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PROPERTIES AND DISTRIBUTION OF PERIPHERALLY EVOKED PRESYNAPTIC HYPERPOLARIZATION IN CAT LUMBAR SPINAL CORD

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SUMMARY

1. The action of peripheral nerve volleys on the polarization of presynaptic terminals of inactive sensory fibres in cat lumbar spinal cord has been investigated by recording (*a*) the dorsal root potential (DRP), (*b*) intracellular changes in polarization of single preterminal axons (PAD or PAH), and (*c*) changes in excitability of populations of preterminal axons.

2. Presynaptic hyperpolarization (positive DRP-PAH) can be evoked by stimulation of muscle group III afferents as well as by volleys in cutaneous Aβ, Aδ and C afferents. These volleys can also produce presynaptic depolarization (negative DRP-PAD).

3. The positive DRP is observed in the decerebrate state and increases in amplitude following spinalization.

4. Picrotoxin blocks the positive DRP at the same dosages required to block the negative DRP. Test negative DRPs are depressed during a conditioning positive DRP. These results are used to support earlier suggestions that the positive DRP results from inhibition of interneurones mediating the negative DRP.

5. Trains of group III stimuli at 20/sec evoke a steady positive DRP. Trains of the same intensity at 200/sec evoke a phasic negative DRP. This frequency dependence is observed for PAD and PAH in single sensory axons.

6. The DRPs recorded from different dorsal root filaments in response to a given stimulus vary widely in the ratio of negative to positive DRP.

7. Intracellular recording from single axons reveals that the same stimuli evoke widely varying ratios of PAD and PAH.

8. Stimulation of FRA evokes PAH > PAD in PBST group I afferents,

PAD > PAH in sural A fibres and intermediate effects in G-S group I units.

9. It is suggested that activation of flexor reflex afferents may selectively potentiate the synaptic efficacy of large muscle afferents mediating the flexor reflex rather than large skin afferents or large afferents from extensor muscles.

INTRODUCTION

Hyperpolarization of presynaptic terminals of inactive sensory fibres can be evoked by stimulation of peripheral nerves (Lloyd, 1952; Mendell & Wall, 1964; Anden, Jukes, Lundberg & Vyklický, 1966; Dawson, Merrill & Wall, 1970; Mendell, 1970; Wagman & Price, 1971; Hodge, 1972), supraspinal centres (Lundberg & Vyklický, 1966; Cangiano, Cook & Pompeiano, 1969; Scibetta & King, 1969; Sessle & Dubner, 1970), and natural activation of certain receptors (Young & King, 1972). This hyperpolarization has been measured most commonly by recording the dorsal root potential (DRP). The DRP is measured from the central end of a cut dorsal rootlet and there is substantial evidence that the negative DRP reflects the extracellular current flow associated with depolarization of presynaptic terminals of certain afferent fibres (Lloyd & McIntyre, 1949; Wall, 1958; Eccles, Eccles & Magni, 1961). The occurrence of a positive DRP has been taken as evidence for the existence of presynaptic hyperpolarization (Mendell & Wall, 1964; Wall, 1964; Anden *et al.* 1966; Lundberg & Vyklický, 1966; Cangiano *et al.* 1969; Dawson *et al.* 1970; Mendell, 1970; Chan & Barnes, 1971; Hodge, 1972). The importance of these processes is related to their postulated role in presynaptic inhibition (negative DRP (Eccles *et al.* 1961)) and presynaptic facilitation (positive DRP (Wall, 1964)).

Most previous studies on the positive DRP have been done by stimulating cutaneous nerves (Mendell & Wall, 1964; Wall, 1964; Dawson *et al.* 1970; Wagman & Price, 1971; Hodge, 1972); in these experiments volleys in large fibres must be blocked to eliminate a large negative DRP which obscures a small positive DRP. Recently, stimulation of muscle nerves has been shown to evoke positive DRPs in a reliable, reproducible manner without need for blocking volleys in large fibres (Mendell, 1970) since volleys in large muscle afferents produce very small negative DRPs (Eccles, Schmidt & Willis, 1962). In this paper further evidence is presented that stimulation of group III fibres in muscle nerves produces positive DRPs which are associated with presynaptic hyperpolarization as measured directly by excitability testing of presynaptic terminals (Wall, 1958) and by intrafibre recording (Koketsu, 1956; Eccles & Krnjević, 1959). Cutaneous nerve stimulation is also shown to evoke PAH. The

variability of the DRP associated with different frequencies of stimulation is explored as is the variability of the positive DRP evoked on different dorsal-root filaments by a fixed stimulus.

Since the DRP is the response of a large population of axons, consistent differences in the behaviour of individual axons to peripheral stimuli might be obscured. In order to study these responses intrafibre records have been obtained from cutaneous and from group I muscle afferents in response to stimulation of a variety of peripheral nerves. These studies have revealed interesting differences between the responses of different kinds of afferent units to peripheral nerve stimulation. The possible functional significance of these differences is considered.

METHODS

Most experiments were performed on unanaesthetized cats made spinal at C1 under temporary ether anaesthesia. Blood supply to the head was occluded and the animals were artificially ventilated. A few experiments (Fig. 1 only) were performed on unanaesthetized animals made decerebrate by mid collicular section. Dorsal root potentials were recorded from small filaments of DR L6 although on occasion DR L7 or DR S1 were used. The electrodes were hooks made of Pt wire and the amplifiers were d.c. or a.c. coupled with a 1 sec time constant. The signals were averaged by a Fabritek no. 1010 digital averager and the resulting display was photographed. Usually 16 sweeps were sufficient to produce a suitable signal-to-noise ratio. The incoming volley was recorded monopolarly from the cord dorsum with the indifferent electrode on back muscles. Nerves prepared for stimulation included the posterior biceps-semitendinosus (PBST), gastrocnemius-soleus (G-S), sural, and plantaris. The tibial, peroneal and sciatic nerves were stimulated in continuity. The temperature of the mineral oil surrounding the spinal cord was kept at 36–38° C.

Intrafibre records were made from afferent fibres in the dorsal part of the spinal cord. Electrodes were filled with 3 M-KCl with resistances of about 15 MΩ in 3 M-KCl. The electrode was inserted into the spinal cord at a point just medial to the dorsal root entry zone and the electrode was slightly angled to advance in a medial to lateral direction. Fibres were identified by orthodromic spikes. The criteria for direct response were: (*a*) short latency response eliminating the possibility of a synaptic delay – this was useful only in the case of large fibres which predominated in these studies, (*b*) one for one following of high frequency stimulation i.e. greater than 500/sec, and (*c*) absence of repetitive discharge following a single shock. The data from a unit was used only if there was a steady membrane potential which was measured indirectly by use of spike amplitudes. The data were judged acceptable only if the spike height was greater than 40 mV. After a fibre was impaled and identified, its response to stimulation of various peripheral nerves was examined. The responses to 16 or 32 stimuli were averaged to improve the signal-to-noise ratio.

RESULTS

Positive dorsal root potential

Fig. 1*D* displays the dorsal root potentials obtained in response to increasing intensities of stimulation delivered to the G-S nerve in a decerebrate animal. Each pair of traces shows the DRP in the bottom trace and

the input volley recorded from the dorsal root entry zone in the top trace. A negative DRP was observed at stimulus strengths up to 7X group Ia threshold; the cord dorsum electrode recorded only the early spike potential associated with activity in group I fibres. When the stimulus intensity reached 8X group Ia threshold, a positive DRP was evoked which had the same threshold as the late negative wave from the cord dorsum. Since this

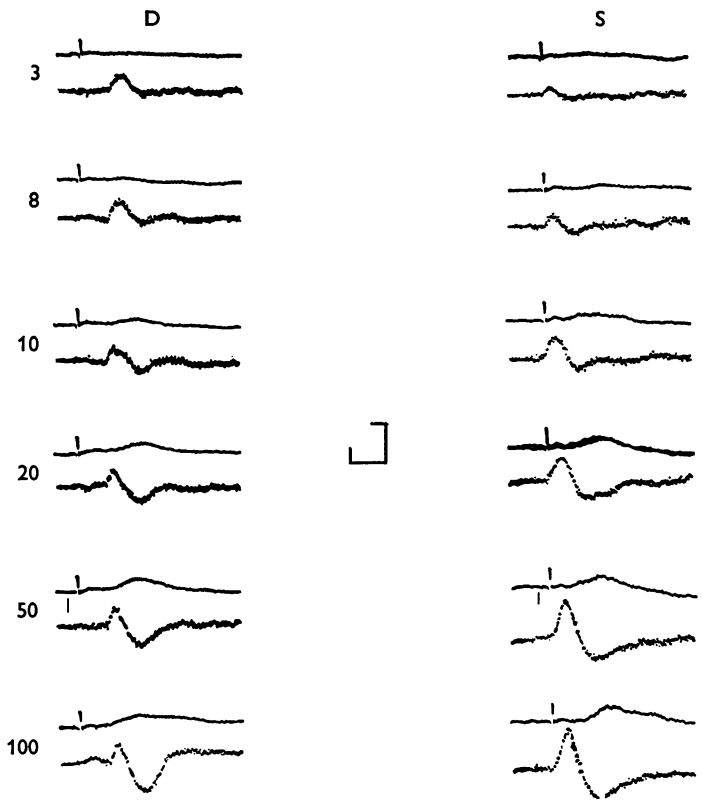

Fig. 1. Left column (D) is from mid collicular decerebrate animal; right column (S) is from same animal after spinalization by section at C1. Stimulation of the gastrocnemius-soleus nerve. The top trace of each pair is recorded from the cord dorsum. The late negative wave is an upward deflexion. The bottom trace is the dorsal root potential recorded from a filament in L6. In this figure and in all others the DRP is the normalized average of 16 or 32 sweeps except where indicated. In this and in all subsequent records the negative DRP is an upward deflexion; the positive DRP is downward. Numbers at left refer to the stimulus strengths in multiples of threshold for the group Ia volley. Stimulus marker for volley only is shown at 50 × group Ia threshold by vertical bar. Time marker is 7 msec for cord dorsum potential, 100 msec for DRP; voltage marker is 80 μV for DRP only. Further discussion in the text.

late negative wave is produced by group III volleys (Bernhard, 1953), it was concluded that activity in the group III fibres but not in group I or group II fibres was responsible for the positive DRP (Mendell, 1970). As the stimulus intensity was raised above 8X group Ia threshold, the amplitudes of the positive DRP and the late negative wave from the cord dorsum were increased but the negative DRP was unchanged.

Following transection of the spinal cord at C1 during temporary ether anaesthesia no DRP was recorded for several minutes during which the ether was blown off by the artificial respiration. After the DRPs reached steady values, a series of identical stimuli was delivered to the gastrocnemius-soleus nerve (Fig. 1S). The group (I+II) negative DRP (3X group I threshold shock) was smaller in the spinal animal than in the decerebrate. This shock produced a very small, long-lasting potential resembling a positive DRP. This was unusual; generally the positive DRP had a well-defined threshold at 7–10X group Ia threshold (Mendell, 1970) which was identical to that for the late negative wave from the cord dorsum (Figs. 1D, 3). Even in Fig. 1S it was clear that a group III volley was necessary to evoke a well defined positive DRP as described by Mendell (1970). As the stimulus intensity was increased above threshold group III strength the negative DRP increased in amplitude to a much greater extent than in the decerebrate state. For a given group III stimulus the positive DRP also was larger in the spinal animal than in the decerebrate. In this animal the enhancement of the positive DRP following spinalization was reflected as a lengthening of its time course; in other preparations an increase in amplitude was observed. An increase in amplitude of the negative DRP V (Lloyd & McIntyre, 1949) and positive DRP VI (Lloyd, 1952) resulting from sural nerve stimulation was also observed following switching from decerebrate to spinal states. Similar results were obtained reversibly by cooling the spinal cord at T12 (Wall, 1967).

Positive DRP and primary afferent hyperpolarization

If the positive DRP results from hyperpolarization of the presynaptic terminals of afferent fibres, it is expected that these terminals should be less excitable by electrical stimulation (Wall, 1958). In order to test this, a stimulating microelectrode was inserted into the spinal cord among fibres of the semitendinosus nerve. These were located by searching for the region from which the maximum antidromic volley recorded monophasically on the peripheral semitendinosus nerve could be evoked. The intensity of the stimulus was then adjusted so that it excited some but not all the group I fibres. The L6 to S2 ventral roots were cut. The height of circles above the base line in Fig. 2 represents the amplitude of the antidromic volley and is therefore a function of the number of fibres stimulated

during the different phases of the negative-positive DRP evoked by a single shock of group III intensity to the gastrocnemius-soleus nerve. During the positive DRP fewer fibres were stimulated indicating a relative hyperpolarization while during the negative DRP the opposite occurred. These results confirm that the late positive DRP was associated with hyperpolarization of presynaptic terminals.

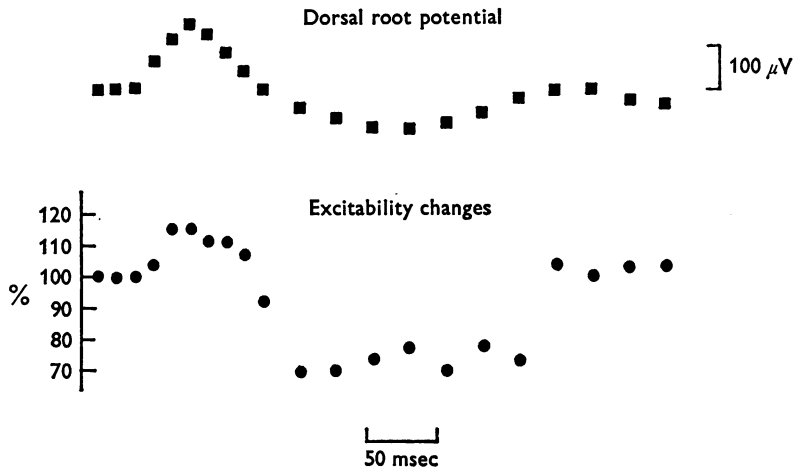

Fig. 2. Excitability testing of group I fibres of the semitendinosus nerve during the DRP evoked by stimulation of the gastrocnemius-soleus nerve at group III strength. Bottom graph (●) plots the height of the monophasic compound action potential recorded from the semitendinosus nerve in response to shocks of constant intensity delivered through a metal microelectrode to the presynaptic terminals of these fibres in the spinal cord at various intervals following the conditioning stimulus to the gastrocnemius-soleus nerve. It is observed that the heights of these potentials change systematically according to the level of the DRP at the time that they are evoked.

A more direct way to examine the association of the positive DRP with presynaptic hyperpolarization is to explore whether stimuli which evoke the positive DRP also produce primary afferent hyperpolarization (PAH). In Fig. 3 the DRPs produced by stimulation of the G-S nerve are compared with the changes in polarization recorded intracellularly from a single group I afferent fibre of the PBST nerve in response to the same stimuli. In the intracellular records (PAD-PAH) the transmembrane potential change is obtained by subtracting the field potential recorded extracellularly (below) from the intracellular potential. In row *A* a stimulus of maximal group I and group II strength but below group III threshold evokes only a negative DRP and a primary afferent depolariza-

tion (PAD). In row *B* the stimulus strength is increased to excite the group III fibres as shown by the appearance of the late negative wave (arrow) in the CDP record. A positive DRP now follows the negative DRP and this has the same threshold as the PAH in the intracellular record. A further increase in stimulus strength (row *C*) increases the amplitude of the negative DRP. The positive DRP becomes larger both in amplitude and in duration. PAH is increased in the single fibre but PAD is virtually unchanged (subtracting extracellular from intracellular, but see Discussion) reflecting the small contribution of group III volleys to PAD in group I afferent fibres (Eccles, Magni & Willis, 1962).

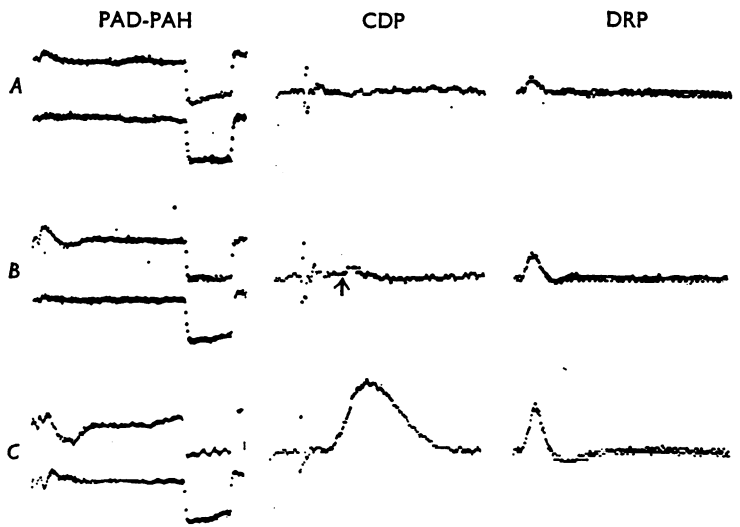

Fig. 3. Association of the positive DRP with PAH. In PAD-PAH column each pair of records displays the intracellular record (top) and extracellular record (bottom) from a PBST group I afferent fibre (AP = 90) in response to single shock stimulation of the G-S nerve at three different strengths. The transmembrane potential is obtained by subtraction of the extracellular record from the intracellular record. The square pulse at the end of each record is 500 μV, 100 msec. In the CDP column each record displays the cord dorsum potential obtained from a monopolar lead on the cord dorsum of L7 (as in Fig. 1) in response to the same stimuli as in the PAD-PAH column and the DRP column. The threshold of the late negative wave reveals that the shock strength in the top record is below group III threshold whereas the shock strength in the middle row is just above group III threshold (arrow denotes late negative wave). The DRP column displays the DRPs recorded from L6 in response to these same shocks. Further discussion in text. All records averaged sixteen times. Calibration pulse in PAD-PAH column represents 100 msec in DRP column and 10 msec in CDP column.

Mechanism for presynaptic hyperpolarization

It has been proposed that the positive DRP results from inhibition of tonic activity of interneurones mediating the negative DRP (Wall, 1964; Lundberg & Vyklický, 1966). If this is so, the amplitude of a test negative DRP should be diminished during a conditioning positive DRP. In Fig. 4*A* the conditioning stimulus is a single shock of group III intensity to the PBST nerve. A test shock of below group III intensity is delivered to the G-S nerve and it evokes only a negative DRP (Fig. 4*B*). In Fig. 4*C* these

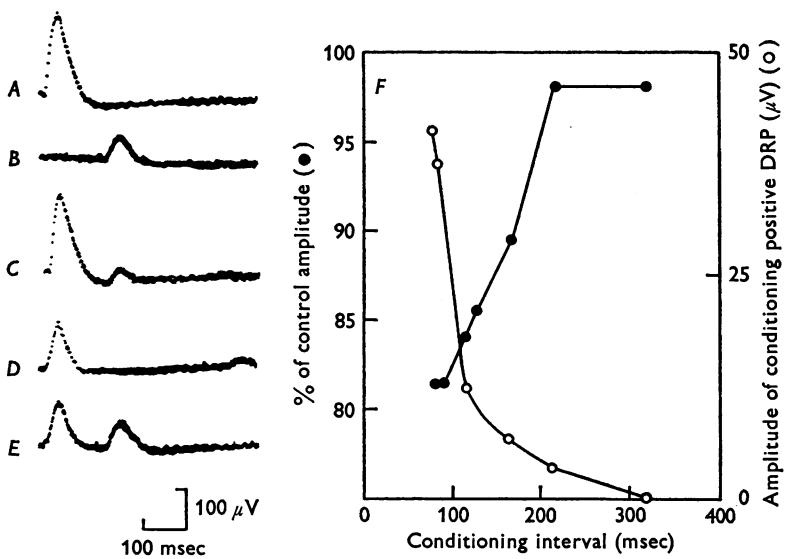

Fig. 4. Depression of a test negative DRP during a conditioning positive DRP. *A*, *B*, *C*, *D*, *E*: averaged records of DRPs recorded from S2 dorsal-root filament. Explanation in text. *F*: time course of positive DRP from L6 beginning 80 msec following conditioning shock at Group (I+II+III) strength to PBST nerve and amount of depression of a test negative DRP during this positive DRP. The conditioning negative DRP and its effect on the test negative DRP is not shown. Further details in text.

stimuli are presented to both nerves with the conditioning PBST nerve being stimulated about 175 msec before the test G-S nerve. The test negative DRP occurs during the conditioning positive DRP, and it is depressed to about 50% of the control value. For these studies the conditioning interval must be at least 80 msec to avoid contamination from the negative DRP produced by the conditioning stimulus. In Fig. 4*D* a 200/sec burst of 4 shocks at below group III intensity delivered to the PBST nerve results in only a negative DRP. In Fig. 4*E* it is shown that

this conditioning shock does not depress the amplitude of the same test negative DRP as in Fig. 4*B*. It is concluded that the depression of the test negative DRP is associated with the positive DRP rather than with the preceding negative DRP. In Fig. 4*F* the time course of the depression of a test negative DRP during a conditioning positive DRP is compared to the time course of the positive DRP itself. Both the conditioning and test DRPs are evoked by group III intensity shocks to the PBST nerve and the delay between them is varied. The time course of the depression roughly parallels that of the positive DRP except at long intervals (320 msec) where a small depression is consistently observed (4 %) in the absence of a positive DRP. This may reflect the lack of sensitivity of the positive DRP as a measure of presynaptic hyperpolarization.

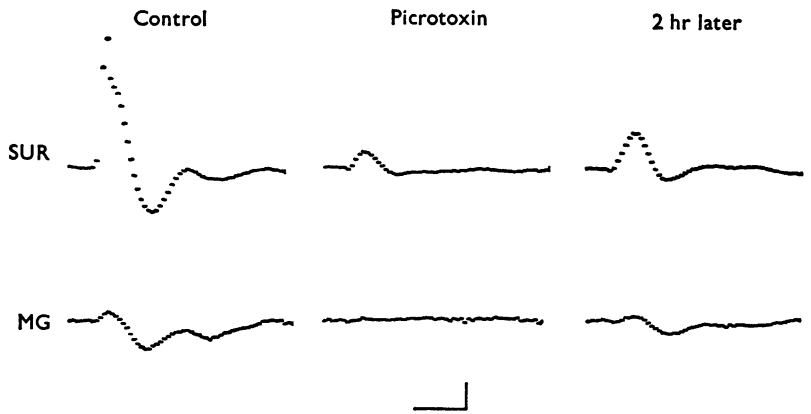

Fig. 5. Action of picrotoxin on the positive DRP. Top row: DRPs produced by stimulation of the sural nerve at maximal A strength. Below: stimulation of the medial gastrocnemius nerve at group III strength. Left, high spinal, unanaesthetized animal. Centre: after administration of picrotoxin at 1·7 mg/kg. Right: 2 hr later. Calibration: 50 μV, 100 msec.

In order to further test the hypothesis that the positive DRP results from inhibition of the interneurones mediating the negative DRP, the action of picrotoxin on the positive DRP has been studied. Picrotoxin has been shown to block the negative DRP (Eccles, Schmidt & Willis, 1963*c*) and its action has been postulated to be exerted at the axo-axonal synapse made by the interneurones on the primary afferent fibres (Eccles *et al.* 1963*c*; Davidoff, 1972). According to the above hypothesis, picrotoxin should block the positive DRP in addition to the negative DRP. In Fig. 5 the upper row displays the DRPs produced by sural nerve stimulation before, immediately after picrotoxin administration (1·7 mg/kg), and 2 hr later. Note that picrotoxin depresses the amplitude of both the positive

and the negative DRPs and that they both begin to recover after 2 hr. The same pattern is observed with the DRPs produced by stimulation of the medial gastrocnemius nerve (bottom row).

Trains of stimuli

Trains of stimuli have been delivered to muscle nerves and the effect of varying the frequency on the DRP has been observed. Fig. 6 demonstrates the results of a typical experiment in which trains of shocks either below or above group III strength are delivered to the G-S nerve at 20/sec and 200/sec. A 20/sec train of stimuli below group III intensity produces

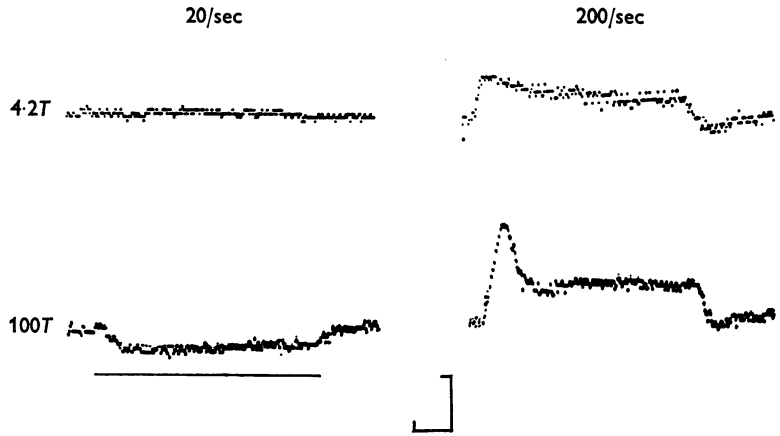

Fig. 6. DRPs evoked by stimulus trains at 20/sec and 200/sec to the gastrocnemius-soleus nerve. Train length is indicated by horizontal line under first column. Negative DRP is an upward deflexion, positive DRP is a downward deflexion.

Top row: stimulus intensity at 4·2 × group Ia threshold is adequate to stimulate group I and group II afferent fibres.

Bottom row: stimulus intensity 100 × group Ia threshold is adequate to stimulate group I, group II and group III afferent fibres. Calibration: 50 μV, 120 msec.

little or no DRP reflecting the small DRP observed after single shocks of this strength. When the frequency of the train is raised to 200/sec, a steady negative DRP is observed. When group III fibres are also stimulated, a 20/sec train of stimuli results in a steady positive DRP which is often preceded by a transient negativity. Trains of group III stimuli at 200/sec evoke a large transient negative DRP which adapts to a smaller steady negative DRP. Comparison with the DRPs produced by 200/sec trains just below group III threshold reveals that the chief effect of the group III 200/sec train is a transient negative DRP; the steady negativity results mainly from activity in peripheral group I and group II fibres.

The experiment of Fig. 6 raises the question of whether the positive and negative DRPs evoked by group (I + II + III) stimuli at different frequencies represent the responses of a single set of afferent fibres or of two groups of afferent fibres, one which depolarizes in response to high-frequency peripheral stimulation and the other which hyperpolarizes in response to lower frequency stimulation. In the experiment of Fig. 7 intrafibre recording from a PBST group I afferent fibre reveals that single shock

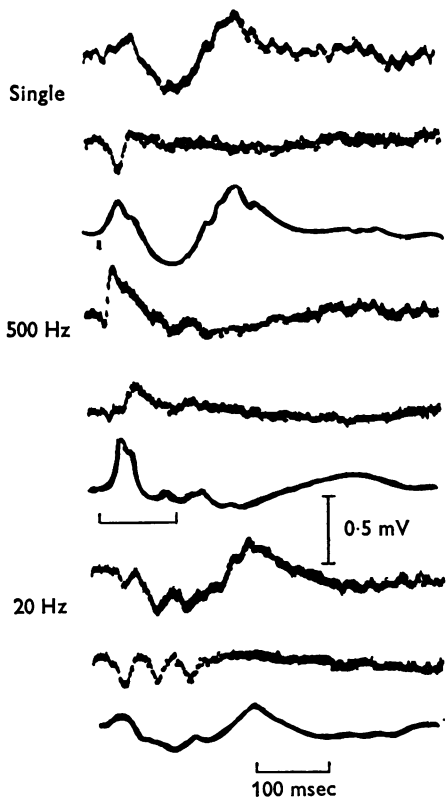

Fig. 7. Effect of frequency of stimulation on the response of a single afferent fibre. PBST afferent fibre, AP = 90 mV. Stimulation of G-S nerve at group (I + II + III) strength using a single shock (Single), a 100 msec train at 500 Hz (500 Hz), and a 100 msec train at 20 Hz (20 Hz). Each group of three traces represents the intracellular record (top), the extracellular record in response to the same stimulus (middle), and the transmembrane potential obtained by graphical subtraction of the extracellular record from the intracellular record (bottom). The vertical line under the transmembrane potential in response to Single marks the approximate location of the stimulus. The horizontal bar under the transmembrane potential in response to 500 Hz denotes the time and duration of the train for 20 Hz and 500 Hz.

stimulation of the G-S nerve at group (I + II + III) strength results in a brief PAD of 215 μV (but, see Discussion) followed by a longer lasting PAH of 250 μV amplitude. A 340 μV PAD is observed following the PAH; this late PAD is occasionally observed both in intrafibre and in DRP recording. During a 100 msec train of shocks at 500 Hz at the same intensity, a transient PAD of 350 μV amplitude is observed. This is succeeded by a small PAH (50 μV) which lasts until the stimulus train is ended. When 3 shocks at 20 Hz (100 msec train) of the same intensity are delivered to the G-S nerve, a very brief PAD gives way to a steady PAH of maximum amplitude 170 μV during the train. The second and third shocks of the train evoke much smaller PADs than the first shock although these probably prevent the PAH from attaining the same amplitude observed with single shock stimulation. At the end of the train the PAH declines and a PAD is observed similar to that following a single shock. The results of this experiment suggest that the response of a passive afferent fibre during trains of stimuli to a peripheral nerve depends very much on the stimulus frequency; PAD is brought out by high frequencies, and PAH by low frequencies.

Variability of the DRP

In most experiments the DRP has been recorded from a single DR filament (usually caudal L 6) throughout the experiment. It is known that the negative DRP is largest in filaments closest to the entry zone of the stimulated root and is very much smaller in filaments two segments away (Barron & Matthews, 1938; Eccles *et al.* 1962). In order to examine the variability of the positive DRP, a number of small DR filaments have been cut and their DRPs recorded. These experiments have revealed that the filament displaying the maximum positive DRP is usually different from the one showing the maximum negative DRP. For example, in Fig. 8 group (I + II + III) stimulation of the G-S nerve which enters through caudal L 7 and rostral S 1 segments produces large negative DRPs in L 7 and S 1 and much smaller ones in L 6 and L 5. The positive DRPs evoked by the same stimulus are largest in L 5 and L 6, absent in S 1 and very small in L 7. A similar pattern is observed for the DRPs produced by maximal stimulation of sural A fibres (i.e. Aβ + Aδ) (Fig. 8). In this preparation stimulation of the peripheral C fibres in the sural nerve evokes a distinct late positive DRP. This late wave has a latency of 160 msec which, allowing for a central delay of 25 msec for the positive DRP (Mendell, 1970) and a conduction distance of 175 mm indicates a peripheral conduction velocity of 1·3 m/sec which is well within the range of conduction velocity for C fibres (Gasser, 1950). This late positive DRP appears to have the same distribution as the positive DRP of shorter latency produced by

G-S and sural stimulation, being absent in S1 and comparatively large at more rostral levels. Fig. 8 also illustrates the general though not universal finding that the amplitudes of the negative and positive DRPs tend to be reciprocally related. Also, for the stimuli used, i.e. sural, PBST, and G-S, the positive DRP tends to be larger in more rostral segments (L6 and L5) than in more caudal segments (L7 and S1). This may be the consequence of the distribution of the negative DRP which is larger in L7 and S1 than in L5 and L6 for these stimuli. Occasional exceptions to these generalizations are observed, e.g. large negative and positive DRPs in an S2 filament with no positive DRP in L6 or L5. In some preparations a search of L5, L6, L7, S1 and S2 filaments yields none which exhibit a positive DRP in response to stimulation of the usual array of limb nerves.

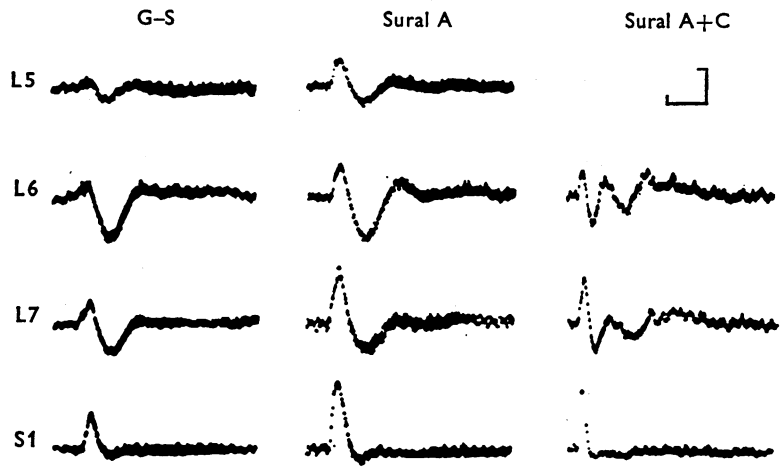

Fig. 8. DRPs evoked in different DR filaments in the same preparation by fixed stimuli. Calibration 100 μV; 100 msec (G-S and sural A), 200 msec (sural A+C).

Cutaneous evoked PAH

Fig. 8 demonstrates that positive DRPs are evoked by volleys in both myelinated and non-myelinated fibres of the sural nerve. In Fig. 9 it is shown that these volleys produce PAH in primary afferent fibres. The response of a PBST group I fibre to volleys in sural myelinated fibres is shown in Fig. 9–1 through Fig. 9–4. In Fig. 9–1 it is seen that a volley in the large Aβ fibres results in a small PAD (obtained by subtracting the EC from the IC record, but see Discussion) followed by a small PAH. The amplitude and duration of this PAH is increased as the stimulus intensity is raised to stimulate more Aβ fibres (Fig. 9–2) and the Aδ fibres (Fig. 9–3 and 9–4). In Fig. 9–2 it is difficult to rule out a small contribution from Aδ fibres which may be obscured by the diphasic Aβ volley. It seems certain,

however, that activity in both the Aβ and the Aδ fibres is responsible for PAH in this primary afferent fibre. When the stimulus strength is increased to excite the non-myelinated fibres (Fig. 9–6 to 9–8), later components of PAH are observed (C-PAH). There is little change in the initial PAH wave resulting from stimulation of the myelinated afferents (taking account of the change in sweep speeds). In Fig. 9–6 it is seen that a small volley in C fibres (arrow) does not evoke a late PAH; the C-PAH requires activation of either a particular group of C fibres or of a certain minimum

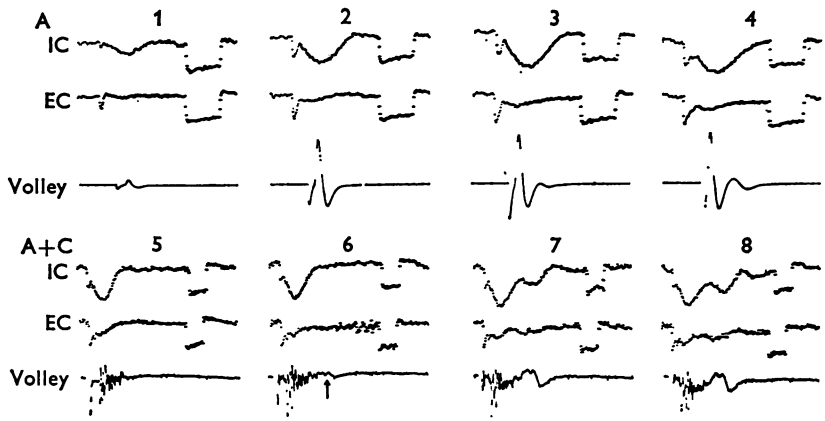

Fig. 9. PAH evoked by stimulation of the sural nerve. PBST group I afferent fibre. Action potential 80 mV. Stimulation of sural nerve at increasing strengths (1 to 8). Each numbered group of three traces (1 to 8) consists of an averaged intracellular record (IC), an averaged extracellular record (EC) and the volley recorded from the sural nerve (Volley). The conduction distance for the volley record is about 30 mm. In 1, 2, 3, 4, the calibration pulse is 500 μV, 100 msec for the averaged IC and EC records. The same pulse gives a 4 msec calibration for the volley. In 5, 6, 7, 8 the calibration pulse is also 500 μV, 100 msec for the averaged IC and EC records but 10 msec for the volley. The initial upward deflexion of the A component in the volley traces of 5, 6, 7 and 8 is too faint to be seen clearly and has been eliminated. The complicated deflexions in these traces before the smooth C wave (arrow in 6) represent repetitive firing of the A fibres and possibly a dorsal root reflex.

number of C fibres which is reached in Fig. 9–7. A further slight increase in stimulus intensity results in a second late component of PAH (Fig. 9–8). The latency of the C-PAH is about 215 msec, which is longer than the estimated 190 msec conduction time from popliteal fossa to spinal cord. This suggests a long central delay (25 msec) for C-PAH which is consistent with the delays found for PAH produced by group III afferents of muscle nerves (Mendell, 1970).

Distribution of PAD and PAH

In nine preparations intrafibre records have been obtained from a variety of afferent fibres. The aim of these experiments has been to establish patterns of projection from various afferent systems on to different afferent fibres particularly with respect to PAH. The responses of thirty-six units to stimulation of the sural, G-S and PBST nerves have been examined. The stimuli have been at maximal A fibre strength for the sural nerve and at a level of group III stimulation to PBST and G-S nerves sufficient to produce a maximal late negative wave on the cord dorsum

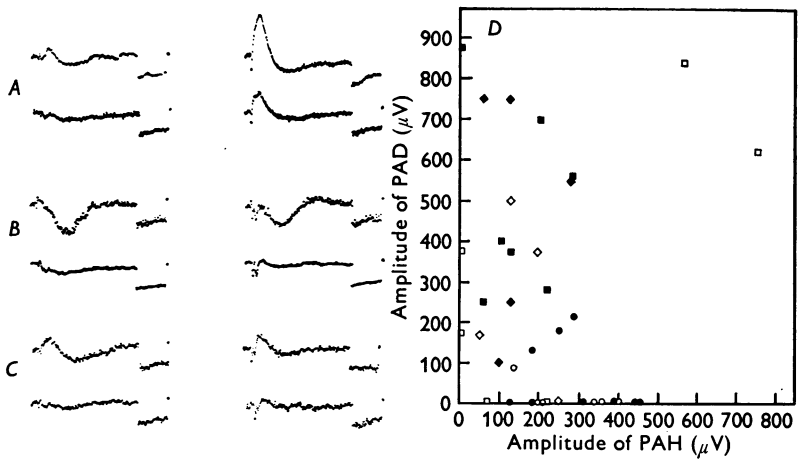

Fig. 10. Responses of primary afferent units to stimulation of different limb nerves. Stimuli as discussed in the text. *A*, *B*, *C*: three afferent units from the same preparation. *A:* sural unit in response to G-S stimulation (left) and PBST stimulation (right). *B:* PBST group I unit in response to G-S stimulation (left) and sural stimulation (right). *C:* PBST group I unit in response to G-S stimulation (left) and sural stimulation (right). Calibration pulse 500 μV, 100 msec. *D:* responses of twenty-one identified units (PBST group I, G-S group I and sural Aβ) to stimulation of peripheral nerves. ●, record PBST, stimulate G-S; ○, record PBST, stimulate sural; ■, record G-S, stimulate PBST; □, record G-S, stimulate sural; ◆, record sural, stimulate PBST; ◇, record sural, stimulate G-S.

(cf. Fig. 1). Of the thirty-six units, twenty-eight have developed PAH in response to stimulation of one or more nerves whereas eight have not hyperpolarized in response to the stimuli used. Examining the responses of individual fibres to stimulation of the afferent nerves, one observes a wide variety of responses ranging from PAD with no PAH to the exact opposite, i.e. PAH with no PAD (Fig. 10*D*). These extremes can be observed in the same preparation in two different units in response to the

same stimulus. A total of sixty responses have been examined in the thirty-six units of the population under consideration; of these sixteen are pure PAD (i.e. PAH = 0) and eighteen are pure PAH. The remaining twenty-five responses are quite varied but there are only eight responses which consist of a large PAD and large PAH where 'large' is arbitrarily defined as greater than 200 μV. A similar tendency has been indicated for DRPs, examples of which are shown in Fig. 8.

In Fig. 10*A*, *B* and *C* the responses of three afferent fibres from the same preparation are displayed. In *A* a fibre identified as a sural unit with Aβ conduction velocity shows a small PAD (170 μV) and a very small PAH (50 μV) in response to stimulation of maximal group III intensity of the gastrocnemius-soleus nerve (left) whereas maximal group III stimulation of the PBST nerve gives a much larger PAD (550 μV) and PAH (275 μV) (right). In *B* the same intensity stimulus to the gastrocnemius-soleus nerve evokes only PAH (455 μV) from a PBST group I fibre; the sural maximal group *A* fibre stimulus (right) also evokes pure PAH (360 μV). The central delay for PAH is about 15–20 msec for the gastrocnemius-soleus stimulus and somewhat longer for the sural stimulus. Both of these are comparable to the central delay for the positive DRP (Mendell, 1970). Row *C* is from another group I PBST fibre, and the same group III gastrocnemius-soleus stimulus as above evokes PAD of 135 μV and PAH of 180 μV (left) whereas the same sural stimulus as in *B* produces PAD of 90 μV and PAH of 135 μV (right).

PAH has been observed in group I afferents from PBST and G-S as well as in cutaneous afferent fibres (Fig. 10). Fig. 10*D* gives in detail the responses of identified sural Aβ, PBST group I and G-S group I fibres to maximal stimulation of myelinated fibres of various nerves. PBST units tend to develop PAH > PAD, sural units generate PAD > PAH and G-S units produce PAD > PAH or PAH > PAD in response to single shocks of sufficient strength to stimulate all myelinated fibres in various peripheral nerves. It is difficult to interpret these results from a functional point of view because of the heterogeneity of the stimulating nerves, particularly in the case of the muscle nerves. Another problem is the wide variability in the results making statistical comparison difficult. It is known, however, that PAD in muscle group I afferents is evoked mainly by muscle group I volleys and not by volleys in the myelinated flexor reflex afferents (FRA), i.e. muscle group II and group III fibres and cutaneous fibres (Eccles, Magni & Willis, 1962). PAD in cutaneous afferents is evoked mainly by FRA volleys and not by group I muscle volleys (Eccles, Schmidt & Willis, 1963*b*). Combining this information with that of Fig. 10*D* reveals that FRA stimulation produces mainly PAH in PBST group I afferents and primarily PAD in cutaneous afferents. The situation

in G-S group I afferents is mixed with cutaneous FRA volleys evoking PAD > PAH in most cases whereas FRA volleys from PBST produce PAH > PAD. This difference in the response of PBST and G-S group I fibres to sural nerve volleys might be accounted for by a larger percentage of group Ib fibres in the G-S population than in the PBST population (Eccles, Magni & Willis, 1962). Alternatively G-S units may be intermediate between sural and PBST units in their response to single FRA volleys. At this time no choice can be made between these alternatives.

DISCUSSION

The present results confirm the association of the positive DRP with hyperpolarization of presynaptic terminals (PAH). The evidence is twofold: first, during the positive DRP a decrease in excitability of intraspinal sensory axons is demonstrated, and secondly, PAH can be recorded intracellularly from preterminal sensory axons. The intracellularly recorded PAH has the same threshold as the positive DRP both being activated by volleys in muscle group III fibres. Furthermore, PAH has a central delay of about 25 msec similar to that for the positive DRP (Mendell, 1970). Finally the duration of PAH in response to a single shock of group III strength is of the order of 100 msec which is similar to that of the positive DRP evoked by the same shock intensity.

The extracellular fields associated with PAH are negative but smaller in amplitude than intracellularly measured PAH. These recordings have been made within 1 mm of the cord dorsum which is at some distance from the terminals of these afferents. By analogy with PAD (Eccles, Magni & Willis, 1962) it can be argued that PAH is generated in the axon membrane at some distance from the recording electrode presumably near the terminals; the field potential represents extracellular current flow from this region to the electrode locus. Systematic field analysis is necessary to prove this point. Because the extracellular potential is always smaller than the intracellular potential it seems unlikely that PAH is a passive phenomenon resulting from currents generated by activity in neurones or glia. The association between glial potentials and DRPs is unlikely (Somjen, 1970). The short latency potentials of Figs. 3, 7 and 9 have been scored as PAD on the formal basis of graphical subtraction. Since the extracellular fields are larger than the intracellular ones, this PAD may result passively from currents generated by other structures. This possible over-estimate of 'active' PAD does not alter the conclusions concerning the differences in response of PBST group I units and cutaneous units to stimulation of the FRA.

PAH is evoked by activity in group III fibres of muscle nerves but not

by activity in proprioceptive group I and group II fibres. Stimulation of C fibres of muscle nerves has been shown to evoke PAH (Mendell, 1970). Cutaneous sural nerve volleys in Aδ and C fibres evoke positive DRPs and PAH (Figs. 8 and 9); evidence has also been presented that volleys in large cutaneous afferents may produce PAH (Fig. 9-2) as reported by Hodge (1972). The common feature of these afferents is that their stimulation evokes the flexion reflex (Lloyd, 1943; Hagbarth & Naess, 1950); the group II fibres also fit this classification but their activity results in PAD, not PAH. Under certain conditions stimulation of fibre groups giving PAH in these experiments can also evoke a negative DRP and PAD (Franz & Iggo, 1968; Zimmermann, 1968; Jänig & Zimmermann, 1971). The frequency of peripheral stimulation may be important in determining whether PAD or PAH will be the dominant effect (Figs. 6 and 7). The origin of this frequency dependence is at present unknown but it is consistent with the previous observation that the second of two negative DRPs is potentiated at conditioning intervals below 20 msec and depressed at intervals above 20 msec (Eccles, Schmidt & Willis, 1963*a*). Similar frequency dependence has been observed with supraspinal stimulation (Lundberg & Vyklický, 1966). Therefore it may be premature to associate the negative or positive DRP with activity in any group of afferent fibres (Mendell & Wall, 1964; Franz & Iggo, 1968; Zimmermann, 1968; Burke, Rudomin, Vylický & Zajac, 1971).

The increase in the group III muscle and cutaneous evoked positive and negative DRPs after switching from a decerebrate to a spinal preparation indicates a supraspinal control over these spinal DRP mechanisms (Carpenter, Engberg, Funkenstein & Lundberg, 1963). The occasional failure to observe any increase in the amplitude of the positive DRP following spinalization (Fig. 1) may result because this component of the positive DRP is already maximal in the decerebrate state. Under these conditions the potentiation of the positive DRP would be manifested only as an increase in duration (Fig. 1) which is equivalent to an increase in the late component of the positive DRP (Figs. 2 and 9). The potentiation of the negative DRP following spinalization (Fig. 1) might also prevent potentiation of the positive DRP through some complex interaction of a negative DRP on a subsequent positive DRP (see below).

Several investigators have shown depression of a test negative DRP during and following a conditioning negative DRP (Eccles, Kostyuk & Schmidt, 1963; Jänig & Zimmermann, 1971). During the conditioning DRP, occlusion with the test negative DRP or presynaptic inhibition of the afferent pathway evoking the test negative DRP have been postulated to account for the decrease in amplitude of the test negative DRP (Eccles, Kostyuk & Schmidt, 1963). In the anaesthetized animal depression of the

test negative DRP is observed for some time following the conditioning negative DRP, and it has been proposed that this reflects long lasting homosynaptic depression (Curtis & Eccles, 1960) in some interneuronal element common to both the conditioning and the test negative DRP (Eccles, Kostyuk & Schmidt, 1963). This assumes that the negative DRP is mediated by interneurones (Eccles, Kostyuk & Schmidt, 1962; Wall, 1962; Rudomin, 1966). The present studies on unanaesthetized animals indicate that this depression which occurs following the conditioning

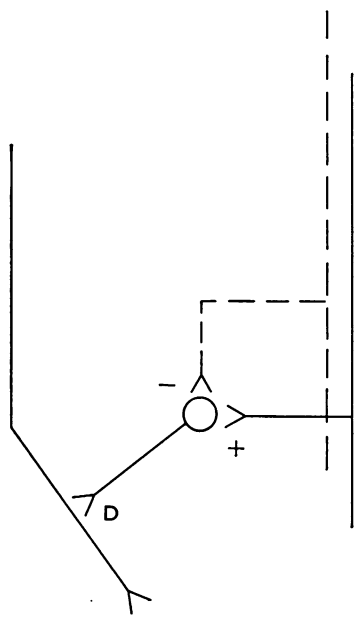

Fig. 11. Schematic diagram of a possible neuronal mechanism for the positive DRP. The solid interneurone has only a depolarizing action (D) on the presynaptic terminal (left). Its activity can be increased by its solid (+) input (negative DRP) and decreased by its dashed (−) input (positive DRP). The interneurone is meant to represent the action of a large internuncial chain.

negative DRP may be associated with the positive DRP (Fig. 5). It has been proposed (Mendell & Wall, 1964; Wall, 1964; Lundberg & Vyklický, 1966) that the positive DRP results from inhibition of tonically active interneurones mediating the negative DRP (Fig. 11) and this could account for the depression of a test negative DRP (Lund, Lundberg & Vyklický, 1965). In the anaesthetized animal these interneurones would not discharge spontaneously and so their inhibition would not result in a positive DRP (Wall, 1964); a test negative DRP occurring during this inhibition would

still be depressed. The action of picrotoxin in blocking both the negative and positive DRPs is quite consistent with the view that PAH is inhibition of interneurones which generate PAD. Picrotoxin is believed to block the axo-axonal depolarizing synapse (Eccles, Schmidt & Willis, 1963*c*; Davidoff, 1972), and so a block at this synapse would depress both the negative and positive DRP. Although the results of these experiments are consistent with current widely accepted interneuronal mechanisms for PAD and PAH, they cannot be said to prove the validity of these models. No firm conclusions concerning the neuronal mechanism responsible for PAH and the action of various drugs on this system can be made until further data is available concerning the identity, the properties and the connexions of the interneurones which are presumed to mediate these actions.

Since the time course latency and threshold of PAD and PAH in different fibres match those of the negative and positive DRPs evoked by the same stimulus, it is likely that the sum of the extracellular currents resulting from PAD and PAH of the constituent fibres of a filament contribute at least a major share of the DRP recorded from that filament (Lloyd & McIntyre, 1949). There is no direct evidence that other processes might not contribute to the DRP. The chief variability in PAD and PAH in different fibres in response to a given stimulus concerns the relative amplitudes of the components, not their time courses (Fig. 10); the same is true for the negative and positive DRPs in different dorsal root filaments (Fig. 8). Thus the DRP provides a good indication of the time course of PAD and PAH in different afferent fibres but not of the relative amplitudes of PAD and PAH for any fibre in that filament. The inverse relationship between the amplitude of the PAD and PAH and also between the negative and positive DRPs indicates the possibility that PAD diminishes the amplitude of a subsequent PAH similar to the effect that PAD evoked by A fibres has on the PAD evoked by C fibres (Jänig & Zimmermann, 1971). Since exceptions to this generalization are observed both for the DRP and PAD-PAH, this hypothesis seems unlikely. An alternate possibility is that the amplitudes of PAD and PAH are independent but vary in a systematic way along the rostro-caudal extent of the cord (Fig. 8). The variability in the ratio of negative and positive DRPs emphasizes the necessity for recording from several dorsal rootlets throughout the lumbar enlargement before concluding whether or not a preparation develops a positive DRP.

The absence of positive DRPs from some preparations suggests that the amount of PAD and PAH produced in an afferent fibre is variable and that the presynaptic hyperpolarization is evoked only in preparations in which a certain 'functional state' exists in the spinal cord. In accord with

the model of Fig. 11 one might speculate that such a 'state' is the presence of tonic activity in interneurones mediating the negative DRP (Wall, 1964).

The absence of PAD from some units in response to maximal group (I+II+III) volleys in muscle nerves is of interest because it has been previously shown (Eccles, Magni & Willis, 1962) that group I volleys on the average evoke PAD in group Ia and group Ib afferent fibres. However, it must be noted that the relative contribution of various muscle afferent systems to PAD has been assessed from the effects of high frequency bursts of afferent volleys which greatly potentiate PAD (Eccles, Magni & Willis, 1962). Single shock stimulation at group I strength evokes little or no PAD in many afferent fibres (Eccles, Magni & Willis, 1962), and since the graph of Fig. 10 is made up exclusively of results from single shocks, there is no conflict on this issue.

The classification of afferent units based on the relative amounts of PAD and PAH evoked in different kinds of afferent fibres (Fig. 10*D*) is valid only for single shocks to the peripheral nerves at maximal A fibre strength for the sural nerve and at group III intensity for the G-S and PBST nerves. A different picture might emerge at stimulus intensities which are just above threshold for group III fibres (Fig. 3). Furthermore bursts of shocks at 20/sec or at 200/sec might favour the appearance of PAH or PAD respectively (Fig. 7). Natural activation of the receptors associated with skin afferents and group III muscle afferents might also alter the classification resulting from Fig. 10. Finally, the choice of stimulating nerves may have been a fortunate one; activation of other nerves may produce PAD-PAH responses which are very different from those evoked by stimulation of sural, G-S and PBST. With single shock stimuli a trend is observed for muscle group I afferents, particularly from PBST rather than from G-S in these experiments, to develop PAH which is large compared to PAD in response to stimulation of the FRA. Cutaneous units develop PAD which is large with respect to PAH in response to single shocks to the FRA of both muscle (Fig. 10) and skin (Hodge, 1972). It is emphasized that nothing can yet be said concerning the absolute values of PAH in different kinds of afferent fibres but only that the relative amounts of PAD and PAH vary systematically in response to FRA stimulation.

These experiments confirm that stimulation of flexor reflex afferents can evoke PAH. The phenomenon of PAH has been used by Melzack and Wall (1965) as an element in a 'gate' theory for pain perception. Contrary to the expectations of that theory these experiments have revealed that FRA stimulation evokes mainly PAH in presynaptic terminals of proprioceptive afferents and PAD in presynaptic terminals of cutaneous afferents. Assuming that PAH is related to presynaptic facilitation, stimulation of

the FRA potentiates the effects of proprioceptors involved in the subsequent motor response rather than the effects of the FRA afferents themselves as implied by the theory of Melzack & Wall (1965). This suggests that the PAH phenomenon may not be related to pain perception as proposed by these authors. These conclusions must be tentative until more is known concerning the effects of natural stimulation on the polarization of presynaptic terminals of both large and small afferent fibres.

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REFERENCES

ANDEN, N. E., JUKES, M. G. M., LUNDBERG, A. & VYKLICKÝ, L. (1966). The effects of DOPA on the spinal cord. 3. Depolarization evoked in the central terminals of ipsilateral IA afferents by volleys in the flexor reflex afferents. *Acta physiol. scand.* **68**, 322–336.

BARRON, D. H. & MATTHEWS, B. H. C. (1938). The interpretation of potential changes in the spinal cord. *J. Physiol.* **92**, 276–321.

BERNHARD, C. G. (1953). The spinal cord potentials in leads from the cord dorsum, in relation to peripheral source of afferent stimulation. *Acta physiol. scand.* **29**, suppl. 106, 1–29.

BURKE, R. E., RUDOMIN, P., VYKLICKÝ, L. & ZAJAC, F. E. III (1971). Primary afferent depolarization and flexion reflexes produced by radiant heat stimulation of the skin. *J. Physiol.* **214**, 185–214.

CANGIANO, A., COOK, W. A., JR & POMPEIANO, O. (1969). Cerebellar inhibitory control of the vestibular reflex pathways to primary afferents. *Archs ital. Biol.* **197**, 341–364.

CARPENTER, D., ENGBERG, I., FUNKENSTEIN, H. & LUNDBERG, A. (1963). Decerebrate control of reflexes to primary afferents. *Acta physiol. scand.* **59**, 424–437.

CHAN, S. H. H. & BARNES, C. D. (1971). Presynaptic facilitation: positive dorsal root potentials evoked from brain stem reticular formation in lumbar cord. *Brain Res.* **28**, 176–179.

CURTIS, D. R. & ECCLES, J. C. (1960). Synaptic action during and after repetitive stimulation. *J. Physiol.* **150**, 374–398.

DAVIDOFF, R. A. (1972). Gamma-aminobutyric acid antagonism and presynaptic inhibition in the frog spinal cord. *Science, N.Y.* **175**, 331–333.

DAWSON, G. D., MERRILL, E. G. & WALL, P. D. (1970). Dorsal root potentials produced by stimulation of fine afferents. *Science, N.Y.* **161**, 1385–1387.

ECCLES, J. C., ECCLES, R. M. & MAGNI, F. (1961). Central inhibitory action attributable to presynaptic depolarization produced by muscle afferent volleys. *J. Physiol.* **159**, 147–166.

ECCLES, J. C., KOSTYUK, P. G. & SCHMIDT, R. F. (1962). Central pathways responsible for depolarization of primary afferent fibres. *J. Physiol.* **161**, 237–257.

ECCLES, J. C., KOSTYUK, P. G. & SCHMIDT, R. F. (1963). Presynaptic inhibition of the central actions of flexor reflex afferents. *J. Physiol.* **161**, 258–281.

ECCLES, J. C. & KRNJEVIĆ, K. (1959). Potential changes recorded inside primary afferent fibres within the spinal cord. *J. Physiol.* **149**, 274–287.

ECCLES, J. C., MAGNI, F. & WILLIS, W. D. (1962). Depolarization of central terminals of group I afferent fibres from muscle. *J. Physiol.* **160**, 62–93.

ECCLES, J. C., SCHMIDT, R. F. & WILLIS, W. D. (1962). Presynaptic inhibition of the spinal monosynaptic reflex pathway. *J. Physiol.* **161**, 282–297.

ECCLES, J. C., SCHMIDT, R. F. & WILLIS, W. D. (1963*a*). The location and the mode of action of the presynaptic inhibitory pathways on the group I afferent fibres from muscle. *J. Neurophysiol.* **26**, 506–522.

ECCLES, J. C., SCHMIDT, R. F. & WILLIS, W. D. (1963*b*). Depolarization of the central terminals of cutaneous afferent fibres. *J. Neurophysiol.* **26**, 646–661.

ECCLES, J. C., SCHMIDT, R. F. & WILLIS, W. D. (1963*c*). Pharmacological studies on presynaptic inhibition. *J. Physiol.* **168**, 500–530.

FRANZ, D. N. & IGGO, A. (1968). Dorsal root potentials and ventral root reflexes evoked by non-myelinated fibres. *Science, N.Y.* **162**, 1140–1142.

GASSER, H. S. (1950). Unmedullated fibres originating in dorsal root ganglia. *J. gen. Physiol.* **33**, 651–690.

HAGBARTH, K. E. & NAESS, K. (1950). Reflex effects of tetanic stimulation of different afferent fibre systems in the hindlimb of the cat. *Acta physiol. scand.* **21**, 336–361.

HODGE, C. J., JR. (1972). Potential changes inside central afferent terminals secondary to stimulation of large- and small-diameter peripheral nerve fibres. *J. Neurophysiol.* **35**, 30–43.

JÄNIG, W. & ZIMMERMANN, M. (1971). Presynaptic depolarization of myelinated afferent fibres evoked by stimulation of cutaneous C fibres. *J. Physiol.* **214**, 29–50.

KOKETSU, K. (1956). Intracellular potential changes of primary afferent nerve fibres in spinal cords of cats. *J. Neurophysiol.* **19**, 375–392.

LLOYD, D. P. C. (1943). Neuron patterns controlling transmission of ipsilateral hindlimb reflexes in cat. *J. Neurophysiol.* **6**, 293–314.

LLOYD, D. P. C. (1952). Electrotonus in dorsal roots. *Cold Spring Harb. Symp. quant. Biol.* **17**, 203–219.

LLOYD, D. P. C. & MCINTYRE, A. K. (1949). On the origins of dorsal root potentials. *J. gen. Physiol.* **32**, 409–443.

LUND, S., LUNDBERG, A. & VYKLICKÝ, L. (1965). Inhibitory action from the flexor reflex afferents on transmission to IA afferents. *Acta physiol. scand.* **64**, 345–355.

LUNDBERG, A. & VYKLICKÝ, L. (1966). Inhibition of transmission to primary afferents by electrical stimulation of the brain stem. *Archs ital. Biol.* **104**, 86–97.

MELZACK, R. & WALL, P. D. (1965). Pain mechanisms: a new theory. *Science, N.Y.* **150**, 971–979.

MENDELL, L. M. (1970). Positive dorsal root potentials produced by stimulation of small diameter muscle afferents. *Brain Res.* **18**, 375–379.

MENDELL, L. M. & WALL, P. D. (1964). Presynaptic hyperpolarization: a role for fine afferent fibres. *J. Physiol.* **172**, 274–294.

RUDOMIN, P. (1966). Pharmacological evidence for the existence of interneurones mediating primary afferent depolarization in the solitary tract nucleus of the cat. *Brain Res.* **2**, 181–183.

SCIBETTA, C. J. & KING, R. B. (1969). Hyperpolarizing influence of trigeminal nucleus caudalis on primary afferent preterminals in trigeminal nucleus oralis. *J. Neurophysiol.* **32**, 229–238.

SESSLE, B. & DUBNER, R. (1970). Presynaptic hyperpolarization of fibres projecting to trigeminal brain stem and thalamic nuclei. *Brain Res.* **22**, 121–125.

SOMJEN, C. G. (1970). Evoked sustained focal potentials and membrane potential of neurones and of unresponsive cells of the spinal cord. *J. Neurophysiol.* **33**, 562–582.

WAGMAN, I. H. & PRICE, D. D. (1971). Pre- and post-synaptic effects of dorsal horn cells of M. Mulatta due to A and C fibre input. In *Proceedings, International Union of Physiological Sciences*, **9**, 591.

WALL, P. D. (1958). Excitability changes in afferent fibre terminations and their relation to slow potentials. *J. Physiol.* **142**, 1–21.

WALL, P. D. (1962). The origin of a spinal cord slow potential. *J. Physiol.* **164**, 508–526.

WALL, P. D. (1964). Presynaptic control of impulses at the first central synapse in the cutaneous pathway. In *Physiology of Spinal Neurones: Progress in Brain Research*, ed. ECCLES, J. C. & SCHADE, J. P., vol. 12, pp. 98–112. Amsterdam, New York: Elsevier.

WALL, P. D. (1967). The laminar organization of dorsal horn and effects of descending impulses. *J. Physiol.* **188**, 403–423.

YOUNG, R. F. & KING, R. B. (1972). Excitability changes in trigeminal primary afferent fibres in response to noxious and non-noxious stimuli. *J. Neurophysiol.* **35**, 87–95.

ZIMMERMANN, M. (1968). Dorsal root potentials after C-fibre stimulation. *Science, N.Y.* **160**, 896–898.