the composition of the perfusing solution are indicated in the Results.

Intracellular recording technique

Intracellular recordings were performed with micropipettes filled with 3 M-K acetate having d.c. resistances of 90–110 MΩ. Stable intracellular recordings from single dorsal horn neurones could be maintained during multiple solution changes for as long as 5 h. Electrical properties of dorsal horn neurones were determined by means of a high-input impedance bridge amplifier (WP Instruments, M707) allowing current injections of the order of 0·05–3·0 nA through the recording

electrode. Synaptic activation of the dorsal horn neurones was obtained with co-axial stainless-steel stimulating electrode positioned on the dorsal roots.

RESULTS

The results described in this paper were obtained from forty-six neurones located in the superficial parts of the spinal dorsal horn. The various parameter estimates for immature rat dorsal horn neurones are given in Table 1. For forty-six studied units the resting potential varied, between −54 to −86 mV, with the mean value of −70·3 mV ± 6·3 (S.D., $n = 46$).

TABLE 1. Passive and active membrane properties of immature rat dorsal horn neurones

Property	n	Mean ± S.D.
Input resistance (R_N)	16	98·1 ± 58·7 MΩ
Membrane time constant (τ)	17	10·4 ± 5·1 ms
Membrane potential	46	70·3 ± 6·3 mV
Na spike		
Overshoot	24	25·0 ± 7·8 mV
Spike duration	25	1·4 ± 0·5 ms
Threshold	24	25·8 ± 8·4 mV

Passive membrane properties

The passive electrical properties of dorsal horn neurones were investigated using intracellularly injected depolarizing and hyperpolarizing current pulses. The input resistance was calculated for sixteen cells from the slope of the current–voltage relationship. The mean values for input resistance and time constant are given in Table 1. The measured values for the input resistance (range: 48–267 MΩ) and the time constant (range: 4–19 ms) varied widely from cell to cell.

Direct and synaptic activation

Fig. 1 illustrates examples of intracellularly recorded action potentials from the soma of a dorsal horn neurone of a 14-day-old rat following direct (Fig 1 *A–E*) and orthodromic (Fig. 1 *F*) stimulation. In Fig. 1 *A* a single action potential of approximately 70 mV amplitude and a duration of 1·4 ms is initiated. Further increase in the current strength (Fig. 1 *B–E*) reduced the latency of the first action potential and evoked repetitive discharge. The repetitive firing usually occurred from a slow plateau-type depolarizing local response (Fig. 1 *C–E*). In Fig. 1 *E* the maximal firing frequency calculated for the first interspike interval was 165/s, and was reduced for the second interspike interval to 110/s. As reported previously (Haapanen *et al.* 1958; Hunt & Kuno, 1959) the spike is often followed by a brief hyperpolarization relative to the firing level. In Fig. 1 *F* the responses of another dorsal horn cell in a 12-day-old rat to a single dorsal root afferent volley are shown. An excitatory synaptic potential (e.p.s.p.) is evoked which was subthreshold for initiation of an action potential (third trace from above). The properties of this potential were examined by using hyperpolarizing (−0·2 to −1·2 nA) and depolarizing (0·2 − 0·4 nA) square current pulses of increasing strength and about 80 ms duration injected in

0·2 nA steps across the cell soma. With hyperpolarizing currents, as seen in Fig. 1 *F*, the amplitude of the synaptic potential was elevated, while depolarizing current pulses evoked repetitive action potentials.

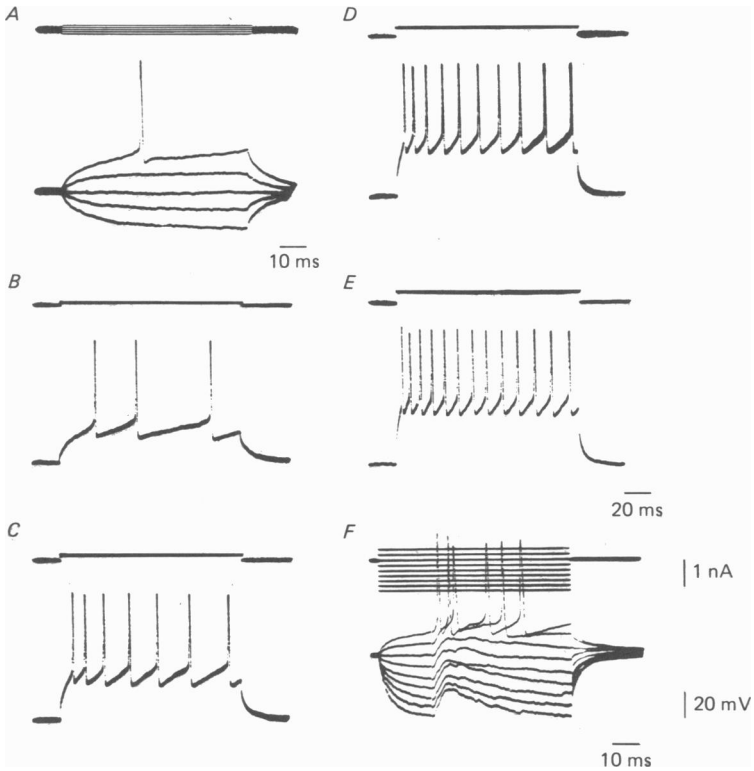

Fig. 1. Direct and synaptic activation of two dorsal horn neurones in a spinal cord slice preparation of a 14-day-old rat (*A–E*) and a 12-day-old rat (*F*). Resting membrane potential was −71 mV (*A–E*) and −66 mV (*F*). Upper traces in all Figures show applied current pulses, lower traces voltage response. In *A* intracellular responses to depolarizing (upward) and hyperpolarizing (downward) current pulses applied in 0·05 nA steps across the cell soma are shown. In *B–E* depolarizing square current pulses of increasing strength injected in 0·1 nA steps evoked repetitive firing. The repetitive discharge occurred from a slow plateau-type depolarizing local response. In *F* synaptic and action potentials recorded from another dorsal horn neurone following stimulation of a dorsal root. Synaptic and action potentials superimposed on intracellularly injected current pulses. Note marked increase in amplitude of synaptic potentials with hyperpolarizing pulses (downward traces) and appearance of repetitive discharge with depolarizing pulses (upward traces).

The high-threshold Ca action potential

Addition of tetraethylammonium chloride (TEA) to the Ringer solution slowed the rate of repolarization of the action potential in a dorsal horn neurone of a 12-day-old rat slice, prolonged the spike duration, and enhanced the after-hyperpolarization (Fig. 2*B*). Following the addition of tetrodotoxin (TTX) to the TEA-containing medium, in order to block the Na conductance, the fast action potential shown in

Fig. 2*B* was completely blocked. However, the cell responded to the larger depolarizing current pulses (range: 1·7–2·0 nA) with a slow regenerative depolarization followed by a slow hyperpolarization. An example of two slow regenerative spikes is shown in Fig. 2*C–D*. These slow spikes were all-or-none in character and were seen in almost all tested cells. The latency of onset and duration of these spikes varied with the amount of stimulus current and the frequency of stimulation. Their latency shortened

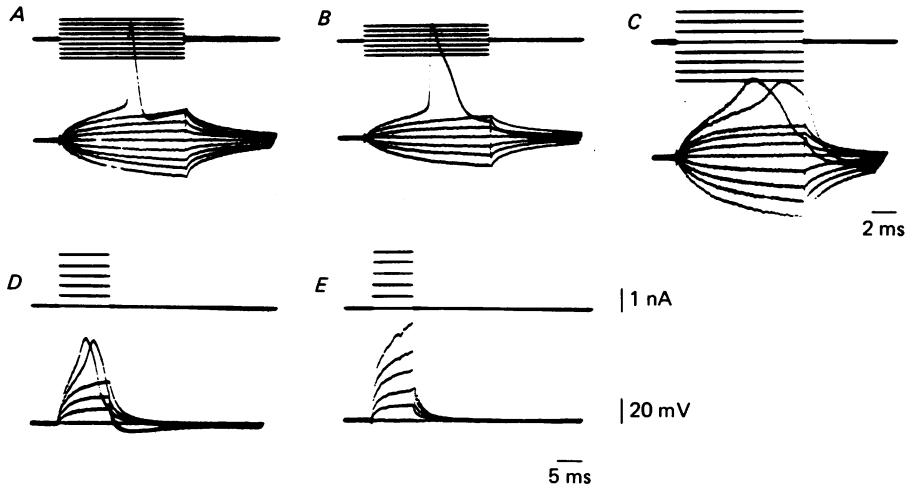

Fig. 2. Effect of TTX, TEA and Ca-free Ringer solution on action potentials evoked by depolarizing current pulses of increasing intensity injected in 0·2 nA (*A–B*), 0·4 nA (*C–D*) and 0·5 nA (*E*) steps across the cell soma of a dorsal horn neurone of a 12-day-old rat. Passive responses to subthreshold depolarizing and hyperpolarizing current pulses also shown. Initial resting potential was −66 mV. *A*, control response to direct stimulation in normal Ringer solution. *B*, action potential recorded after addition of 10^{-3} M-TEA. *C–D*, TTX-resistant action potentials recorded following addition of 10^{-6} M-TTX to the TEA-containing medium. Note higher threshold and slower rate of rise of TTX-resistant potentials if compared with a control. *E*, TTX-resistant action potentials disappeared when Ca was removed from TEA + TTX-containing Ringer solution.

as current intensity was raised. These slow action potentials could be distinguished from the fast action potentials by their higher threshold, smaller amplitude, and longer spike duration and after-hyperpolarization (Table 2).

The Ca-dependence of the slow TTX-resistant action potentials is illustrated in Fig. 2*E* where it can be seen that removal of Ca from the bathing medium containing TTX and TEA made depolarizing current pulses, which triggered the slow action potentials shown in Fig. 2*C–D*, ineffective. Furthermore bath application of a Ringer solution containing 5×10^{-3} M-Ca potentiated the high-threshold Ca spike.

Blockade of the high-threshold spike (Fig. 3*C* or *D*) by Co ions in a dorsal horn neurone of an 18-day-old rat is illustrated in Fig. 3*E*. Upon washing the slice with a Co-free medium, a slow regenerative depolarizing response could again be elicited (Fig. 3*F*). After removal of TTX and TEA from the perfusing solution a partial recovery of the Na spike is seen in Fig. 3*G*.

TABLE 2. Some characteristics of the high-threshold and the low-threshold Ca action potentials*

	n	Mean ± S.D.
High-threshold spike		
Membrane potential	9	62·2 ± 7·4 mV
Spike height	9	32·7 ± 11·1 mV
Spike duration	7	13·1 ± 7·7 ms
Threshold	9	49·8 ± 8·9 mV
Magnitude of after-hyperpolarization	8	26·6 ± 7·6 mV
Duration of after-hyperpolarization	7	114·0 ± 83·0 ms
Low-threshold		
Membrane potential	4	73·3 ± 2·4 mV
Spike height	4	13·8 ± 3·1 mV
Spike duration	4	26·5 ± 4·0 ms
Threshold	4	22·4 ± 2·9 mV

* All measurements done in a Ringer solution containing TTX + TEA.

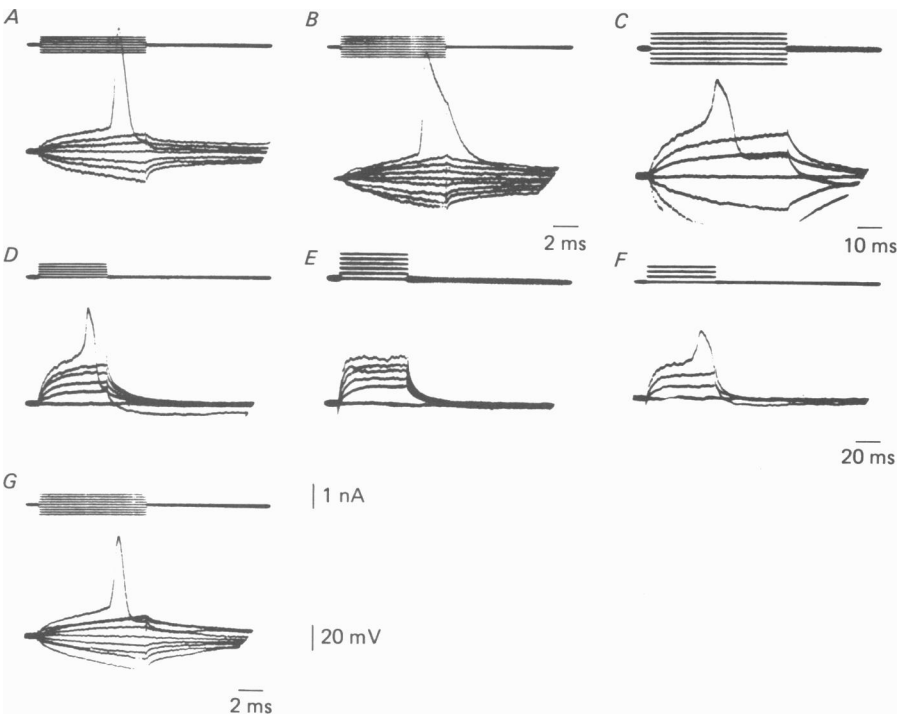

Fig. 3. Blockade of TTX-resistant spikes by cobalt ions in a dorsal horn neurone of an 18-day-old rat. Resting potential was −62 mV. *A*, control response to direct stimulation in normal Ringer solution. *B*, action potential following addition of 2×10^{-3} M-TEA. *C–D*, TTX-resistant action potentials following addition of 4×10^{-6} M-TTX. *E*, TTX-resistant spike disappeared when Co chloride (2×10^{-3} M) was added to bathing medium. *F*, partial recovery of TTX-resistant spike after removal of cobalt. *G*, partial recovery of Na spike following removal of TTX and TEA from perfusing solution.

The low-threshold Ca action potential

Besides the high-threshold Ca-dependent spike already described, the neuronal membrane of dorsal horn cells with resting potentials more negative than 65 mV appears to be capable of generating a low-threshold TTX-resistant action potential having some properties similar to that recently described in the inferior olivary neurones by Llinás & Yarom (1981*a*, *b*). As shown in Fig. 4 direct stimulation of a

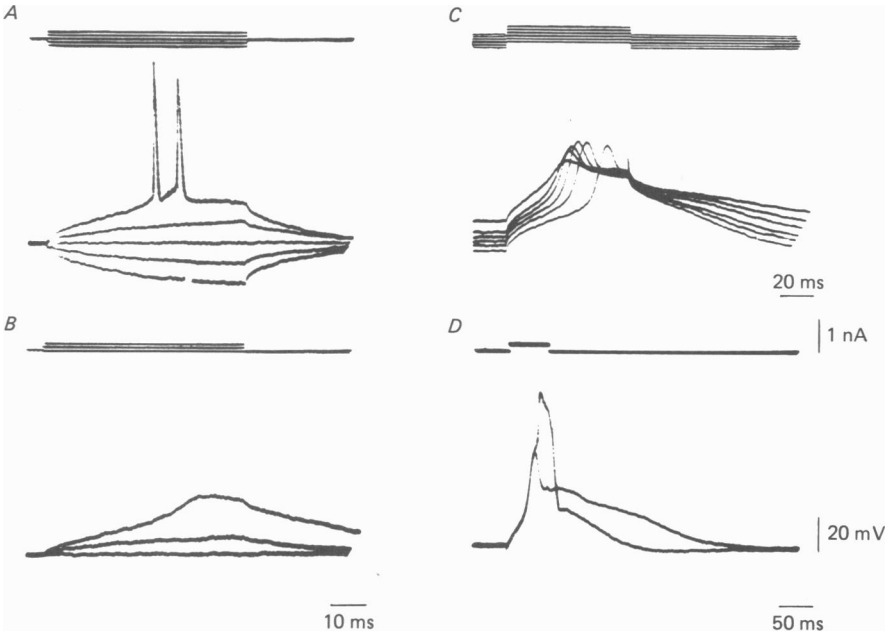

Fig. 4. The low threshold TTX-resistant spike and TTX-resistant Ba action potentials in a dorsal horn neurone of an 11-day-old rat. Initial resting potential was −77 mV. *A*, control responses to direct stimulation. *B*, addition of 3×10^{-6} M-TTX into the bath abolished fast spikes and uncovered a low-threshold electroresponsiveness. In *C–D*, TTX-resistant action potentials following addition of Ba (10^{-3} M) into the perfusing solution containing TTX (3×10^{-6} M) and TEA (5×10^{-3} M). *C*, low-threshold Ba action potentials recorded at resting level (−64 mV) and at increasing levels of membrane hyperpolarization (range: 64–82 mV). Note increased rate of rise and amplitude of these potentials with increasing hyperpolarization. *D*, co-activation of low-threshold and high-threshold Ba potentials. Note that the two spikes can be generated during the same stimuli. The presence of a Ba local response is also evident.

dorsal horn cell in an 11-day-old rat by a depolarizing current pulse of 0·2 nA and 60 ms duration evoked two Na spikes which were preceded by a slow depolarization (Fig. 4*A*). Although addition of TTX into the bath abolished Na spikes, the low-threshold membrane electroresponsiveness remained (Fig. 4*B*). The rate of rise of this all-or-none low-threshold depolarizing potential is usually increased following membrane hyperpolarization.

The ionic nature of this low-threshold TTX-resistant potential was further investigated by adding Ba to the medium containing TTX and TEA (Fig. 4*C–D*).

It is known that Ba moves through the Ca channel more rapidly than Ca (Werman & Grundfest, 1961; Hagiwara, 1973; Hagiwara, Fukuda & Eaton, 1974) and does not activate the Ca-dependent K conductance (Krnjević, Pumain & Renaud, 1971; Eckert & Lux, 1976). Fig. 4*C* illustrates the low-threshold depolarizing responses elicited in a dorsal horn neurone by depolarizing current pulses applied at resting level (−64 mV) and at increasing levels of membrane hyperpolarization (−64 to −82 mV).

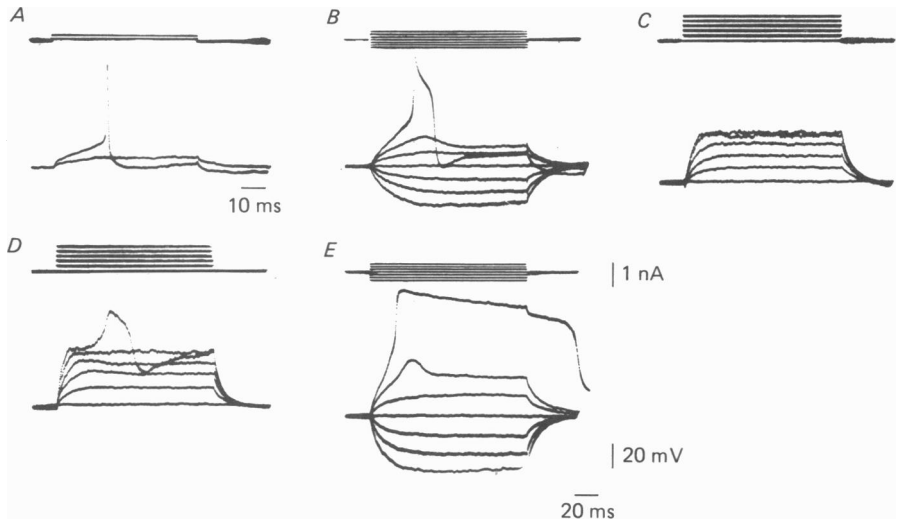

Fig. 5. Both, high-threshold and low-threshold TTX-resistant spikes are blocked by cobalt. 10-day-old rat. Resting potential was −62 mV. *A*, control response. *B*, co-existence of both, low-threshold and high-threshold potentials in a medium containing TTX (5×10^{-6} M) and TEA (5×10^{-3} M). *C*, both responses are abolished by Co (5×10^{-3} M). *D*, *E*, recovery following removal of cobalt from TTX and TEA-containing Ringer solution. Note also in *E* (66 min since removal of Co) the difference in the time course between the low-threshold and the high-threshold potentials.

As hyperpolarization increases, the amplitude and the rate of rise of the low threshold Ba spikes are increased. Estimation of the rate of rise of these low threshold spikes as a function of membrane hyperpolarization shows that plateau is reached in this cell at about −80 mV. The co-activation of the low-threshold and the high-threshold Ba spikes and a slow plateau phase following the low-threshold action potential are seen in Fig. 4*D*. In the presence of Ba the high-threshold spike was of longer duration, while the decay phase of the low-threshold spike was not modified by Ba.

The co-existence of two types of Ca-dependent action potentials in a dorsal horn neurone of a 10-day-old rat slice preparation perfused with normal Ringer solution containing TTX and TEA is shown in Fig. 5*B*. Both responses were abolished if the slice was bathed in a 5 mM-cobalt-containing medium (Fig. 5*C*). When cobalt was removed, both potentials, although not fully recovered, could again be evoked (Fig. 5*D*).

Recovery and co-existence of both the high-threshold and the low-threshold Ca-dependent potentials 66 min after perfusion with a Co-free TTX- and TEA-

containing Ringer solution are illustrated in Fig. 5*E*. Note the striking difference in the time course of the two potentials: whereas the low-threshold potential has a relatively fast repolarizing phase even under conditions of a reduction of the voltage-dependent K conductance, the high-threshold electroresponsiveness is prolonged. Similar behaviour of two types of Ca spikes in the inferior olivary neurones in the presence of TEA has been attributed to the inactivation properties of the low-threshold Ca conductance and the lack of fast inactivation for the high-threshold Ca spike (Llinás & Yarom, 1981*b*).

DISCUSSION

The data presented illustrate that satisfactory intracellular recordings from immature rat dorsal horn neurones *in vitro* could be maintained for as long as 5 h, the time often required when testing the influence of various ionic environments on cell membrane properties. This is in itself an encouraging finding, since obtaining stable recordings from the superficial parts of the spinal dorsal horn *in vivo* has been technically very difficult (Cervero *et al.* 1977; Yaksh *et al.* 1977; Hayashi *et al.* 1978; Kumazawa & Perl, 1978; Bennett *et al.* 1979). This *in vitro* preparation, in addition has provided new information about the ionic mechanisms underlying some of the electrophysiological properties of dorsal horn neurones.

Passive membrane properties

Large variation was found in the input resistance (R_N), and the membrane time constant (τ) of dorsal horn neurones of 9–18-day-old rats. In addition it is significant that the R_N values for the rat dorsal horn cells cover a higher range (48–267 MΩ) than has been reported for mouse spinal cord neurones in primary dissociated cell culture (Ransom *et al.* 1977) and cat dorsal horn neurones, as recorded *in vivo* (Zieglgänsberger & Tulloch, 1979). This generally higher R_N value of immature rat dorsal horn neurones may be attributed, at least in part, to the small size of cells and the recording temperature of 33 ± 1 °C, although possible differences in actual membrane properties (specific membrane resistivity) and developmental characteristics of dendritic trees should be also considered. Anatomical studies of the superficial parts of the spinal dorsal horn have shown the preponderance of small-sized neurones (Rexed, 1952; Réthelyi & Szentágothai, 1973; Sugiura, 1975; Beal & Cooper, 1978; Gobel, 1979; Bennett *et al.* 1979). In addition our preliminary investigation of immature rat dorsal horn neurones intracellularly labelled with horseradish peroxidase *in vitro* (Nedeljkov & Randić, 1982) has confirmed that the majority of cells are small with fewer dendritic branches. This morphological finding is consistent with our present physiological findings of high cell input resistance and of effective electrical accessibility to dendritic synaptic sites from a somatically positioned micro-electrode.

The values calculated for the membrane time constant of developing dorsal horn neurones (range: 4–19 ms) are somewhat higher than the value of 5–9·5 ms obtained for mouse spinal cord neurones in cell culture (Ransom *et al.* 1977).

Voltage-dependent action potentials

In rats 9–18 days old all dorsal horn neurones tested could generate fast action potentials in response to direct and orthodromic stimulation. In comparison with the data obtained in adult animals, where intracellular recordings were performed *in vivo*, (Haapanen *et al.* 1958; Hunt & Kuno, 1959; Eccles *et al.* 1960; Cervero *et al.* 1977) the action potentials of rat dorsal horn neurones *in vitro* showed the mean value for 'overshoot' of about 25 mV and the mean amplitude of approximately 69 mV in relation to the firing level. These values for the spike height and 'overshoot' are larger than those observed *in vivo*, possibly reflecting the more stable recording conditions *in vitro*. However, the spike duration of approximately 1·4 ms obtained in immature dorsal horn cells is longer than the values of 0·5–1·0 ms recorded in adult dorsal horn neurones. The latter result may be accounted for by several factors such as immaturity of the spike generation mechanisms and the lower recording temperature.

The finding that the action potentials were reliably blocked by tetrodotoxin indicates that a voltage-dependent Na conductance, as in other neurones, plays an important role in generation of fast action potentials evoked in immature dorsal horn neurones either by direct or orthodromic stimulation. The latter finding is in agreement with the observations made on mouse spinal cord neurones in primary dissociated cell culture (Ransom *et al.* 1977; Heyer *et al.* 1981).

Voltage-dependent Ca conductances

High-threshold Ca spike. When the fast Na and K conductances are blocked by perfusion of the spinal cord slices with TTX and TEA, depolarizing current pulses of long duration often elicit a slow regenerative depolarizing potential followed by a slow after-hyperpolarization. The mechanism underlying the generation of this response appears to be a voltage-dependent Ca conductance change because this regenerative inward current is TTX-resistant, it requires the presence of extracellular Ca, and it is blocked by Co and Mn, the ions known to block Ca conductances. Since the appearance of these Ca-dependent action potentials requires that the membrane of the dorsal horn neurones be depolarized to a higher level (range: 35–58 mV from rest) than is necessary to evoke Na spike (range 14–38 mV), the term 'high threshold Ca spike', as recently proposed by Llinás & Yarom (1981*a*) is adopted in this paper.

Ca-dependent action potentials of similar properties have been recently demonstrated in mouse spinal cord neurones in cell culture (Heyer *et al.* 1981), and also in frog motoneurones (Barrett & Barrett, 1976), cerebellar Purkinje cells (Llinás & Hess, 1976; Llinás & Sugimori, 1980*a*, *b*), CA1 and CA3 hippocampal pyramidal neurones (Schwarzkroin & Slawsky, 1977; Wong, Prince & Basbaum, 1979) and inferior olivary neurones (Llinás & Yarom, 1980*a*, *b*). While we have no direct evidence for the site of generation of the high-threshold Ca potentials in the immature rat dorsal horn neurones, the dendritic localization in hippocampal pyramidal cells (Schwarzkroin, & Slawsky, 1977; Wong *et al.* 1979), cerebellar Purkinje cells (Llinás & Sugimori, 1979, 1980*b*) and inferior olivary neurones (Llinás & Yarom, 1981*a*, *b*) has been demonstrated.

Low-threshold Ca electroresponsiveness

It has been recently reported that following membrane hyperpolarization above −70 mV, inferior olivary neurones may generate Ca-dependent potentials in response to direct or synaptic stimulation (Llinás & Yarom, 1981*a*). In immature rat dorsal horn neurones having resting potentials more negative than −65 mV a low threshold electroresponsiveness of similar properties to those of inferior olivary neurones following direct stimulation was observed. Because this low-threshold potential is reversibly blocked by Co, an agent known to block Ca conductance, and because it is enhanced in the presence of Ba, an ion which moves easily through Ca channels (Hagiwara & Byerly, 1981), we suggest that the low-threshold spike may be generated by an inward Ca current. As in inferior olivary neurones this low-threshold Ca conductance exhibits inactivating properties as suggested by the perfusion experiments with Ba and TEA (Fig. 5*E*). However, a direct proof for the inactivation would require use of the voltage-clamp technique.

In several types of central neurones (cf. motoneurones: Barrett & Barrett, 1976; hippocampal pyramidal cells: Traub & Llinás, 1979; Wong *et al.* 1979; Purkinje cells: Llinás & Sugimori, 1980*b*; inferior olivary neurones: Llinás & Yarom, 1981*b*, and mitral cells: Mori, Nowycky & Shepherd, 1981) Ca current is believed to modulate a slow K conductance which in turn controls the repetitive firing. Thus the presence of voltage-dependent Ca channels in developing rat dorsal horn neurones may be an important mechanism for regulating the normal and pathological firing behaviour of these cells. In addition, certain putative chemical messengers (adrenaline, noradrenaline, 5-hydroxytryptamine, γ-aminobutyric acid, somatostatin and enkephalins) have been found to modulate voltage-dependent Ca currents (Dunlap & Fischbach, 1978; Mudge, Leeman & Fischbach, 1979). This control of the Ca channel by neurotransmitters is potentially of great importance as a mechanism to explain plasticity and modulation at central synapses.

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REFERENCES

Barrett, E. F. & Barrett, J. N. (1976). Separation of two voltage-sensitive potassium currents, and demonstration of a tetrodotoxin-resistant calcium current in frog motoneurones. *J. Physiol.* **255**, 737–774.

Beal, J. A. & Cooper, M. H. (1978). The neurons in the gelatinosal complex (laminae II and III) in the monkey (Macaca Mulatta): a Golgi study. *J. comp. Neurol.* **179**, 89–122.

Bennett, G. J., Hayashi, H., Abdelmoumene, M. & Dubner, R. (1979). Physiological properties of stalked cells of the substantia gelatinosa intracellularly stained with horseradish peroxidase. *Brain Res.* **164**, 285–289.

Cervero, F., Molony, V. & Iggo, A. (1977). Extracellular and intracellular recordings from neurones in the substantia gelatinosa Rolandi. *Brain Res.* **136**, 565–569.

Dunlap, K. & Fischbach, G. D. (1978). Neurotransmitters decrease the calcium component of sensory neurone action potentials. *Nature, Lond.* **276**, 837–839.

ECCLES, J. C., ECCLES, R. M. & LUNDBERG, A. (1960). Types of neurone in and around the intermediate nucleus of the lumbosacral cord. *J. Physiol.* **154**, 89–114.

ECKERT, R. & LUX, H. D. (1976). A voltage-sensitive persistent calcium conductance in neuronal somata of *Helix*. *J. Physiol.* **254**, 129–151.

FRANK, K. & FUORTES, M. G. F. (1956). Unitary activity of spinal interneurones of cats. *J. Physiol.* **131**, 424–435.

GOBEL, S. (1979). Neural circuitry in the substantia gelatinosa of Rolando: anatomical insights. In *Advances in Pain Research and Therapy*, ed. BONICA, J., LIEBESKIND, J. C. & ALBE-FESSARD, D. G., pp. 175–195. New York: Raven Press.

HAAPANEN, L., KOLMODIN, G. M. & SKOGLUND, C. R. (1958). Membrane and action potentials of spinal interneurons in the cat. *Acta physiol. scand.* **43**, 315–348.

HAGIWARA, S. (1973). Ca-spike. *Adv. Biophys.* **4**, 71–102.

HAGIWARA, S. & BYERLY, L. (1981). Calcium channel. *A. Rev. Neurosci.* 4, 69–125.

HAGIWARA, S., FUKUDA, J. & EATON, D. C. (1974). Membrane currents carried by Ca, Sr, and Ba in barnacle muscle fiber during voltage clamp. *J. gen. Physiol.* **63**, 564–578.

HAYASHI, H., PRICE, D. D., DUBNER, R. & RUDA, M. A. (1978). Comparative analysis of the response properties of layer I and II/III neurons in the monkey spinal cord dorsal horn. *Pain Abstr.* Second World Congress on Pain, Montreal, Canada **1**, 239.

HEYER, E. J., MACDONALD, R. L., BERGEY, G. K. & NELSON, P. G. (1981). Calcium-dependent action potentials in mouse spinal cord neurons in cell culture. *Brain Res.* **220**, 408–415.

HONGO, T., JANKOWSKA, E. & LUNDBERG, A. (1966). Convergence of excitatory and inhibitory action on interneurones in the lumbosacral cord. *Exp. Brain Res.* **1**, 338–358.

HUNT, C. C. & KUNO, M. (1959). Properties of spinal interneurones. *J. Physiol.* **147**, 346–363.

KRNJEVIĆ, K., PUMAIN, R. & RENAUD, L. (1971). Effects of Ba^{2+} and tetraethylammonium on cortical neurones. *J. Physiol.* **215**, 223–245.

KUMAZAWA, T. & PERL, E. R. (1978). Excitation of marginal and substantia gelatinosa neurons in the primate spinal cord: indications of their place in dorsal horn functional organization. *J. comp. Neurol.* **177**, 417–434.

LLINAŚ, R. & HESS, R. (1976). Tetrodotoxin-resistant dendritic spikes in avian Purkinje cells. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2520–2523.

LLINAŚ, R. & SUGIMORI, M. (1979). Calcium conductances in Purkinje cell dendrites: their role in development and integration. *Prog. Brain Res.* **51**, 323–334.

LLINAŚ, R. & SUGIMORI, M. (1980*a*). Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *J. Physiol.* **305**, 171–195.

LLINAŚ, R. & SUGIMORI, M. (1980*b*). Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *J. Physiol.* **305**, 197–213.

LLINAŚ, R. & YAROM, Y. (1981*a*). Electrophysiology of mammalian inferior olivary neurones *in vitro*. Different types of voltage-dependent ionic conductances. *J. Physiol.* **315**, 549–567.

LLINAŚ, R. & YAROM, Y. (1981*b*). Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurones in vitro. *J. Physiol.* **315** 569–584.

MILETIĆ, V. & RANDIĆ, M. (1980). Extra- and intracellular recordings from dorsal horn interneurons in the rat spinal cord slice preparation. *Pharmacologist* **22**, 204.

MILETIĆ, V. & RANDIĆ, M. (1981). Neonatal rat spinal cord slice preparation: postsynaptic effects of neuropeptides on dorsal horn neurons. *Dev. Brain Res.* **2**, 432–438.

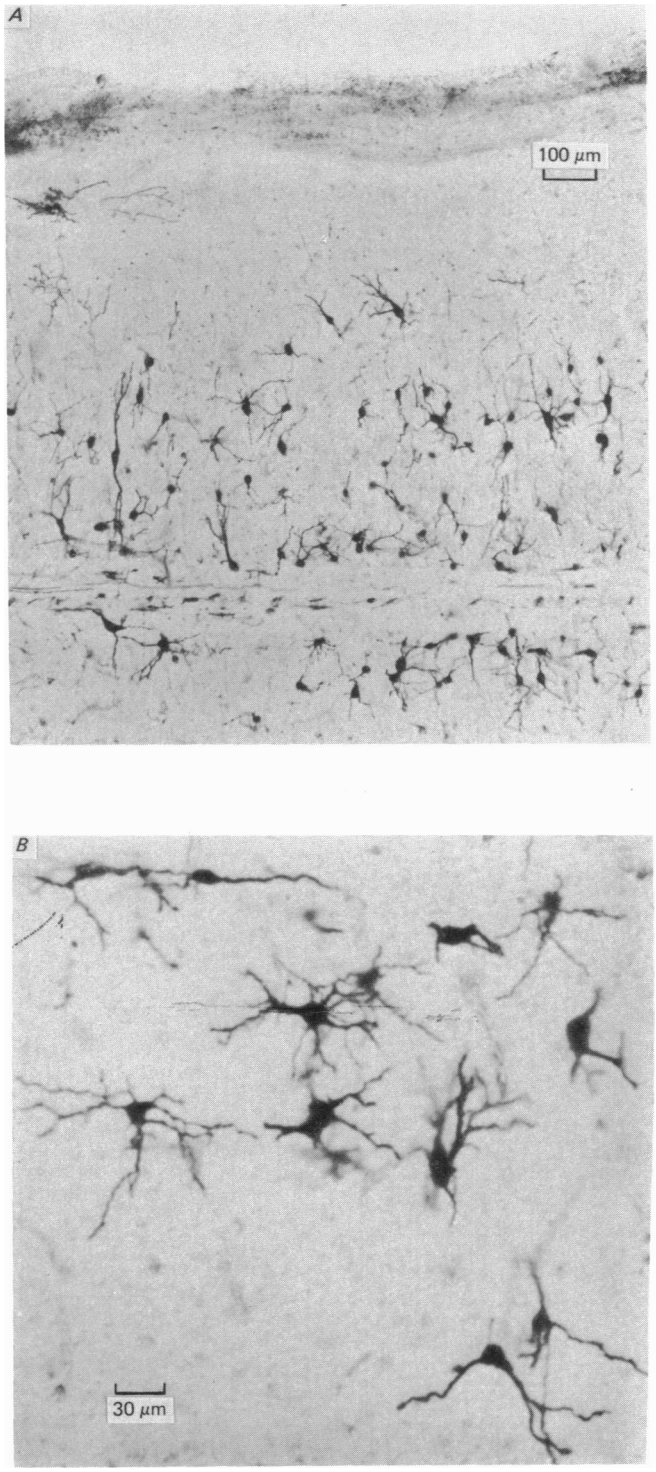
MORI, K., NOWYCKY, M. C. & SHEPHERD, G. M. (1981). Electrophysiological analysis of mitral cells in the isolated turtle olfactory bulb. *J. Physiol.* **314**, 281–294.

MUDGE, A. W., LEEMAN, S. E. & FISCHBACH, G. D. (1979). Enkephalin inhibits release of substance P from sensory neurons in culture and decreases action potential duration. *Proc. natn. Acad. Sci. U.S.A.*, **76**, 526–530.

MURASE, K. & RANDIĆ, M. (1981). Intracellular recording from dorsal horn neurons in the rat spinal cord slice preparation. *Neurosci. Abstr.* **7**, 529.

MURASE, K. & RANDIĆ, M. (1982). Calcium-dependent action potentials of rat spinal dorsal horn neurons *in vitro*. *Neurosci. Abstr.* **8** (in the Press).

MURASE, K., NEDELJKOV, V. & RANDIĆ, M. (1982). The actions of neuropeptides on dorsal horn neurons in the rat spinal cord slice preparation: an intracellular study. *Brain Res.* **234**, 170–176.


A
100 μm
B
30 μm

NEDELJKOV, V. & RANDIĆ, M. (1982). Intracellular staining of γ-aminobutyric acid-responsive cells in rat spinal dorsal horn *in vitro*. *Neurosci. Abstr.* **8** (in the Press).

RANSOM, B. R., NEALE, E., HENKART, M. BULLOCK, P. N. & NELSON, P. G. (1977). Mouse spinal cord in cell culture. I. Morphology and intrinsic neuronal electrophysiological properties. *J. Neurophysiol.* **40**, 1132–1150.

RÉTHELYI, M. & SZENTÁGOTHAI, J. (1973). Distribution and connections of afferent fibres in the spinal cord. In *Handbook of Sensory Physiology*, vol. II, *Somatosensory System*, ed. IGGO, A. pp. 207–252. Berlin, Heidelberg, New York: Springer-Verlag.

REXED, B. (1952). The cytoarchitectonic organization of the spinal cord in the cat. *J. comp. Neurol.* **96**, 415–466.

SCHWARTZKROIN, P. A. & SLAWSKY, M. (1977). Probable calcium spikes in hippocampal neurons. *Brain Res.* **135**, 157–161.

SUGIURA, Y. (1975). Three dimensional analysis of neurons in the substantia gelatinosa Rolandi. *Proc. Japan Acad.* **51**, 336–341.

TRAUB, R. & LLINÁS, R. (1979). Hippocampal pyramidal cells: significance of dendritic ionic conductances for neuronal function and epileptogenesis. *J. Neurophysiol.* **42**, 476–496.

WERMAN, R. & GRUNDFEST, H. (1961). Graded and all-or-none electrogenesis in arthropod muscle. II. The effects of alkali-earth and onium ions on lobster muscle fibres. *J. gen. Physiol.* **44**, 997–1027.

WONG, R. K. S., PRINCE, D. A. & BASBAUM, A. I. (1979). Intradendritic recordings from hippocampal neurons. *Proc. natn. Acad. Sci. U.S.A.* **76**, 986–990.

YAKSH, T. L., WALL, P. & MERRILL, E. G. (1977). Response properties of substantia gelatinosa neurones in the cat. *Neurosci. Abstr.* **3**, 495.

ZIEGLGÄNSBERGER, W. & TULLOCH, I. F. (1979). Effects of substance P on neurones in the dorsal horn of the spinal cord of the cat. *Brain Res.* **166**, 273–282.

EXPLANATION OF PLATE

A–B, Golgi–Cox impregnated dorsal horn neurones in a 150 μm thick horizontal section of lumbosacral spinal cord of a 12-day-old rat.