J. Physiol. (1978), **279**, *pp.* 437–455
With 11 *text-figures*
Printed in Great Britain

MECHANISMS OF POST-SYNAPTIC EXCITATION IN AMPHIBIAN MOTONEURONES

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(*Received 18 October 1977*)

SUMMARY

1. Post-synaptic excitation produced in motoneurones of the isolated perfused frog spinal cord by different monosynaptic inputs and by ionophoretically applied glutamate was analysed with intracellular recording technique.

2. Ca^{2+}-deficient, high Mg^{2+} (5–20 mM) media or addition of Mn^{2+} (2 mM) or Co^{2+} (5 mM) reversibly abolished chemically mediated e.p.s.p.s derived from medullary reticular formation, ventral and lateral columns, but not the short-latency, rapidly rising e.p.s.p.s derived from dorsal roots or muscle nerves, suggesting electric coupling between some primary afferents and spinal motoneurones. This conclusion is consistent with the dynamic properties of dorsal root e.p.s.p.s, their small sensitivity to cooling, and with results of correction of intracellular records made for contribution of extracellular field potential. E.p.s.p.s evoked by ventral root stimulation were also insensitive to Ca^{2+}-lack and presence of 5–10 mM-Mg^{2+}.

3. As the post-synaptic membrane was made more negative the amplitude of electrotonic dorsal root e.p.s.p.s was increased, and it was decreased by depolarizing currents. No reversal of the early part of the electrotonic e.p.s.p. was observed, although the presence of the local response would account for the occasional reversal of its later phase seen with depolarization.

4. When hyperpolarizing and depolarizing currents were applied to motoneurones in which chemically mediated e.p.s.p.s of the reticular cells, the ventral and lateral columns, were evoked, the actual reversal of the early part of e.p.s.p. was not observed, and there was no correlation between the sensitivity of the e.p.s.p.s to injected currents and their time course. The positive values of the extrapolated reversal potentials and the effects of changes in ionic content of perfusing media suggest that synaptically released transmitter triggers off the Na permeability of the subsynaptic membrane.

5. The amplitude of depolarization produced by ionophoretically applied glutamate depends non-linearly on membrane potential and the curvature of this dependence differs from that seen with chemically mediated e.p.s.p.s. The asymptotic nature of this relationship is explicable by a dependence of the membrane conductance change upon the membrane voltage.

6. The results of conductance measurements during the glutamate induced depolarization, the values of apparent reversal potentials and their dependence on

external Na^+ and K^+ and internal Cl^- is explicable by the opening post-synaptic channel gates for Na^+ and closing post-synaptic channel gates for K^+.

7. Chemical and electrical transmission in the amphibian cord is discussed in relation to recent anatomical findings.

INTRODUCTION

The junctional mechanisms at most excitatory synapses in the central nervous system of vertebrates remain far from certain. This may be due to several reasons. Usually it is difficult to achieve the effective control of presynaptic terminals and to select specific monosynaptic projections converging onto the same cell. The spatial distribution of relevant synapses on the somatodendritic membrane, the nature of the excitatory transmitter substances and the ionic fluxes involved in their action are not yet known. Controlled changes in the extracellular ionic concentrations of neurones which are studied *in situ* cannot be made.

The motoneurones of the isolated spinal cord of the frog offer a neuronal system in which the different identifiable monosynaptic inputs can be compared in the same cell and can be studied in a controlled ionic environment over long periods. It was demonstrated that monosynaptic excitation of amphibian motoneurones may be brought about by stimulation of the lateral columns and dorsal roots (Fadiga & Brookhart, 1960), the brain stem reticular formation and the ventral columns (Shapovalov & Shiriaev, 1973; Cruce, 1974) and the ventral roots (Kubota & Brookhart, 1963; Grinnell, 1966). The post-synaptic depolarization may be also produced by one of the putative neurotransmitters, L-glutamate (Sonnhof, Linder, Grafe & Krumnikl, 1975). Therefore, the post-synaptic excitation produced by different pre-synaptic pathways and by ionophoretically applied substances may be compared under similar experimental conditions.

The experiments described here demonstrate that some monosynaptic actions evoked by stimulation of dorsal and ventral roots may be electrically mediated, compare the effects of transmembrane polarization on electrotonic and chemical e.p.s.p.s with different time course and present some evidence about the ionic mechanisms of post-synaptic excitation produced synaptically and by micro-ionophoresed glutamate.

A brief preliminary account of some of the results of this work has been published previously (Shapovalov & Shiriaev, 1975; Velumian & Shapovalov, 1975).

METHODS

Preparation. The experiments were performed on specimens of *Rana ridibunda*. The preliminary surgery was performed under ether anaesthesia. The spinal cord and medulla were removed together with the ventral and dorsal roots of the ninth to tenth segments and occasionally with the muscle nerve of the hind limb. The spinal cord was hemisected to reduce diffusional barriers, mounted in a small chamber (volume of the chamber with connected tubes, 3 ml.), and perfused with a steady flow (2–4 ml./min) of oxygenated Ringer solution.

Except when the effects of temperature changes were being studied, the bath temperature was maintained within 1 or 2° between 16 and 18 °C and was monitored using a thermistor probe. The modified solutions were applied to the spinal cord by turning a stopcock which replaced the flow of normal Ringer solution with the test solution and vice versa. The composition of the

normal Ringer solution was (mM): NaCl 99–114; KCl 2·0; $CaCl_2$ 2·0; $NaHCO_3$ 6·0; glucose 5·5; with NaH_2PO_4 0·75 and Na_2HPO_4 2·0 to achieve a pH of 7·4–7·6. Variations in the composition of the testing solutions are indicated in the appropriate sections of Results and included abolition of Ca^{2+}, addition of 5–20 mM-$MgCl_2$, 5·0 mM-$CoCl_2$, 2 mM-$MnCl_2$ or 1·6 mM-EGTA. Osmoticity of solutions was kept constant. Ca^{2+} was also varied sometimes by including more or less than normal amount of this ion in solution without changing the concentration of the other constituents. A properly maintained preparation readily survived more than 24 h.

Intracellular recording technique. Conventional recording equipment was used. Intracellular recordings were made from lumbar motoneurones using bevelled micropipettes filled with 3 M-KCl or 2 M-K citrate, with resistances between 10 and 40 MΩ. Usually a high DC gain was used. Impaled cells were identified by their antidromic action potential in response to ventral root stimulation. Only motoneurones that had a stable resting potential of 55 mV or higher were used. Usually the microelectrode remained in a motoneurone while the solutions were changed.

A calomel electrode with agar bridge (prepared with 2 M-KCl) was used as an indifferent electrode and was connected through a calibrator unit to the earth. A similar electrode was used for connexion of the microelectrode with the input amplifier. The voltage response of the cell was frequently digitized using a DIDAC-4000 computer. An extracellular average was also recorded of the voltage response to presynaptic stimulation. This average was later subtracted by computer from the intracellular average to correct the distortion of intracellular response by extracellular field.

Current could be applied through the bridge circuit to evoke an action potential, to change the transmembrane potential and to monitor the input conductance changes during post-synaptic depolarization. Injected currents were measured by the voltage drop across a 1000 MΩ resistor. The input resistance of motoneurones was calculated from the changes in the amplitude of the antidromic action potential superimposed on current steps (Frank & Fuortes, 1956) or using the technique of injecting small current pulses into the motoneurone and measuring the resulting voltage changes. L-glutamate was administered ionophoretically through an extracellular microelectrode (tip diameter 2–3 μm) to motoneurones whose activity was recorded intracellularly. The inter-tip distance between recording and injecting electrodes was 20–30 μm. Glutamate efflux was controlled by passing a current through the microelectrode connected in series with a 1000 MΩ resistor. Stimulation of dorsal and ventral roots or the muscle nerves of the hind limb was accomplished through gold wires. Bipolar tungsten electrodes were used to stimulate the medullary reticular formation, ventral columns and lateral columns of the spinal cord.

RESULTS

Electrically mediated monosynaptic e.p.s.p.s. As at other peripheral and central synaptic junctions with a chemical mode of transmission the effect of Ca^{2+} on evoked transmitter release occurs also at synapses of frog motoneurones (Katz & Miledi, 1963; Grinnell, 1966; Dambach & Erulkar, 1973; Barrett & Barrett, 1976). Ca-free media in the presence of Mg^{2+} (5–20 mM) completely and reversibly abolished all polysynaptic responses produced by stimulation of both dorsal root afferents and descending pathways. Monosynaptic e.p.s.p.s evoked from the reticular formation, ventral and lateral columns were also blocked after 15–30 min perfusion of the hemicords with Ca^{2+}-deficient, high Mg^{2+} solutions, this effect being accentuated by addition of 1·6 mM-EGTA. The same was true of experiments in which the spinal cord was soaked in Ringer solution with a normal Ca^{2+} content but in the presence of 5 mM-Co^{2+} or 2 mM-Mn^{2+}. When the bath Ca^{2+} was reduced to zero with 5–20 mM-Mg^{2+} or 1·6 mM-EGTA and 5 mM-Co^{2+} or 2 mM-Mn^{2+} present in the solution and 6–8 hr allowed to elapse, it was still possible, however, to elicit e.p.s.p.s with either primary afferents (dorsal root, n. ischiadicus or muscle nerves of the hind limb) or ventral root stimulation.

The full effect of Ca-free solutions or Mg^{2+}, Co^{2+} or Mn^{2+} ions on the synaptic

activity was achieved after 20–30 min of perfusion. Following this period the remaining e.p.s.p.s reached a steady state which was maintained for many hours.

Comparison of the sections reticular formation and dorsal root 9 of Fig. 1 shows that both the monosynaptic e.p.s.p. evoked by reticular formation stimulation and the polysynaptic portion of response evoked by the dorsal root volley are abolished when the spinal cord is perfused with Ringer solution containing 2 mM-Mn^{2+}. Throughout the perfusion, however, the fast short-latency dorsal root e.p.s.p. persisted. A return to normal Ringer solution brought about full recovery of the monosynaptic and polysynaptic responses eliminated by addition of Mn^{2+}.

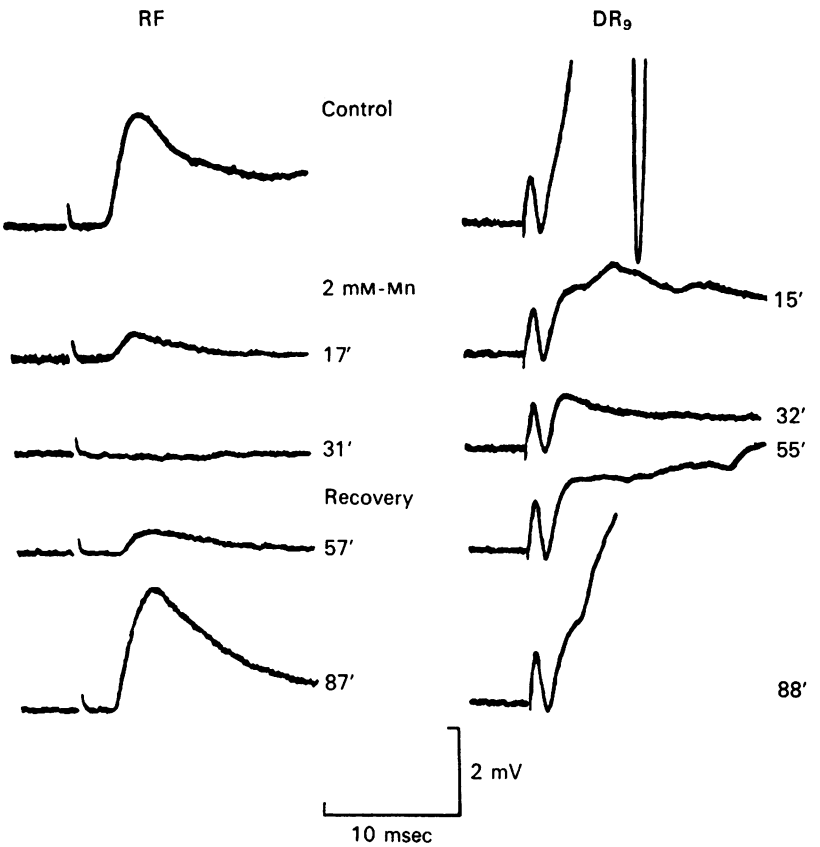

Fig. 1. Effect of addition of Mn^{2+} on reticular formation (RF) and dorsal root 9 (DR_9) e.p.s.p.s recorded from the same motoneurone. Top traces are control records. Numbers indicate the time (min) following the beginning of perfusion with Ringer solution containing 2 mM-Mn^{2+} and following readmitting of normal Ringer solution.

The activity of presynaptic fibres from the dorsal root was practically unaffected by reduction in bath Ca^{2+} or by Mg^{2+} (5–10 mM) and Mn^{2+} (2 mM) as evidenced by the amplitude and duration of the presynaptic spike. However, Co^{2+} (5 mM) or high Mg^{2+} (20 mM) induced a decrease and prolongation of the presynaptic spike.

The spontaneous miniature synaptic potentials which can be recorded from spinal motoneurones in normal Ringer solution could still be seen after suppression of evoked responses by Ca lack and by elevations of the bath Mg^{2+} as shown previously

(Katz & Miledi, 1963; Dambach & Erulkar, 1973). In Ringer solution containing 5 mM-Co^{2+} the frequency of miniature potentials may even be increased after the complete suppression of all chemically mediated synaptic activity, as at the frog neuromuscular junction (Shapovalov, 1962; Weakly, 1973).

The resistance to Ca-lack and to Mg^{2+}, Co^{2+} and Mn^{2+} ions of the dorsal and ventral root e.p.s.p.s would be explained if they were a result principally of electrical transmission. Further evidence indicating the electrical coupling between primary afferents and spinal motoneurones is provided when the post-synaptic responses are freed from distorting field effects as recorded after withdrawing the microelectrode to a position just outside the cell. When the recording electrode is at the motor nucleus region, stimulation of the dorsal root produces a characteristic focal potential representing the potential field initiated by action potentials in the afferent fibres

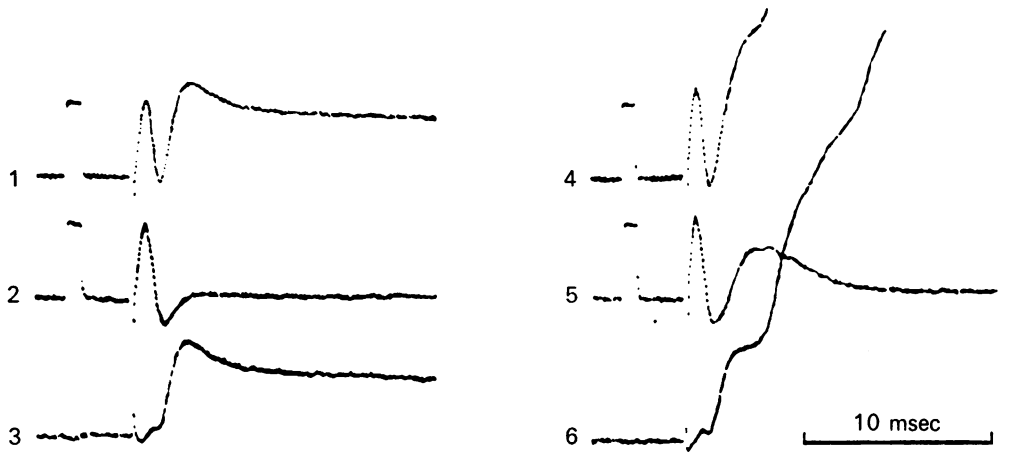

Fig. 2. Relationship between intracellularly and extracellularly recorded monosynaptic dorsal root e.p.s.p.s recorded from the same motoneurone after chemical synapses had been blocked by Mn^{2+} (1–3) and 80 min after normal Ringer solution was reapplied (4–6). Averaged intracellular records (1 and 4), extracellular records (2 and 5) and the result of subtraction of the extracellular response from the intracellular one. Calibration pulse 1 mV, 1 msec (subtracted by computer in 3 and 6).

(presynaptic component) and the post-synaptic activity generated by motoneurones and internuncial cells. The extracellular field potential change begins with diphasic positive-negative signs of the presynaptic volley approaching and eventually reaching presynaptic terminals. In normal Ringer solution the presynaptic spike is followed by the positive post-synaptic response frequently masking the preceding negativity due to the presynaptic volley. When the chemically mediated post-synaptic activity is suppressed by Ca-lack or by Mg^{2+}, Co^{2+} ions the negative phase of the presynaptic field potential is always clearly evident.

Fig. 2 (1–3) illustrates the voltage responses of a lumbar motoneurone to the dorsal root volley digitized at 33 points/msec and an average built up by the computer over 20 sweeps. The chemical transmission is blocked by Mn^{2+} ions and the intracellular average reveals both the presynaptic spike and the Mn^{2+} resistant e.p.s.p. An extracellular average shows the typical biphasic presynaptic response and no signs of post-synaptic positivity. When the extracellular average is subtracted from the

intracellular average, the intracellular minus extracellular response reveals no significant distortion by extracellular field. Occasionally the corrected e.p.s.p. begins 0·1–0·3 msec earlier and its rising phase remained practically the same as before correction.

The records of Fig. 2 (4–6) show the potentials recorded from the same motoneurone following repeated intracellular impalement. The recovery of chemically mediated transmission was achieved as the normal Ringer solution was readmitted. It may be seen that the amplitude of the intracellularly recorded monosynaptic e.p.s.p. is increased and the extracellular response reveals a typical post-synaptic positivity as described by Fadiga & Brookhart (1960). As a consequence of an opposite type of distortion produced by the negative and positive phases of the extracellular field potential after subtracting the extracellular potential from intracellular average the rise time of the obtained monosynaptic response becomes shorter, its amplitude decreases and it is separated by a prominent notch or plateau from a later presumably polysynaptic response. The amplitude of the corrected monosynaptic response in normal Ringer solution is equal to the amplitude of the corrected e.p.s.p. recorded in Mn^{2+}-Ringer suggesting that the apparent decrease produced by Mn^{2+} may be due to the abolition of the positive component of the extracellular field but not because the partial suppression of the monosynaptic response in the cell recorded from.

Similar correction of intracellular records performed in cords treated with heavy doses of pentobarbital (1·2–1·6 mM) in order to avoid polysynaptic contamination reveals the separation of apparently homogenous monosynaptic e.p.s.p. in the initial, presumably electrical, and the later, presumably chemical components (Fig. 3, 1–3). In normal Ringer solution correction of intracellular responses occasionally reveals the absence of any monosynaptic e.p.s.p. although the intracellular recording indicates an early component appearing with a monosynaptic latency (Fig. 3, 4–6). In fact such monosynaptic e.p.s.p. 'seen' by the intracellular electrode may be a pure reflexion of the positive phase of the extracellular field potential. Usually the initial portion of the positive field potential nearly coincides with or even starts after the peak of the electrically mediated e.p.s.p. and the extracellular negativity of the presynaptic spike coincides with its rising phase.

If the onset of the positivity of the extracellularly recorded post-synaptic response corresponds to the beginning of the intracellularly recorded monosynaptic e.p.s.p. (Fadiga & Brookhart, 1960) then the time lag between the onset of the electrically and chemically mediated monosynaptic e.p.s.p.s is 0·5–1·4 msec ($0·93 \pm 0·056$ msec, $n = 16$). This value fits well with the estimated synaptic delay for the chemically mediated monosynaptic e.p.s.p. in amphibian cord (Brookhart & Fadiga, 1960). These findings suggest that primary afferents may be connected with spinal motoneurones via both electrical and chemical synapses.

Another line of evidence suggesting that dorsal root e.p.s.p. may result from combined electrical and chemical synaptic action was obtained from experiments in which muscle nerves of the hind limb were stimulated. The records of Fig. 4 show an all-or-none early postsynaptic depolarization with a latency about 1 msec shorter than that of a usual monosynaptic e.p.s.p.

Stimulation of the deep branch of the peroneal nerve produced a typical monosynaptic e.p.s.p. with weak stimuli, but with slightly stronger stimulation the early

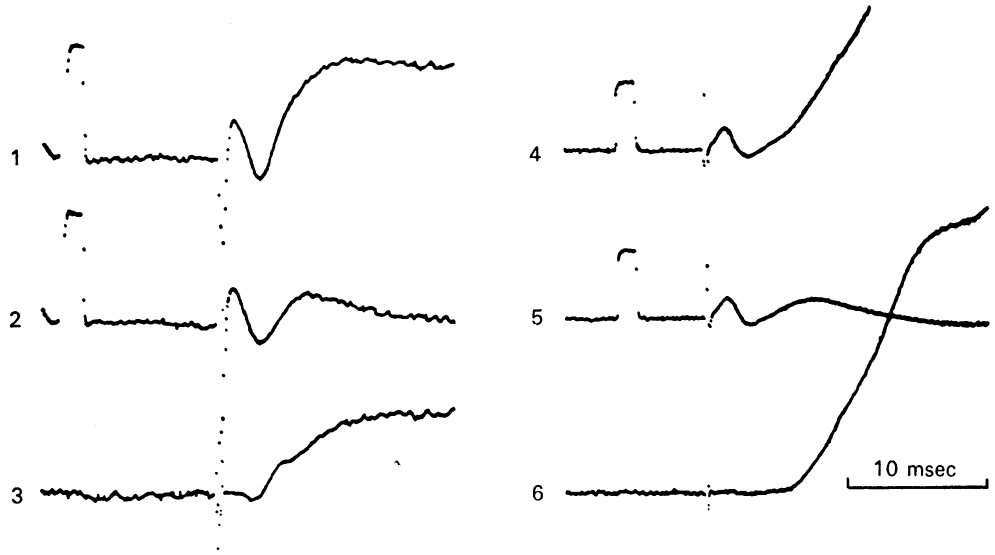

Fig. 3. Relationship between intracellular and extracellular recorded dorsal root e.p.s.p.s evoked in two different motoneurones: 1–3, after 30 min perfusion with Ringer solution containing 1·2 mM-pentobarbitone; 4–6, in normal Ringer solution. Averaged intracellular records (1 and 4), extracellular records (2 and 5) and the result of subtraction of the extracellular field from the intracellular response (3 and 6). Calibration pulse 2 mV, 1 msec (subtracted by the computer in 3 and 6).

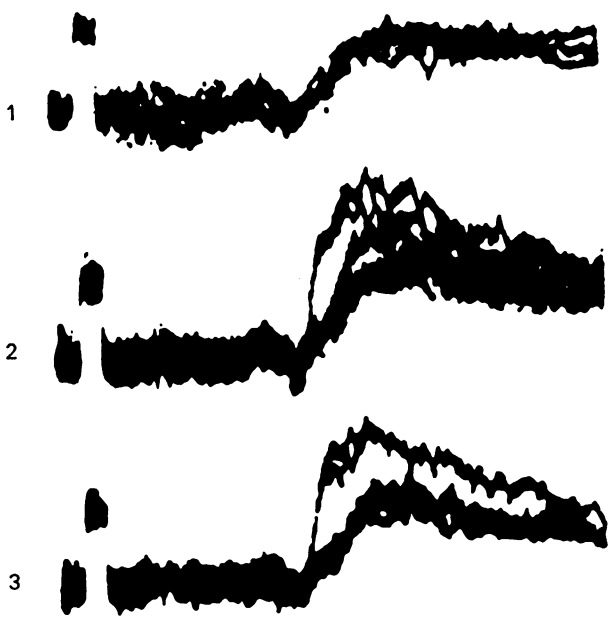

Fig. 4. Intracellular records from a motoneurone of responses generated by an afferent volley in the peroneus nerve. 1–3, effect of progressive increase in peroneus volley. Intensity of stimulation current 0·05 mA (1), 0·08 mA (2) and 0·1 mA (3). Calibration pulses 1 mV, 1 msec.

potential appears with a discrete latency jump. This early component actually begins from the presynaptic field potential and its rising phase is markedly faster than that of the later monosynaptic component. Moreover, some differences in thresholds suggests that each component of the monosynaptic e.p.s.p. is produced by activation of different presynaptic fibres.

Many other properties of the early dorsal root e.p.s.p.s resistant to the absence of Ca^{2+} are consistent with an electrical mode of transmission. These e.p.s.p.s have short latency (1·0–2·1 msec, mean $1{\cdot}57 \pm 0{\cdot}083$ msec) and fast time course. The rise time is in the range 0·9–2·0 msec (mean $1{\cdot}45 \pm 0{\cdot}007$ msec, $n = 19$). The decay may vary in different motoneurones. They can follow without block of reinforcement high frequency stimulation (up to 100 per sec) and are relatively insensitive to changes in temperature.

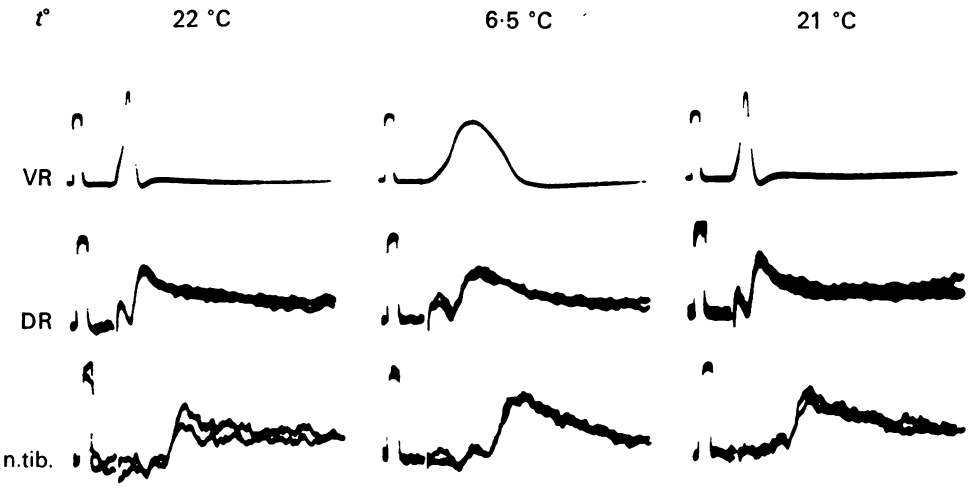

Fig. 5. Comparison of the effect of reduction of temperature on the e.p.s.p.s derived from the dorsal root (DR_9) and tibial nerve and on the antidromic action potential recorded from the same motoneurone. Calibration pulse 1 msec, 50 mV for the ventral root (VR), 2 mV for dorsal root (DR) and 1 mV for n. tibialis.

The effects of temperature were investigated by allowing the spinal cord to equilibrate at a particular $t°$ for at least 5–10 min before records were made. The temperature was varied over a range from 22 to 4 °C, and records at the extremes of this range were repeated in order to assess whether or not they were reversible. In Fig. 5 are illustrated the effects of changes in temperature on the latency, time course and amplitude of e.p.s.p.s evoked in the same motoneurone by stimulation of DR_9 and n. tibialis (all other roots were cut). All records were made in Ca-deficient solution in the presence of 5 mM-Mg^{2+}, when chemical transmission was blocked. At 6·5 °C the amplitude of the e.p.s.p.s remained nearly the same, as at room temperature.

The increase in latency of e.p.s.p.s and the prolongation of both the presynaptic spike and the antidromic action potential are quite obvious. The effects of temperature are completely reversible. In experiments actually not taken up here it was found that in the cold the amplitude of chemically evoked e.p.s.p.s declined, the

latency and rise time were prolonged drastically. These findings are consistent with the difference between effects of cooling on chemical and electrical synapses at leech motoneurones (Nicholls & Purves, 1972).

Obviously, the conclusion about electrical coupling between dorsal root afferents and spinal motoneurones must lead to the proposal of the absence of a reversal potential for the dorsal root e.p.s.p.s resistant to Ca-lack (Bennett, 1972). Therefore an investigation was made of the effects of transmembrane polarization on the electrical dorsal root e.p.s.p.s.

The records of Fig. 6 show the amplitude for such an e.p.s.p. for a variety of depolarizing and hyperpolarizing currents and calculated values of the membrane potential. At the resting membrane potential (-72 mV) the e.p.s.p. had a rise time of 1·6 msec and a time constant of decay 5·3 msec, which is similar to the time constant of the motoneurone membrane (4·8 msec), suggesting a proximal location of the synaptic input.

Hyperpolarization augmented the electrically mediated e.p.s.p. as shown for example in Fig. 6. Similar effects of hyperpolarization have been observed at electrotonic junction in crayfish septate axon (Watanabe & Grundfest, 1961) and giant motor synapses (Furshpan & Potter, 1959). This effect may be a result of an increase in spike height on the presynaptic side due to electrotonically spreading hyperpolarization or, more likely, because of rectification in the junctional membrane.

The effect of depolarizing currents on the electrical e.p.s.p. was more dramatic, but as the cell was depolarized the initial portion of the e.p.s.p. did not reverse even at a membrane potential of $+114$ mV. It should be pointed out that on depolarizing the cell an active or local response was observed quite often to accompany the e.p.s.p. (Fig. 6). This local response could increase the peak amplitude of the e.p.s.p. and prolong its time course on moderate depolarization, as illustrated by the effect of 5 nA depolarizing currents. With larger depolarizing currents a marked undershoot occurred, producing a biphasic behaviour of the e.p.s.p. However, unlike the i.p.s.p., the undershoot due to local response did not increase uniformly with increasing depolarization. The e.p.s.p.s shown in Fig. 6 are all averages and mask the great variety of time courses which occurred with individual e.p.s.p.s triggering off local responses. A similar behaviour of unitary somatic Ia e.p.s.p.s was described by Edwards, Redman & Walmsley (1976) at feline motoneurones.

As contamination of the e.p.s.p. with inhibitory or disynaptic excitatory components is improbable due to suppression of chemical transmission, the biphasic behaviour of the e.p.s.p. because of the local response may explain the biphasic reversal of monosynaptic composite Ia e.p.s.p.s in mammalian motoneurones (Shapovalov & Kurchavyi, 1974; Werman & Carlen, 1976).

Properties of chemically mediated monosynaptic e.p.s.p.s. As the monosynaptic e.p.s.p.s produced by stimulation of the reticular formation, ventral and lateral columns were completely and reversibly abolished by Ca-lack or by addition of Mg^{2+}, Co^{2+} and Mn^{2+} it may be concluded tentatively that they are a result of chemical transmission.

Many of these monosynaptic actions reached a summit about 0·8–1·5 msec after initiation (range from 0·8 to 3·2 msec), thus their rising phase was similar to that of electrically mediated dorsal root e.p.s.p.s, and declined with an approximately

exponential time course. However, the shortest time constant of decay was found to be 6–8 msec, i.e. longer than the time constant of the motoneurone membrane.

In experiments specifically designed for the purpose of comparing the effect of transmembrane polarization on the e.p.s.p.s with different time course, eighty-eight e.p.s.p.s were subjected to membrane potential changes. Of these, a comparison of

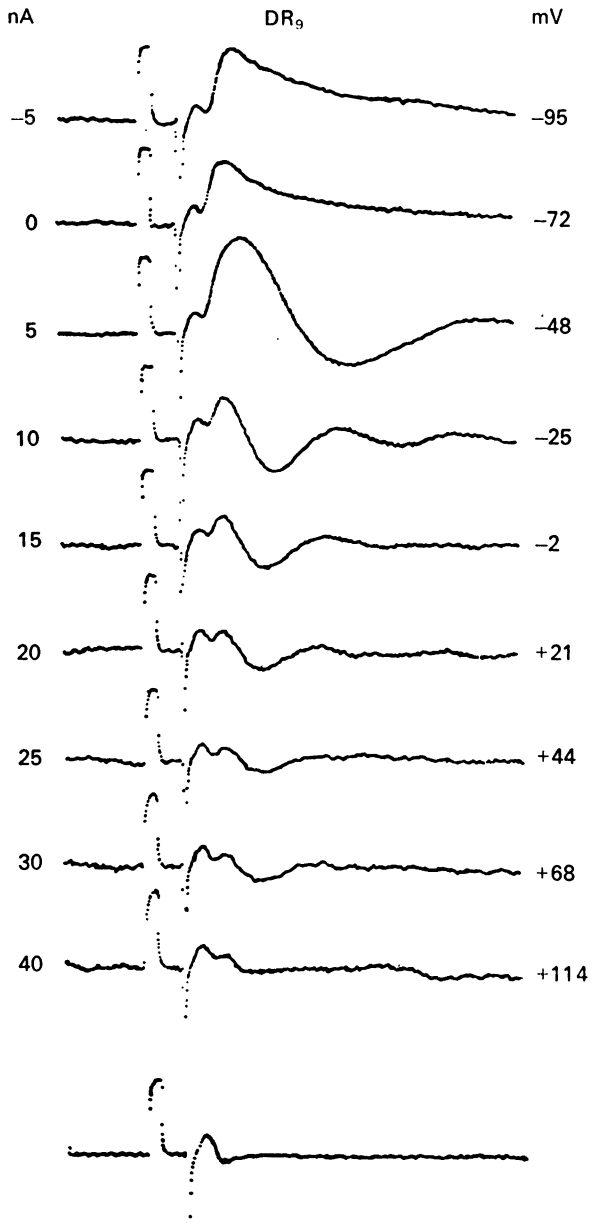

Fig. 6. Averaged dorsal root (DR_9) e.p.s.p. recorded during perfusion in Ca-free solution in the presence of 5 mM-Mg^{2+}. Intensity of the injected currents and the membrane potential is shown beside each record. The extracellular record at the bottom was obtained after the electrode had been removed a small distance from the cell. Calibration pulse 1 msec, 2 mV.

the effects of polarizing currents on two different monosynaptic inputs converging onto the same cell was accomplished in sixteen motoneurones, and in three cases three different inputs were examined in the same cell.

Fig. 7 gives an example of reticular formation, of dorsal and ventral column e.p.s.p.s recorded from the same cell. The reticular formation and ventral column monosynaptic e.p.s.p.s are of approximately the same size, but the duration of their rising and falling phases differs considerably. With hyperpolarization there is an increase in the ventral column e.p.s.p. peak amplitude and a decrease in the decay time both of reticular formation and ventral column e.p.s.p.s. The peak amplitude of

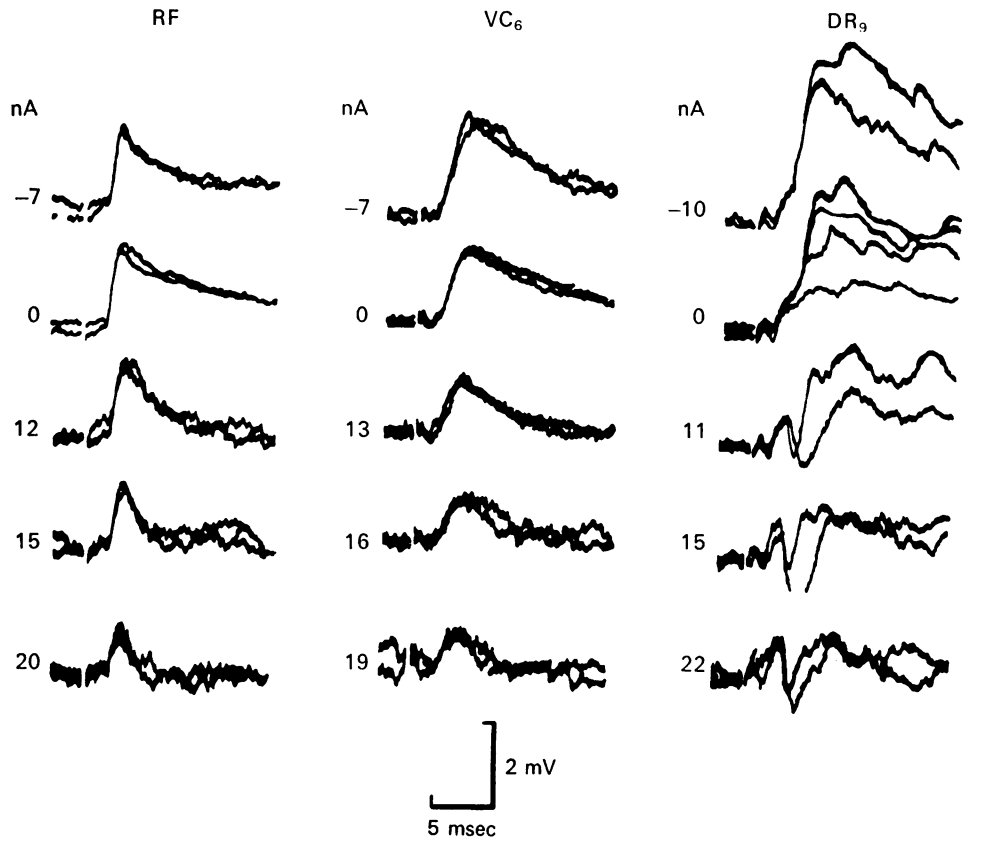

Fig. 7. Effect of hyperpolarizing and depolarizing currents on the e.p.s.p.s of the reticular formation, the ventral column and dorsal root, recorded from the same cell. Intensity of injected currents is shown beside each record.

both e.p.s.p.s decreases with increasing depolarizing current and their decay is also decreased. However, the faster reticular formation e.p.s.p.s were not more affected by depolarizing currents than were the slower e.p.s.p.s produced by ventral column volleys. Moreover, the slower responses were frequently even more sensitive to depolarization when compared with the faster e.p.s.p. in the same cell. Similar results were obtained in mammalian motoneurones receiving monosynaptic inputs from supraspinal centres and from muscle afferents (Shapovalov & Kurchavyi, 1974)

and when composite (Werman & Carlen, 1976) and unitary Ia e.p.s.p.s with different time courses were compared (S. J. Redman, personal communication).

In no case was the monosynaptic e.p.s.p. completely reversed but was usually decreased in size and the later phase was frequently inverted.

The records of Fig. 7 further indicate that depolarizing currents effectively reverse the polysynaptic component of the dorsal root e.p.s.p. but do not affect its monosynaptic component (which in this case may probably reflect partly an extracellular field potential).

The inability to demonstrate an actual reversal of the chemically mediated monosynaptic e.p.s.p.s does not imply that a reversal potential does not exist, but would

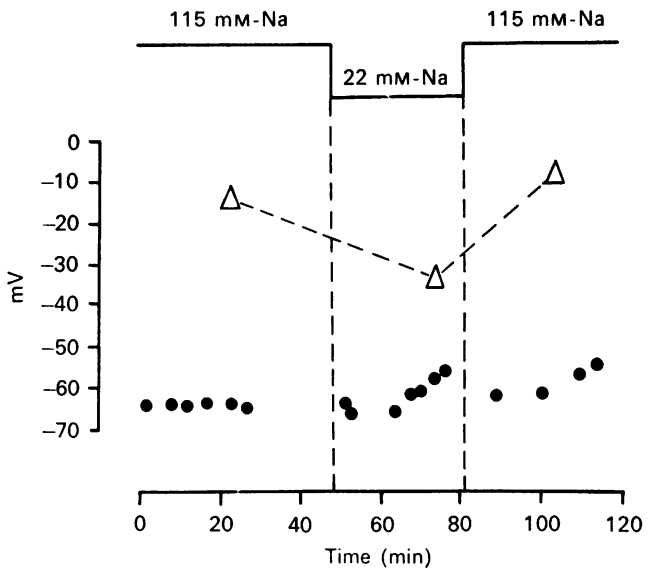

Fig. 8. Effect of partial removal of external Na^+ on the apparent reversal potential of reticular formation e.p.s.p. Duration of perfusion with modified solution is indicated by bar. Ordinate: membrane potential in mV (filled circles), reversal potential (open triangles). Abscissa: time (min).

suggest that their tonic mechanism is quite different from that of the end-plate potential at the neuro-muscular junction.

When the linear relationship between the amplitude of the monosynaptic e.p.s.p. and the membrane potential was extrapolated it was found that the values of the reversal potential are shifted in most cases in the positive side from zero. In fact, in many motoneurones the values of the reversal potentials were more than +100 mV. Although the determination of reversal potential by extrapolation may be invalid because of the possible errors introduced by nonlinear properties of the nonsynaptic membrane and spatial distribution of the synapses, it may indicate the sensitivity of monosynaptic actions to injected currents.

When reticular formation ventral and lateral column e.p.s.p.s were compared in different motoneurones, as in the same cell, there was no correlation between the sensitivity of e.p.s.p.s to depolarizing currents and their rise time.

The positive values of the extrapolated reversal potentials point to Na^+ as the major current carrier in the excited post-synaptic membrane.

In an attempt to obtain more information about the ionic mechanisms of post-synaptic excitation produced by descending volleys several successful attempts were made to change the ionic composition of the perfusing solution during the course of a single penetration. The procedure was to examine the effect on the reversal potential of variation in the concentration of Na^+, K^+ and Cl^- ions in the solution bathing the spinal cord.

Fig. 8 shows that about fivefold reduction of external Na^+ was followed by a conspicuous shift of the reversal potential of the reticular formation e.p.s.p. in the negative direction. On returning to the normal Ringer solution there was a rapid recovery of the reversal potential. Similar results were obtained in seven other motoneurones. Changing external K^+ between 2.0 and 0·2 mM did not alter appreciably reversal potential in most cells, though the amplitude and reversal of the post-spike hyperpolarization was affected in the expected direction. Total substitution of external Cl^- for sulphate did not affect the reversal potential of monosynaptic e.p.s.p.s.

In a few motoneurones the reversal potential of the depolarizing p.s.p.s was found to be approximately -40 to -70 mV, and the actual reversal of the signs of the initial phase was readily achieved. However, these responses may have been i.p.s.p.s since they were highly sensitive to the external Cl^- concentration.

Properties of glutamate induced responses. Analysis of post-synaptic responses produced synaptically and by ionophorezed glutamate has not as yet been sufficient to prove the identity of this substance and the excitatory transmitter in the mammalian spinal cord (Curtis *et al.*, 1972). However, it would be of interest to compare some properties of the glutamate induced depolarization and the e.p.s.p.s as recorded above.

When glutamate was applied by ionophoretic ejection into the vicinity of motoneurones recorded from it consistently caused depolarization, the minimal duration of which was about 100 msec. Responses evoked by this aminoacid remained virtually unchanged when chemically mediated synaptic transmission was blocked by Ca-lack or by addition of external Mg^{2+}, Co^{2+} or Mn^{2+} in the bath, indicating that applied substance directly affects the motoneuronal membrane.

Fig. 9 plots changes in glutamate response and monosynaptic reticular formation e.p.s.p. of the same cell with different amounts of polarizing current. A relation between the magnitude of the glutamate responses and the membrane potential demonstrates that they increase with hyperpolarization and decrease with depolarization. However, the relationship is approximately linear only over a restricted range of membrane potential, and considerable deviation from linearity appears for strong depolarizations. This deviation from linearity and the asymptotic nature of the curve do not allow the determination of the reversal potential. No signs of the actual reversal could be observed in all thirty-nine cells studied. The steep portion of the relationship between glutamate depolarization and the membrane potential in Fig. 9 may be extrapolated to about -30 mV. However, the actual reversal is absent even at $+60$ mV. Assuming the reversal potential for glutamate response to be indicated by the most negative value of the membrane potential at which the response firstly achieved zero (-7 mV for the cell illustrated in Fig. 9), reversal

potentials were in the range 0–51 mV (mean $-26{\cdot}2 \pm 2{\cdot}3$ mV, $n = 39$), most negative estimates were obtained from cells with resting potentials greater than -65 mV.

It may be assumed that the depolarization produced by glutamate is due to an effect predominantly on the somal membrane of motoneurones. Therefore the deviation from linearity may be tentatively explained by the proposal that the glutamate induced membrane conductance change is dependent also upon the membrane voltage itself.

The relationship between the membrane potential and the amplitude of the monosynaptic reticular formation e.p.s.p. recorded from the same cell is much more linear but also does not allow for an extrapolation to a clear reversal potential. Therefore it is difficult to compare the reversal potentials of the e.p.s.p. and the glutamate response.

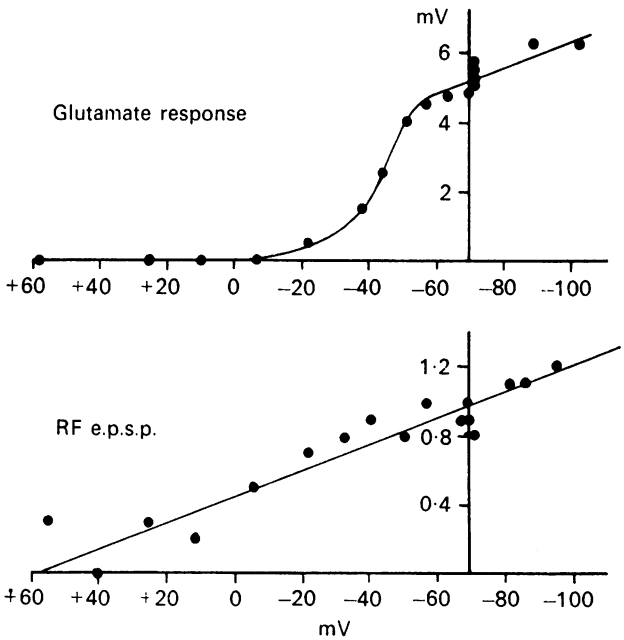

Fig. 9. The plot of glutamate response peak amplitude against membrane potential (above) and of reticular formation (RF) peak amplitude against membrane potential (bottom). Both post-synaptic effects were recorded from the same motoneurone.

The mechanism of the depolarization produced by glutamate may be investigated by its ionic dependence.

Fig. 10 illustrates the effects of both Na^+ removal and K^+ addition on the reversal potential of glutamate response recorded from the same cell. When the hemicord was perfused by low-Na Ringer solution containing 9 mM-Na^+ and 99 mM-choline chloride the extrapolated reversal potential was shifted in the negative direction. On the return to the normal Ringer solution the reversal potential was approximately the same as in the control state. Modifying the external concentration of K^+ ions influence also the reversal potential shifting it in a positive direction (Fig. 10), there was a complete recovery in normal Ringer solution. It may be concluded therefore that the glutamate induced depolarization was due to a transient change in the

membrane permeability to both Na^+ and K^+ ions. Injections of Cl^- ions into three cells with 20 nA hyperpolarizing currents passed through 3M-KCl-filled microelectrode during 2 min caused no apparent shifts of reversal potential for glutamate responses.

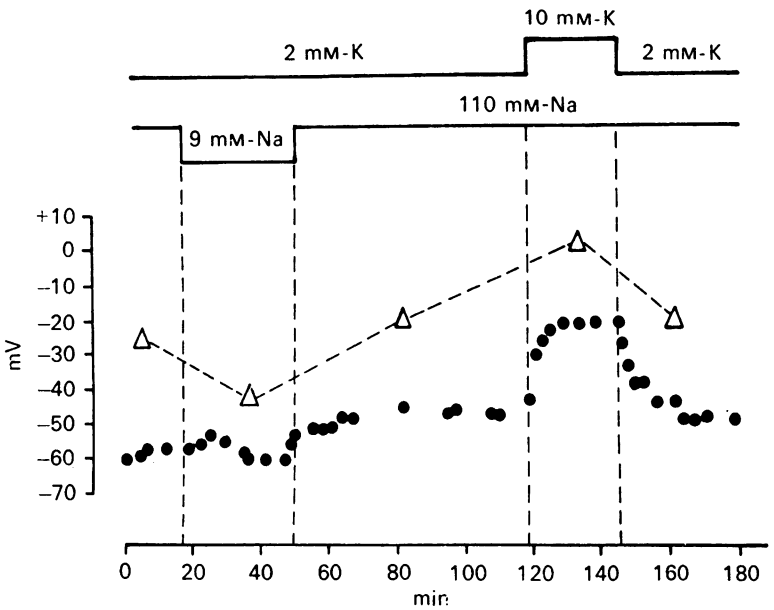

Fig. 10. Effects of partial removal of external Na^+ ions and fivefold increase of external K^+ ions on the apparent reversal potential of a glutamate response. Note a prompt depolarization of about 25 mV produced by an increase of K^+ in the perfusate (△, extrapolated values of reversal potential, ●, resting membrane potential). Duration of perfusion with modified solution is indicated by bar above.

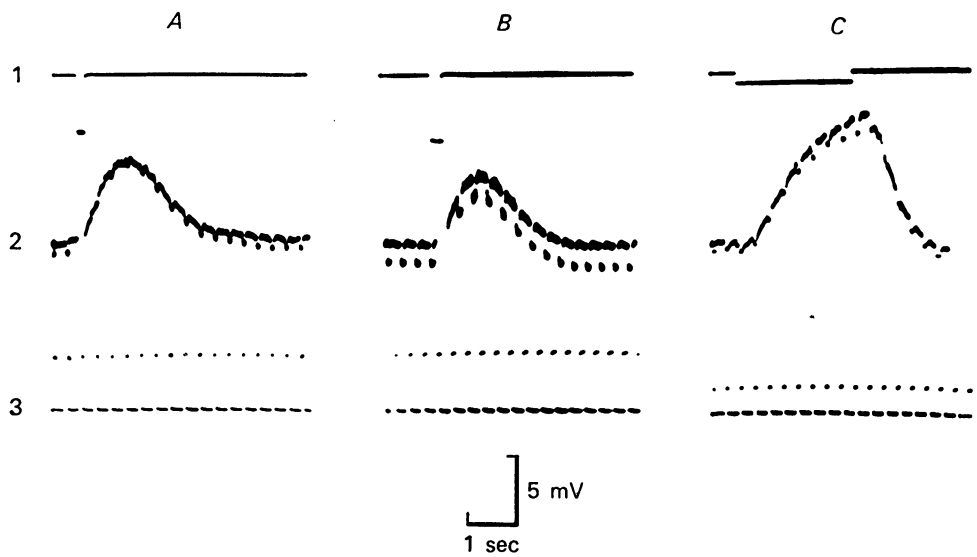

Fig. 11. Conductance changes in three different motoneurones (*A*, *B* and *C*) during depolarizing responses induced by ionophorezed glutamate. 1: records of electro-phoretic current (110 nA for *A*, 130 nA for *B* and 20 nA for *C*). 2: glutamate response. 3: current pulses. Intensity of polarizing current pulses: 2 nA for *A*, *B* and 1 nA for *C*.

The membrane conductance changes during glutamate responses were examined by the intracellular application of square current pulses through the recording inner barrel. The decrease in voltage pulse height during the glutamate responses indicated a decreased cell input resistance. Only in some motoneurones (nine cells) during glutamate applications there was a remarkable reduction in the polarizing potential changes caused across the cell membrane by the current pulses. In nine cells no conductance changes and in seven cells even decrease in conductance was determined during glutamate induced depolarization. Three different experiments of this kind are illustrated in Fig. 11. Similar results were reported recently by Engberg, Flatman & Lambert (1975) on feline motoneurones.

No correlation was noted between the presence or absence of conductance change and the amplitude and the duration of the response. The decrease in conductance cannot occur unless the membrane becomes less permeable to some ion species. It may be supposed therefore that the glutamate induced depolarization was due to a transient increase in the membrane permeability to Na^+ and decrease to K^+.

DISCUSSION

As changes in Ca^{2+} concentration or the addition of Mg^{2+}, Co^{2+} or Mn^{2+} do not affect the short-latency monosynaptic e.p.s.p.s evoked by dorsal root volleys, the resulting synaptic potential may involve the electrotonic junctions between primary afferents and spinal motoneurones. This conclusion is consistent with the properties of these e.p.s.p.s (very short latency, specific interrelation with extracellular field potential, the ability to follow high frequency stimulation without block or reinforcement, the small sensitivity to changes in $t°$ and with the existence of tight or gap junctions in amphibian cord (Charlton & Gray, 1965; Sotelo & Taxi, 1970), including its motor nuclei (Shapovalov, 1977; Motorina, 1978).

The fast rising phase of all electrotonic dorsal root e.p.s.p.s and the rapid decay of many of them suggest the proximal location of relevant synapses. It should be noted in this connexion that although it was believed for a long time that in the frog the dorsal root fibres may establish synaptic articulations exclusively with distal dendrites of motoneurones (Chambers, Sprague & Liu, 1960; Fadiga & Brookhart, 1960) recent anatomical evidence shows that collaterals of dorsal root fibres make synaptic contacts on the proximal dendrites and on the somata of frog motoneurones (Szekely, 1976).

The direct synaptic projections from the reticular formation, ventral and lateral columns excite lumbar motoneurones via chemically transmitting synapses. Although the corresponding e.p.s.p.s have different time courses a substantial number of them have a rising phase in the range of 0·8–1·5 msec, as with the electrotonic e.p.s.p.s.

It is remarkable that the monosynaptic e.p.s.p. evoked by stimulation of various long projecting pathways and generated by differing junctional mechanisms behave in a similar way to current injection. Apparently, very much the same physiological effect can be achieved by different means. From the functional point of view this implies that the amplitude of the direct synaptic actions exerted by projecting pathways is relatively independent of other excitatory inputs converging onto the same cell.

It has been widely assumed that e.p.s.p.s with fast time courses are located closer to the neurone soma where the recording electrode is located and are most sensitive to trans-membrane polarization. This is the explicit and implicit assumption of both the abstract mathematical model (Rall, 1967; Jack, Noble & Tsien, 1975; Redman, 1976) and analogue model of the motoneurones (Kurchavyi, Motorina & Shapovalov, 1973). The major qualification to this notion is the need to assume the identical junctional and ionic mechanisms of e.p.s.p. generation at different synapses.

Although cable models of neurones have proved to be very useful tools in developing an understanding of synaptic events, the assumption of uniform mechanisms at different synapses requires critical reassessment. Contradiction between results obtained on the model and on mammalian motoneurones (Shapovalov & Kurchavyi, 1974; Werman & Carlen, 1976; S. I. Redman, personal communication) and in the present study may be tentatively explained by different properties of various excitatory synaptic inputs. Evidence exists that different junctional mechanisms can occur even at different synapses of primary afferents on the same motoneurone (Fig. 4.)

It is possible for the synapses of all pathways studied to end upon similar regions of the motoneurone membrane and yet to generate e.p.s.p.s of different time courses if the duration of post-synaptic currents vary.

The apparent sensitivity of electrotonic e.p.s.p.s to transmembrane polarization may be due to the fact that the polarizing currents passing backwards across electrical synapses change the amplitude of the presynaptic action potential or to rectification in the junctional membrane. The biphasic reversal of some electrotonic e.p.s.p.s in Ca^{2+}-free, high Mg^{2+} solutions cannot be due to contamination of the postsynaptic response by inhibitory component or a delayed, chemically mediated excitatory component with a reversal potential well within range of the induced depolarization, but may be caused by active response generated by the nonsynaptic membrane. At chemically transmitting synapses a variable contribution to synaptic current from different ions may affect the sensitivity of resulting e.p.s.p.s to transmembrane polarization.

Results obtained in the present work suggest that glutamate can open ionic gates to Na^+ and close the ionic gates to K^+. In the present study we could not observe the actual reversal of glutamate responses seen in cat motoneurones (Curtis *et al.*, 1972; Ziegelgänsberger & Puil, 1973). However, the duration of ionophoresis and resulting responses in mammalian motoneurones were much longer and may involve a hyperpolarizing phase which hinders the direct comparison. The chemically mediated postsynaptic effects produced by stimulation of different presynaptic pathways may involve participation of various transmitter substances and different ionic conductances. The inability to demonstrate a reversal of the early part of chemically mediated e.p.s.p.s suggests that they are generated by a transient membrane permeability change to ion species the equilibrium potential for which is near the Na or even Ca equilibrium potential. However, it is also possible that the membrane conductance change is dependent also upon the membrane voltage itself. Undoubtedly, further work on the problem of defining the mechanism of e.p.s.p. generation and the actual site of e.p.s.p. transmission is needed. Until more precise information can be obtained, it would be very difficult to generalize about the relationship between the

time course, location and the sensitivity to membrane potential change of different e.p.s.p.s.

We would like to thank Dr J. J. B. Jack (Oxford) for kindly reading the manuscript and his careful criticism, Professor D. R. Curtis (Canberra) for suggested improvements in English, Dr S. J. Redman (Clayton) for allowing us to quote unpublished results from his laboratory, Dr Z. A. Tamarova for allowing us to use the data presented in Fig. 4 of the present work, and T. A. Gromova for the skilful technical assistance and the photographic work.

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