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HYPERPOLARIZATION OF FROG PRIMARY AFFERENT FIBRES CAUSED BY ACTIVATION OF A SODIUM PUMP

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SUMMARY

1. In the isolated frog spinal cord repetitive stimulation of a lumbar dorsal root produced a sustained negative potential recorded from an adjacent inactive dorsal root by sucrose gap techniques. This negative potential was followed by a positive potential, an indication that the dorsal root terminals were hyperpolarized. Increasing the duration of the tetanus applied to the active root increased the amplitude and duration of the after-hyperpolarization which could be up to 6 mV and 3 min respectively.

2. The hyperpolarization presumably reflected an increased rate of active sodium pumping. Since it was reversibly reduced by metabolic inhibitors (dinitrophenol, NaCN) and cooling (Q_{10}, 2·6) it was clearly dependent upon intact metabolic activity. In addition, a variety of procedures used to inhibit sodium pumps (including application of ouabain, elimination of potassium from the superfusate, and partial substitution of lithium for sodium ions) significantly and reversibly decreased the potential.

3. The hyperpolarization was not dependent upon intact chemical synaptic transmission since it could survive prolonged immersion of the cord in Ringer solution containing manganese or magnesium ions.

4. It is suggested that the hyperpolarization of inactive fibres resulted from a decreased extracellular potassium concentration in the dorsal horn produced as a result of a pumping mechanism which extruded sodium and transported potassium inwards by dorsal root fibres directly activated by the tetanus.

INTRODUCTION

When afferent volleys enter the mammalian spinal cord through a dorsal root, a number of events transpire. The volleys usually give rise to a slow depolarization (primary afferent depolarization; p.a.d.) of afferent terminals of active fibres located in the same dorsal root and of inactive fibres in adjacent dorsal roots. Because p.a.d. is electrotonically conducted in primary afferent fibres, its existence can be measured as a negative potential recorded from a dorsal root – the dorsal root potential (d.r.p.).

Dorsal root potentials are usually recorded by means of two electrodes placed upon a root, one close to the spinal cord (proximal lead) and the other at a distance toward the cut end of the

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root (distal lead). It is customary to express the sign of the potential in reference to the proximal lead. A negativity of the proximal electrode indicates depolarization of the intraspinal portion of the afferent fibres and is recorded upward; positivity of the proximal electrode denotes hyperpolarization of the proximal portion of the afferent fibres and is recorded downward.

In addition to p.a.d., afferent volleys can also hyperpolarize terminals of inactive afferent fibres. In fact, more than 25 years ago Lloyd (1952) first reported that there was a small, but long-lasting, positive potential (the so-called DRP_{vl}) following the larger negative d.r.p. Since then it has been shown that single or repetitive stimuli applied to peripheral nerves, particularly if the stimuli excite thin myelinated and unmyelinated afferents (Mendell & Wall, 1964; Mendell, 1973), can produce either a positive d.r.p. or else a diphasic one with a large positive component. Although there is no direct evidence for the hypothesis, it is thought that such positive d.r.p.s (an indication that primary afferent terminals have been hyperpolarized – primary afferent hyperpolarization, p.a.h.) result from reduction of a background tonic presynaptic depolarization (Mendell & Wall, 1964; Mendell, 1972, 1973).

There are also reports of positivity of a dorsal root and hyperpolarization of the active afferent fibres in it following tetanic electrical stimulation (Woolsey & Larrabee, 1940; Lloyd, 1952; Eccles & Krnjević, 1959). This post-tetanic hyperpolarization is most likely caused by the action of an electrogenic Na^+ pump (Kerkut & York, 1971; Thomas, 1972). The relationship (if any) of this type of hyperpolarization to p.a.h. is not known.

In the amphibian spinal cord electrical stimulation of dorsal root fibres results in depolarization of afferent fibre terminals and a negative d.r.p. when d.c.-coupled recording techniques have been used. In contrast to the situation in the mammalian spinal cord, positive d.r.p.s have not been reported. Our present experiments, however, demonstrate that hyperpolarization of the terminals of passive afferent fibres in frog dorsal roots can be produced by tetanic stimulation of adjacent roots. This unique type of hyperpolarization appears to be produced by electrogenic Na^+ pumping. Preliminary findings have been reported (Hackman & Davidoff, 1979).

METHODS

Experiments were performed on adult frogs (*Rana pipiens*, 30–55 g) anaesthetized by cooling on crushed ice. After decapitation and laminectomy the spinal cord was removed, the lumbar cord was hemisected sagittally and one half-cord with attached eighth and ninth ventral and dorsal roots was placed in a sucrose gap apparatus (Barker, Nicoll & Padjen, 1975). In most experiments the ninth dorsal rod was placed across the 3 mm sucrose gap. The cord and only the intramedullary portion of primary afferent fibres was continuously superfused with Ringer solution (NaCl, 114 mM; KCl, 2 mM; $CaCl_2$, 1·8 mM; $NaHCO_3$, 4 mM; glucose 5·5 mM) gassed with 95% O_2/5% CO_2. The temperature was maintained at 15 °C unless stated otherwise.

Calomel electrodes were employed to measure the difference in potential between the spinal cord bath and the distal end of the ninth dorsal root maintained in a pool of Ringer solution. Differential d.c.-coupled recording between the two electrodes was used and the preparation was usually left ungrounded. After amplification the signals were recorded on an oscilloscope and on a Brush 220 pen recorder and led to a Hewlett–Packard model 5480B signal averager. In many cases two to four samples were averaged and plotted on an X–Y recorder. Stimuli were delivered to appropriate roots from an isolated stimulator via Ag–AgCl wire electrodes.

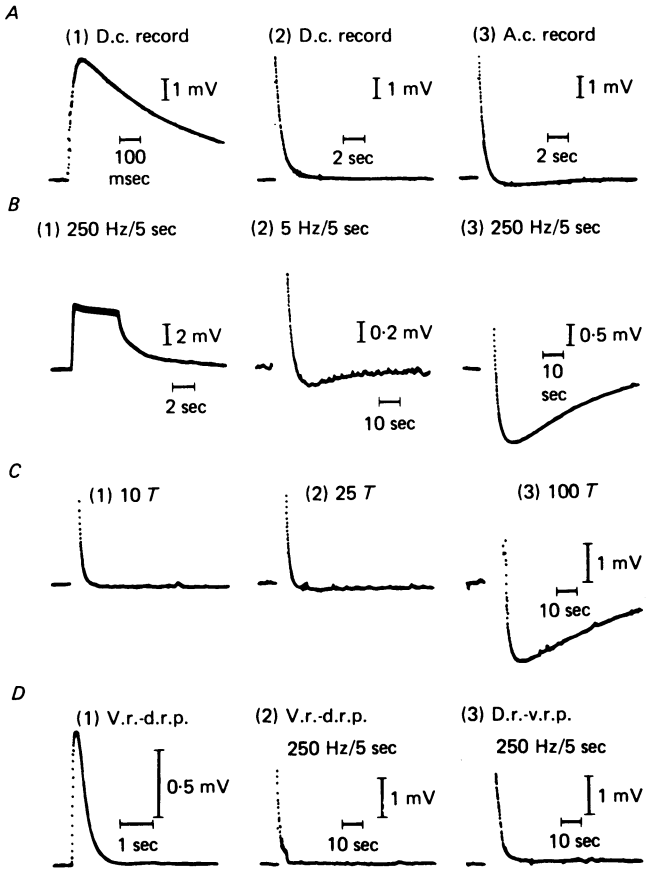

Fig. 1. Dorsal and ventral root potentials (d.r.p.s and v.r.p.s) produced by stimulation of dorsal and ventral roots. *A*, d.r.p. produced by a single supramaximum shock to adjacent dorsal root. A_1, A_2, recorded with d.c. amplification at two different sweep speeds. A_3, recorded with a.c. amplification (time constant 5·3 sec). *B*, d.r.p.s produced by repetitive stimulation of adjacent dorsal root. B_1, Submaximum stimuli delivered at rate of 250 Hz, for 5 sec. B_2, B_3, d.r.p.s produced by supramaximum tetani (5 Hz, 5 sec and 250 Hz, 5 sec respectively). The sustained negative d.r.p. in B_3 (and in many subsequent traces) is out of range of the oscilloscope. *C*, d.r.p.s elicited by graded tetani (250 Hz, 5 sec). Stimulus strength is expressed in multiples of a test stimulus (*T*) that elicited a just detectable d.r.p. D_1, D_2, d.r.p.s produced by single and multiple (250 Hz, 5 sec) supramaximum stimuli delivered to ventral root. D_3, v.r.p. elicited by supramaximum tetanus (250 Hz, 5 sec) of dorsal root.

RESULTS

Dorsal root potentials generated by single and tetanic stimulation. Fig. $1A_1$ illustrates a typical sucrose gap recording of a d.r.p. elicited in response to supramaximum stimulation of an adjacent dorsal root. With a slower oscilloscope sweep speed it can be seen that a hyperpolarization does not follow the depolarization (Fig. $1A_2$). This was true even if stimuli of intensity and duration (30 V, 2 msec) sufficient to excite unmyelinated C fibres were used. However, if as is customary

in many laboratories an a.c.-coupled recording system with a long time constant was employed, then a prominent artifactual positive component was seen (Fig. $1A_3$).

When we applied trains of stimuli to an adjacent root, a negative shift of the base line resulted; this represents a sustained d.r.p. (Fig. $1B_1$; cf. Lloyd, 1952; Holobut &

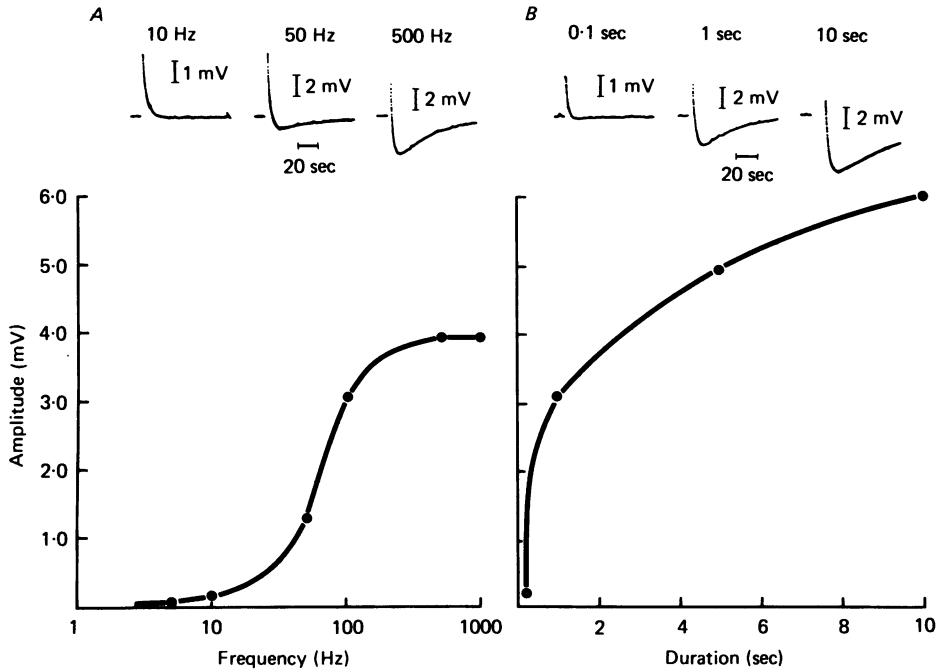

Fig. 2. Effect of increasing frequency and duration of dorsal root tetani on after-hyperpolarization. *A*, examples of hyperpolarizations produced by 1·0 sec tetani delivered at 10, 50 and 500 Hz. Complete series of observations at different frequencies is plotted in the graph. *B*, examples of hyperpolarizations produced by 100 Hz tetani delivered for 0·1, 1·0 and 10·0 sec. Complete series at different durations is plotted in the graph. Abscissae in both graphs show amplitudes of dorsal root hyperpolarizations expressed in mV.

Niechaj, 1973; Mendell, 1973; Lothman & Somjen, 1975; Nicoll, 1979). At the end of the tetanus this potential shift fell off rapidly. Provided that stimuli of sufficient strength were used (usually greater than 2 V and 0·2 msec), the potential shift consistently decayed to a value below the base line (Fig. $1B_{2,3}$). This positive potential (an indication that passive afferent terminals had been hyperpolarized) was elicited on several occasions with as few as 5 to 25 stimuli (Fig. $1B_2$), but generally longer trains were required (Fig. $1B_3$). Sometimes the after-hyperpolarizations were as large as 6 mV in amplitude and 3 min in duration. Furthermore, the magnitude and the duration of the hyperpolarization were a function both of the stimulus intensity (Fig. $1C$) and of the frequency and duration of the stimulus trains (Fig. 2). With repeated stimulation we obtained consistent and reproducible responses if we waited 3 to 5 min between trains.

In addition to stimulating a dorsal root a negative d.r.p. can also be elicited in

the amphibian by antidromic stimulation of the ventral root. We observed no after-hyperpolarization following the negative d.r.p. produced in this manner when we applied either single supramaximal stimuli (Fig. 1 D_1) or trains of various frequencies (1–1000 Hz) and durations (1–20 sec) to the ventral root (Fig. 1 D_2, cf. Nicoll, 1979). Similarly, no hyperpolarization was noted in sucrose gap recordings from the ventral root when the dorsal root of the same segment was repetitively stimulated (Fig. 1 D_3).

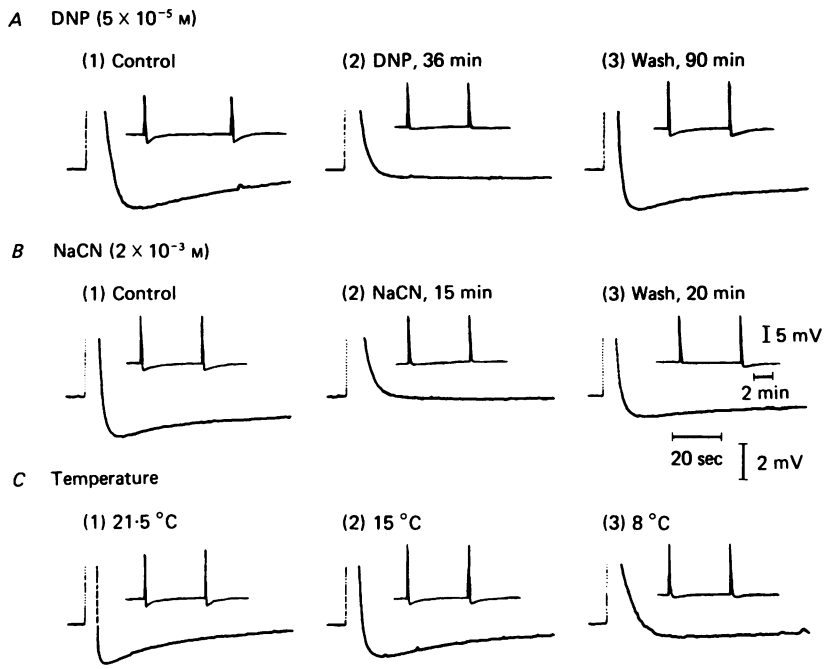

Fig. 3. Effects of metabolic inhibitors and temperature on dorsal root hyperpolarizations. *A*, effect of DNP. Control (A_1) obtained in Ringer solution. Supramaximum tetanus of 250 Hz for 5 sec delivered here and in all subsequent traces in this figure. Insert shows response to same tetanus recorded with rectilinear paper writer. Traces obtained after exposure to DNP (5×10^{-5} M) for 36 min (A_2) and after washing for 90 min (A_3). *B*, effect of NaCN. Control recorded (B_1) and traces recorded 15 min after addition of NaCN (2×10^{-3} M) to superfusing fluid (B_2) and 20 min after washing with normal Ringer solution (B_3). *C*, hyperpolarization obtained at three different temperatures: C_1, 21·5 °C. C_2, 15 °C. C_3, 8 °C.

Effects of metabolic inhibitors and cooling. After-hyperpolarizations following depolarizing stimuli may be produced by several different mechanisms. These include changes in ionic permeability (Jansen & Nicholls, 1973) and activation of ionic pumps (Thomas, 1972). The latter require metabolic energy. In our experiments hyperpolarization of passive afferent terminals was clearly dependent upon intact metabolic activity.

The result of an experiment where 2,4-dinitrophenol (DNP, 5×10^{-5} M) was added to the superfusing solution is seen in Fig. 3*A*. This procedure reduced the rate of return of the depolarization to the base line and reduced the amplitude of the hyperpolarization to 18% of the control value. It did not substantially decrease the

preceding depolarization. Similar results were obtained with NaCN. Application of this compound reduced the hyperpolarization to 7% of the control (Fig. 3*B*). The effects produced by both metabolic inhibitors were reversible (Fig. 3A_3 and B_3).

As illustrated in Fig. 3*C* the hyperpolarization was temperature-sensitive. It was markedly suppressed when the temperature of the superfusate was reduced and augmented when the temperature was raised. Cooling is known to decrease the activity of metabolically driven processes. The after-hyperpolarization was much more sensitive to temperature changes than was the negative potential. A Q_{10} for the after-hyperpolarization was calculated from the changes in peak amplitude for the range of temperatures between 18 and 8 °C and was found to be about 2·6. This is in the range of values reported for ionic pumps in other preparations (Den Hertog & Ritchie, 1969; Brown, Brownstein & Scholfield, 1972).

Ion pump inhibitors. Metabolically dependent hyperpolarizations may reflect pumping of ions across a membrane resistance. There is evidence already that the negative d.r.p. results from a selective influx of Cl^- ions (Nishi, Minota & Karczmar, 1974); therefore the primary afferent fibre terminals must possess an inward Cl^- pump. If such an inward Cl^- pump were electrogenic, then it could be responsible for the hyperpolarization seen after a sustained negative d.r.p. But compounds known to block Cl^- transport such as 4-acetamido-4-isothiocyanostilbene-2,2′-disulphonic acid (SITS), 10^{-4} M (Thomas, 1977), furosemide, 10^{-3} M (Zadunaisky, Lande & Hafner, 1971), and bumetanide, 5×10^{-5} M (McGahan, Yorio & Bentley, 1977) did not affect either the rate of fall of the depolarization or the amplitude and duration of the hyperpolarization (not illustrated). Therefore it does not appear that Cl^- ions play a major role in the production of the hyperpolarization.

In contrast, we found that a variety of procedures used to inhibit Na^+ pumps (Kerkut & York, 1971; Thomas, 1972) did significantly and reversibly reduce the hyperpolarization evoked by a dorsal root tetanus. These procedures included: application of ouabain (10^{-5} M); partial substitution of Li^+ for Na^+ (57 mM); and elimination of K^+ from the external medium. The results are illustrated in Fig. 4 which demonstrates that all three manipulations significantly reduced the peak amplitude of the hyperpolarization and the rate of decay of the depolarization. Of these procedures elimination of K^+ was the least effective presumably because enough K^+ was released from neurones into the extracellular space during activity to maintain pumping. It should be noted that when normal Ringer solution was again used to superfuse the preparation, the dorsal root hyperpolarized in the absence of stimulation (not illustrated). This hyperpolarization presumably resulted from K^+ activation of the Na^+ pump at a time when the concentration of Na^+ inside the terminals was still elevated.

Considering that ouabain, substitution of Li^+ for Na^+, and reduction of external K^+ are demonstrably effective ways to block Na^+ pumps, a hyperpolarizing potential that is eliminated by these three manipulations may be considered to result from activation of such a pump.

Activation of the Na^+ pump. Is the Na^+ pump activated by means of conventional chemical synaptic transmission? Is it possible, for example, that antidromic action potentials produced in passive fibres by the synaptically mediated dorsal root reflex could load these fibres with Na^+ and thereby initiate active Na^+ extrusion? To

ascertain whether or not the Na^+ pump is activated by a synaptic process we superfused the cord in Ringer solution modified by the addition of Mg^{2+} (10–20 mM) or Mn^{2+} (3 mM) ions. These concentrations of divalent cations are sufficient to block chemical synaptic transmission in the isolated frog cord (Erulkar, Dambach & Mender, 1974: Taugner, Sonnhof, Richter & Schiller, 1978).

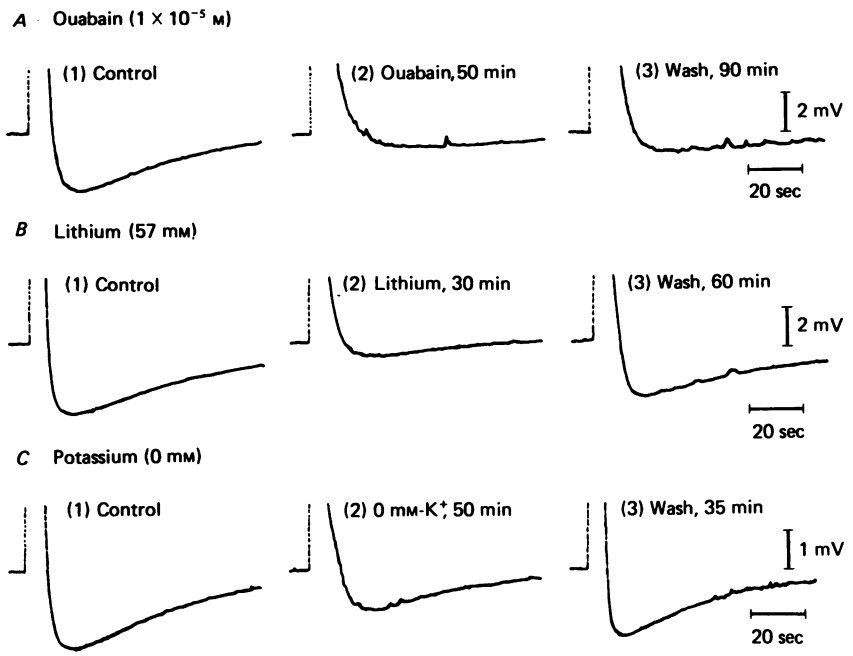

Fig. 4. Effects of ouabain, lithium substitution and potassium-free Ringer solution on dorsal root hyperpolarizations. All tetani were supramaximum and delivered at 250 Hz for 5 sec. *A*, effect of ouabain. A_1, control. A_2, 50 min after application of ouabain (1×10^{-5} M). A_3, 90 min after washing with normal Ringer solution. *B*, effect of Li^+. B_1, control. B_2, recording obtained 30 min after replacing 57 mM-NaCl with equivalent amount of LiCl. B_3, 60 min after washing with normal Ringer solution. *C*, effect of removing K^+ from superfusate. C_1, control. C_2, 50 min after superfusion with Ringer solution from which K^+ has been removed. C_3, 35 min after washing with normal Ringer solution.

The effects of these alterations in the Ringer solution were complex. When large sustained depolarizations and correspondingly large hyperpolarizations of a passive root were elicited by trains of supramaximum stimuli delivered to an adjacent root, addition of Mg^{2+} or Mn^{2+} ions did not significantly affect either the amplitude or the duration of the hyperpolarization (Fig. 5*A*). We interpret this finding as an indication that chemical synaptic transmission is not necessary to produce the hyperpolarization. However, it is difficult to determine what effects the two divalent cations may have had on the sustained depolarizations since the large artifacts produced by the supramaximum stimuli precluded accurate measurement of the degree of depolarization.

If, on the other hand, small depolarizations and small hyperpolarizations were evoked by submaximum stimuli, addition of Mg^{2+} or Mn^{2+} ions significantly reduced

the amplitude of the depolarization and almost abolished the hyperpolarization (Fig. 5*B*). This result might be interpreted as indicating that a synaptic process contributes to the activation of the Na^+ pump, but it should be realized that these cations not only block synapses, but also strongly reduce the amount of K^+ released by a tetanus into the extracellular space of the frog spinal cord dorsal horn (Syková, Shirayev, Kříž & Vylický, 1976). This reduced extracellular K^+ concentration may substantially alter the rate of Na^+ pumping and so decrease the after-hyperpolarization.

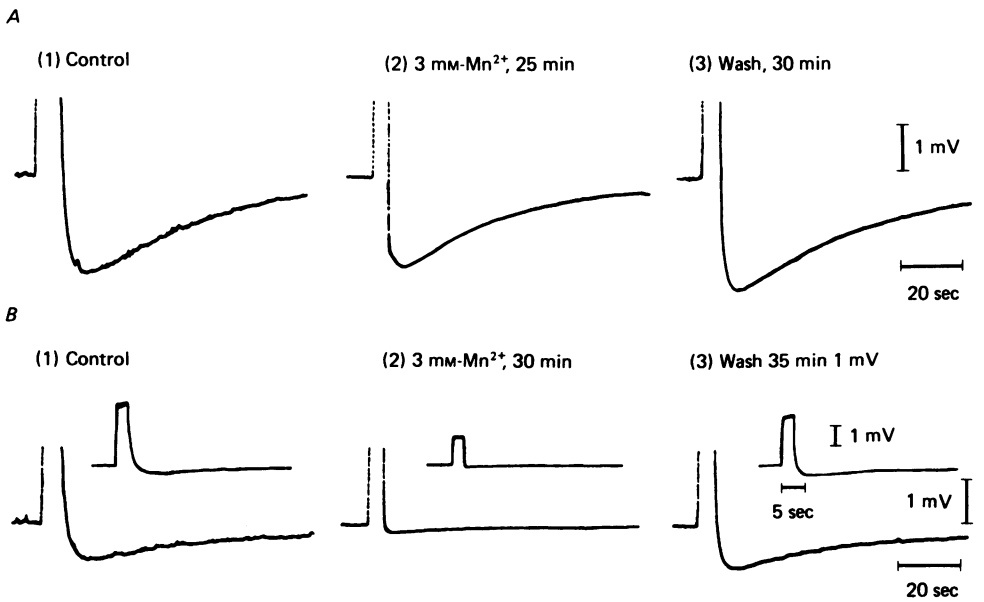

Fig. 5. Effects of manganese on dorsal root hyperpolarizations. A_1, control record. Supramaximum tetanus delivered at 250 Hz for 5 sec. A_2, 30 min after adding Mn^{2+} (3 mM) to Ringer solution. A_3, 30 min after washing with normal Ringer solution. B_1, control record. Submaximum tetanus delivered at 250 Hz for 5 sec. B_2, in Mn^{2+} for 25 min. B_3, after washing for 30 min. Inserts show paper writer records.

Reduced K^+ release might also be expected when supramaximum stimuli were used and the cord superfused with Ringer solution containing Mg^{2+} or Mn^{2+}. But the rate of Na^+ pumping may not be affected in this situation since substantially more K^+ is released by stronger dorsal root stimuli than by weaker stimuli (Cordingly & Somjen, 1978). Thus, despite a decrement in K^+ release in the presence of Mn^{2+} and Mg^{2+}, there may still be sufficient extracellular K^+ to keep the Na^+ pump going at a maximum rate.

DISCUSSION

Hyperpolarization of afferent terminals. The observations reported here show that repetitive stimulation of afferent fibres entering the frog spinal cord is followed by a hyperpolarizing change in the membrane potential of the terminals of the fibres contained in an adjacent passive dorsal root. As indicated in the Introduction,

hyperpolarization of the terminals of active dorsal root fibres following tetanization of the dorsal root has been described previously (Woolsey & Larrabee, 1940; Lloyd, 1952; Eccles & Krnjević, 1959). Evidence is also available to show that such active afferent fibres exhibit a reduced excitability after a tetanus (Koketsu, 1956; Wall & Johnson, 1958). This effect is limited to the stimulated fibres. However, hyperpolarization of passive afferent terminals has only recently been noted (Nicoll, 1979) although the phenomenon can be seen in the illustrations of several reports (e.g. Holobut & Niechaj, 1973; Mendell, 1973).

We have presented evidence to indicate that this after-hyperpolarization reflects an increased rate of active Na^+ pumping. Thus, the hyperpolarization has all the characteristics of phenomena produced by Na^+ pumps in a variety of vertebrate and invertebrate preparations (Kerkut & York, 1971; Thomas, 1972). It is significantly reduced, and in some cases abolished, by applying metabolic inhibitors and ouabain, by cooling, by partially substituting Li^+ for Na^+ or by reducing the external concentration of K^+ ions. Furthermore, the data do not support alternative hypotheses. For example, the observations obtained in experiments with reduced K^+ in the superfusate are inconsistent with a hyperpolarization produced by an increased K^+ conductance (Jansen & Nicholls, 1973). Likewise, the experimental evidence obtained with the use of blockers of Cl^- transport is against an involvement of Cl^- ions in the production of the hyperpolarization.

Extracellular K^+ concentration and hyperpolarization. Data generated through the use of K^+-sensitive micro-electrodes have shown that tetanic stimulation of afferent fibres in mammalian and amphibian spinal cords induces a significant elevation of extracellular K^+ concentration (Krnjević & Morris, 1975; Lothman & Somjen, 1975; Syková *et al.* 1976; Nicoll, 1979). Subsequently the excess K^+ is reduced to its previous level by active uptake into neurones and perhaps glia (see Somjen, 1979 for refs). The active transport of K^+ is presumed to be a consequence of rapid Na^+ pump activity in which there is a direct coupling between extrusion of Na^+ and influx of K^+. The identity of the K^+-clearing mechanism with the Na^+ pump is supported by reports that K^+ uptake is retarded by manipulations which block the Na^+ pump, namely administration of cardiac glycosides (Krnjević & Morris, 1975; Cordingley & Somjen, 1978), cooling (Lewis & Schuette, 1975) and anoxia (Morris, 1974).

It is believed that elevated extracellular K^+ concentration is responsible, at least in part, for the sustained depolarizations seen in passive dorsal root terminals during a tetanus of adjacent roots (Lothman & Somjen, 1975; Nicoll, 1979). If that is the case, then the rate of decay of such potentials can be used as a measure of the rate at which K^+ is cleared from the extracellular space. And indeed, in our experiments the decay of the depolarization was decreased by procedures which have been reported to reduce Na^+ pumping and K^+ clearance, such as cooling and the application of metabolic inhibitors and ouabain. In addition, in the presence of enough Mg^{2+} and Mn^{2+} ions to block chemical synaptic transmission, the decay rate was accelerated. Under these conditions smaller amounts than usual of K^+ were released during the tetanus (Sykova & Vyklický, 1977), and presumably these smaller quantities could be cleared from the extracellular space more rapidly.

Following its recovery from an elevated level, the concentration of K^+ in the dorsal

horn can fall considerably below its prestimulation base line level (Krnjević & Morris, 1975; Lotham & Somjen, 1975; but cf. Nicoll, 1979). This 'undershoot' is thought to indicate a prolonged period of uptake of K^+ by neurones and terminals in which the Na–K coupled pump had been stimulated by a lasting Na^+ load (Krnjević & Morris, 1975). If this were the case, the large Na^+ load would exist in those active fibres and terminals that had been directly stimulated by the tetanus; passive fibres, on the other hand, would not be affected in this way. As a result of Na^+ pumping by these active dorsal root fibres, a decrease in extracellular K^+ concentration in the dorsal horn would follow. This would shift the equilibrium potential for K^+ and thus increase the membrane potential of *all* neuronal elements in the environment, passive and active fibres alike. We postulate that this temporary depletion of extracellular K^+ is responsible for the hyperpolarization seen in passive afferent fibres following a tetanus. This hypothesis requires rather specific close spatial relationships among the fibres of active and passive roots. Anatomical data indicating such a relationship in the frog is lacking at present but, since in the cat spinal cord afferent fibres are apposed in tightly packed bundles (Scheibel & Scheibel, 1969), it would not be surprising if this were the case in the amphibian as well.

Several alternative hypotheses are possible. For example, it is possible that the elevated extracellular K^+ concentration attained during a tetanus directly activates an electrogenic Na^+ pump located in passive fibres. The electrogenic Na^+ pump would then hyperpolarize the passive fibres directly. This hypothesis cannot be dismissed on the basis of data currently available. But, if these fibres have not been loaded with Na^+, the impetus for activation of the pump solely by K^+ is probably small (Rang & Ritchie, 1968). This is borne out by the observation that transient application of elevated concentrations of K^+ to the frog spinal cord produces a depolarization, but a hyperpolarizing phase is not seen during recovery (J. C. Hackman & R. A. Davidoff, unpublished observations).

There are, however, at least three ways in which the passive fibres could accumulate intracellular Na^+. (1) It is possible that antidromic firing by the dorsal root reflex is responsible for Na^+ loading of passive fibres (Nicoll, 1979). But this idea can be rejected by present experiments which showed that the hyperpolarization can remain following abolition of reflex activity by Mn^{2+} and Mg^{2+} ions. (2) Na^+ could move from active to inactive fibres if there were electrical coupling between afferent fibres *via* gap junctions. There are few data favouring such connexions (Grinnell, 1970; Davidoff, 1972), but such junctions are present between frog afferent fibres and motoneurones (Taugner *et al.* 1978). (3) During repetitive stimulation (Weinreich & Hammerschlag, 1975), Ca^{2+}-independent release of glutamic acid from dorsal root fibres occurs. Glutamic acid depolarizes dorsal root terminals, presumably by increasing Na^+ permeability, and the depolarization is invariably followed by a hyperpolarization (Davidoff, 1972; Barker *et al.* 1975). In addition, glutamic acid has already been shown to activate a Na^+ pump in frog motoneurones (Sonnhof, Grafe & Krumnikl, 1976). It is difficult to devise conclusive experiments which would test these last two possibilities, so either (or both together) remain viable hypotheses.

Hyperpolarization and afferent fibre function. Na^+ pumps have previously been identified in a variety of sensory units in both vertebrates and invertebrates (e.g. Eccles & Krnjević, 1959; Nakajima & Takahashi, 1966; Baylor & Nicholls, 1969).

It has been postulated that following impulses, such pumps produce long-lasting alterations in the properties of the sensory units. But in these previously reported cases initiation of pumping and the accompanying hyperpolarization occurred in response to firing of the unit under study. In the present investigation, activity of afferent fibres was shown to produce an increased membrane potential in inactive sensory fibres.

Is such hyperpolarization of passive afferent fibres a laboratory curiosity, or does it have functional significance? In this regard it is important to note that amphibian afferents can fire at appreciable rates (> 100 Hz) for prolonged periods of time if appropriate physiological stimuli are used (Shimada & Yai, 1960; Holloway, 1973). Such afferent activity would easily activate the Na^+ pump and affect the membrane potential of neighbouring fibres. A functional role for the process described here must therefore be postulated. It is difficult to assess the importance of such a role, but hyperpolarization of afferent fibres may affect conduction of impulses in terminals and the release of transmitter from them. If this proves to be the case, the phenomenon is certainly of functional significance.

Could the post-tetanic hyperpolarization account for primary afferent hyperpolarization and positive d.r.p.s? We think not. When compared, the properties of p.a.h. appear to be different from the properties of the after-hyperpolarization. For example, unlike the after-hyperpolarization p.a.h. can be elicited by single afferent volleys (Mendell, 1972, 1973). In addition, positive d.r.p.s can be recorded without any sign of preceding depolarization (Mendell, 1973). Moreover, repetitive stimulation enhances negative d.r.p.s more than positive ones (Mendell, 1972, 1973). However, it does remain possible that the two processes, p.a.h. and the post-tetanic hyperpolarization described here, may work in concert to modulate the over-all input–output relationships of afferent fibres.

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