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THE ACTIONS OF EXCITATORY AMINO ACIDS ON MOTONEURONES IN THE FELINE SPINAL CORD

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SUMMARY

1. Combined recording or ionophoretic electrodes of the concentric type were used to investigate the depolarizing responses of DL-homocysteate (DLH) and L-glutamate in cat lumbar motoneurones.

2. Typically, DLH responses were slow both in onset and recovery, while glutamate responses were fast in onset and recovery and were frequently accompanied by a post-response hyperpolarization.

3. DLH responses (smaller than those necessary to evoke firing) were accompanied by a stable *decrease* in G_M. This decrease was usually more than could be accounted for by anomalous rectification of the membrane.

4. Small glutamate responses were accompanied by either a small decrease, no change or a small increase in G_M. There was a biphasic change in G_M during large responses: G_M decreased during the rising phase and early part of the response plateau and thereafter increased as the depolarization was maintained. It is proposed that the high conductance state during glutamate application (but not the depolarization itself) is a manifestation of glutamate uptake.

5. Firing evoked by DLH was stable during very long applications of the drug. Firing evoked by glutamate was usually of short duration, despite the maintained depolarization.

6. No reversal potential for the DLH responses could be demonstrated, but the responses decreased in size both with hyperpolarization and depolarization of the membrane. A 'null point' of the response in the negative direction was found to be approximately -95 mV.

7. DLH responses were insensitive to changes in the internal Cl concentration. When the external K concentration was increased by K^+ ionophoresis, the DLH responses became smaller. It is concluded that the DLH response is probably mediated via a decrease in K^+ conductance and that the availability of this conductance channel is potential dependent.

8. Changes in the sizes of evoked potentials (e.p.s.p.s, i.p.s.p.s and a.h.p.s) with DLH and glutamate responses were investigated. The size of each of these evoked potentials was inversely related to G_M during the responses; thus they all showed stable increases during DLH responses. E.p.s.p.s recorded during DLH were of longer half-width and time-to-peak than the control, but there was no change in the maximum slope ($V.sec^{-1}$). When e.p.s.p.s decreased in size with glutamate the time-to-peak remained constant.

9. Acidic amino acids have been implicated as natural excitatory transmitters. The consequence of our results for the mechanism of excitatory transmission is therefore discussed.

INTRODUCTION

Dicarboxylic amino acids and their analogues have been shown to depolarize and excite neurones throughout the C.N.S. (Curtis & Johnston, 1974; Tebēcis, 1974). This excitation has been presumed to follow interaction of the agonists with a single common receptor (e.g. Curtis, Duggan, Felix, Johnston, Tebēcis & Watkins, 1972). The depolarization has also been tacitly assumed to be accompanied by an increase in G_M, although there have been relatively few direct measurements of conductance during amino acid induced excitation. The general notion is that these amino acids excite neurones, at least in part, by increasing P_{Na} (Zieglgänsberger & Puil, 1973; Curtis & Johnston, 1974).

The present investigation was precipitated by our finding that the DL-homocysteate evoked depolarization of motoneurones was accompanied by a stable *decrease* in G_M (Engberg, Flatman & Lambert, 1975*a*). We subsequently found qualitative differences between the depolarizations evoked by DLH and by L-glutamate.

The concept that dicarboxylic amino acids were implicated as natural transmitters was initially resisted (Curtis, Phillis & Watkins, 1960; Curtis, 1965). However, supported by histochemical and electrophysiological evidence, glutamate and aspartate have since been proposed to be the natural transmitters of excitation at a variety of synapses in the C.N.S. (Curtis & Johnston, 1974; Krnjević, 1974). More specifically, glutamate has a strong claim to be the transmitter of the Ia e.p.s.p. in motoneurones (Curtis *et al.* 1972; Duggan, 1974). Our results concerning the mechanism of amino acid induced excitation are particularly relevant in this connexion. A variety of studies have shown that the Ia e.p.s.p. behaves in a manner inconsistent with its proposed mediation via the classical increase in membrane permeability (e.g. see Klee, 1975).

Some of the results reported here have been published earlier as brief communications (Engberg *et al.* 1975*a*; Lambert, Flatman & Engberg, 1978).

METHODS

About twenty-five cats of either sex (2–3·5 kg) were used in this study.

Anaesthesia and surgery

In most experiments anaesthesia was induced by an I.P. injection of pentobarbitone (Nembutal®, 35 mg kg^{-1}) and maintained by regular injections of 5–10 mg via a catheter in the femoral vein. In a few cases, where the animals were anaemically decorticated, anaesthesia was induced by a mixture of O_2, N_2O and halothane. After insertion of a tracheal catheter, anaesthesia was maintained during surgery by a gas mixture of O_2, air and ether (from an AGA vapourizer, type ME27). After surgery, anaemic decortication was performed according to the procedure used by Engberg & Ryall (1966) and the anaesthesia discontinued. In these preparations the spinal cord was usually sectioned at T10–T12.

Blood pressure was continuously monitored via a cannula inserted in the femoral artery. When vascular failure threatened, blood volume was supplemented by I.V. infusion of a mixture of Macrodex® and Rheomacrodex®. In more severe cases a solution of noradrenaline (2·5 μg $ml.^{-1}$ in Ringer) was continuously infused. Body temperature was maintained during surgery

by heating the top of the dissecting table and during the recording period by heating through the stand in which the animal was mounted and by exposure to infra-red light as necessary. An I.M. dose of penicillin-procaine NOVO (60,000 u.) was given routinely.

Surgery

A tracheal cannula was inserted routinely. The left hind limb was dissected to isolate the nerves for subsequent stimulation. The nerve groups used were: hamstring (usually subdivided into anterior biceps + semimembranosus (absm) and posterior biceps + semitendinosus (pbst)); sural (sur); medial and lateral gastrocnemius + soleus (g-s); flexor digitorum longus + plantaris (fdl-p); posterior tibial (tib); superficial peroneal (sp); deep peroneal (dp). The spinal cord was exposed by a dorsal laminectomy of L4–L7 inclusive. A longtiudinal incision was made in the dura mater and the ventral roots of L7 and S1 were cut distally from the cord and freed for subsequent stimulation.

After surgery, the cat was immobilized with gallamine triethiodide (Flaxedil®, 20 mg I.V.) Paralysis was maintained with intramuscular injections (40 mg) as required. Intermittent positive pressure ventilation was maintained with a constant volume respirator. Respiration was adjusted to maintain the end tidal CO_2 at about 4 %. A bilateral pneumothorax was routinely performed to reduce respiratory pulsations of the spinal cord.

The cat was suspended in the experimental frame by clamps on vertebrae L3 and T6 and at the pelvis. Pools of warm paraffin oil were made in the left leg and the back. The nerves and ventral roots were mounted on bipolar Ag electrodes for constant voltage stimulation (Eide, 1972) using pulses of 0·05 msec duration. A silver ball cord dorsum electrode was positioned near the entry point of the L7 dorsal root. Nerve stimulating intensities are reported in terms of multiples of the threshold stimulating voltage of the largest fibres in the nerve (T). Before insertion of the micro-electrodes into the cord, a small area of the pia mater was removed with finely ground watchmaker's forceps.

Electrodes

Combined recording/ionophoretic electrodes of the concentric type were used (see Sonnhof, 1973). The tips of the central recording electrodes were broken back to 1·5 μm and were filled by boiling with a solution of 2·5 M-KCl (or 2 M-K acetate) in 1·5 % agar. Electrodes were selected for suitable resistances (3–8 MΩ) and a tip with a bevelled appearance. In some cases electrodes were filled by back pressure with 3 M-KCl. The electrode arrangement is illustrated in Fig. 1.

Recording electrodes were screened by spraying with graphite to within 1 mm of the tip (Engberg, Flatman & Lambert, 1975*b*). The outer ring of six or seven ionophoretic barrels was fabricated essentially as described by Sonnhof (1973). Each barrel contained one glass fibre made from the same glass. After pulling, the electrodes were broken back to 5–8 μm tip diameter. The barrels were filled with the drug solutions given below, and each solution was covered with a small amount of liquid paraffin. A little liquid paraffin was placed in the well of the ionophoretic assembly to act as a lubricant for the central electrode, which was introduced by hand under microscopic control until it protruded beyond the ionophoretic barrels by 40–60 μm. The electrodes were glued together at their shafts by Super Epoxy® glue. The electrodes were usually prepared the day before an experiment and stored at +4 °C in the dark with their tips under liquid paraffin.

The recording electrode was connected via a Ag/AgCl half cell to the input stage of the amplifier, which was in turn mounted directly on a step driven micromanipulator (Eide & Källström, 1968). A thick chlorided Ag wire buried in the muscles of the back served as the indifferent electrode. The principle described by Eide (1968) was used to inject current into the neurones and to compensate for the voltage drop across the recording electrode. The graphite screen was driven at × 1 amplification (band width 10 MHz). Square, negative going current pulses of short duration (usually 3 nA. 10–20 msec) were used to measure G_M. With efficient screening and neutralization of the residual capacitance, the electrode resistance compensation point was unambiguous. Electrode rectification and polarization was controlled for by passing the same currents with the electrode extracellular as had been used during the impalement. It was our experience, however, that the extracellularly recorded electrode properties were not necessarily in agreement with those recorded intracellularly.

Neuronal potentials were monitored on the following apparatus according to requirements:

audibly via an audio amplifier and loud-speaker; two Tektronix oscilloscopes with a storage monitor; medium speed ink jet recording (Elema-Siemens Mingograph Model 81); slow speed pen recording (Radiometer Servograph); digital display of membrane potential.

Ionophoresis

Gold wires connected the drug barrels to floating constant current generators ($Z_0 > 10^9\ \Omega$). Ionophoretic current was monitored by a meter placed in the return lead, which was separate from the recording ground. Drugs were not routinely retained, but if leakage was suspected a retaining potential of 0·5–0·7 V was used. Ejections of the amino acids (as anions) were normally electrically balanced by ejection of Na^+ from a diametrically opposite barrel (experience showed that when adjacent barrels were used, some of the agonist would be carried into the balancing barrel). The following drugs were used in this study: DL-mono-Na homocysteate (0·3 M, pH 7·9–8·1, 20–60 MΩ); L-mono-Na glutamate (1 M, pH 8·0–8·1, 30–65 MΩ); L-mono-Na aspartate (1 M, pH 8·4–8·8, 20–30 MΩ); NaCl (1 M, 8–15 MΩ); KCl (1 M, 10–20 MΩ).

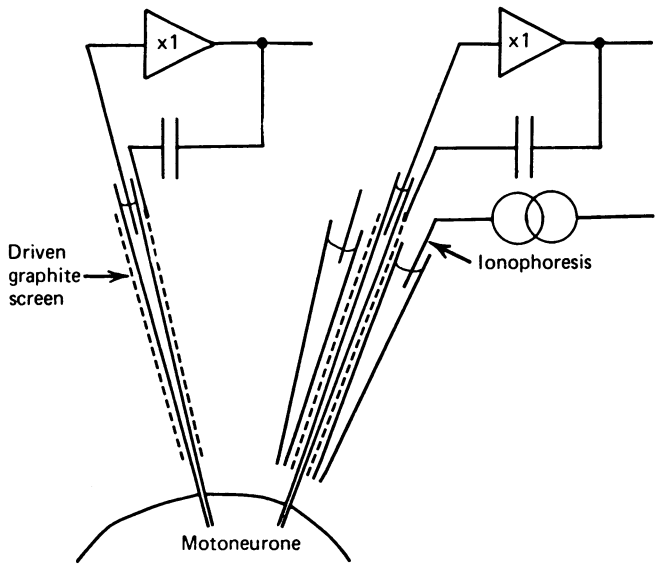

Fig. 1. Micro-electrode arrangements for recording and ionophoresis.

Double manipulator

For the intracellular injection of large currents it was necessary to impale the neurone with two independent electrodes, one to record and one to pass the currents (Fig. 1). The double micromanipulator used was that designed by Engberg, Källström & Marshall (1972). A neurone was first impaled with the usual recording/ionophoretic electrode and an action potential evoked by intracellular stimulation. This acted as a field point source and was used as a target when tracking with the second, single electrode. The tips of the electrodes were previously aligned outside the cord. Peripherally situated neurones in the sacral segments were the most receptive to double impalement.

General notes on the Figures

On the slow time base recordings of the membrane potential the fidelity of the recorder was not good enough to mark transient potentials which lasted for less than a few msec (e.g. the positive-going deflexions of action potentials). However, many of the records shown in the Figures are modulated by slower negative-going deflexions (e.g. a.h.p.s of antidromically evoked spikes, conductance measuring pulses, i.p.s.p.s etc.). No special mention of these deflexions is made unless they are of particular relevance.

Synaptic potentials and conductance measuring pulses were routinely averaged using a

Didac 800 signal averager. At least ten samples were taken both before and during the drug application. Averaging periods are indicated by a heavy black bar below the membrane potential record. In most cases the averaged records taken before and during a drug action are also shown superimposed to facilitate comparison. Most averaged records include a calibration pulse of 1 mV, 1 msec.

Records filmed directly from the oscilloscope were of two or three super-imposed traces. A filled circle adjacent to the membrane potential record marks the time of filming.

Information about the motoneurones given in the Figures includes (wherever possible) the muscle innervated by the neurone, the antidromically evoked action potential (with its first derivative) and the absolute membrane potential.

Ionophoretic applications of the amino acids are marked by arrows above the membrane potential records. Usually, electrically balanced ionophoretic applications were used and are designated by e.g. Na^+ DLH^- along with the current used. With unbalanced applications only one ion is nominated.

Unless otherwise indicated, all the material for the Figures was taken from cats which were anaesthetized with pentobarbitone.

Abbreviations used in the text and Figures

DLH, DL-mono-Na homocysteate; glutamate, L-mono-Na glutamate; aspartate, L-mono-Na aspartate; E_M, membrane potential; G_M, membrane conductance; R_M, membrane resistance; g, individual ionic conductance; a.p., action potential; a.h.p., afterhyperpolarization; e.p.s.p., excitatory post-synaptic potential; i.p.s.p., inhibitory post-synaptic potential; P, membrane permeability.

RESULTS

The results to be presented were obtained from a study which was largely concerned with the investigation of the effects of DLH on spinal motoneurones. Because of their physiological significance, a more limited study of the actions of both L-glutamate and L-aspartate was also performed. Qualitative differences in the actions of the amino acids are especially emphasized.

A. Actions of the amino acids on membrane potential

The responses obtained to the three amino acids are summarized in Table 1. On occasions, a neurone would not respond to one, two or any of the three amino acids. Following small electrode movements, however, responses could then be evoked. This implies that before the movements the access of the amino acids to the neuronal membrane was hindered. Three possibilities exist: the ionophoretic barrels were too far away from the impaled neurone; a diffusion barrier was interposed between the ionophoretic barrels and the neurone (e.g. glia); one or more of the ionophoretic barrels had poor drug releasing properties. There was sometimes a correlation between a particular electrode and the failure to evoke responses. Therefore, the significance of negative results is obscure and they are not included in Table 1.

1. Depolarization and repetitive firing

Typical responses to DLH and glutamate are shown in Fig. 2. These were obtained from the same cell. Neither drug depolarized the cell sufficiently to reach the threshold for firing. The responses have very characteristic shapes. The DLH response is slow both in onset and recovery. The glutamate response, however, is altogether squarer; it rises quickly to a plateau, and the membrane repolarizes rapidly when the drug is turned off. Another common feature of the glutamate response is the

post-response hyperpolarization (Figs. 2 and 13) (see also Zieglgänsberger & Puil, 1973).

The shape of aspartate responses was more similar to that of glutamate than of DLH.

When DLH induced sufficient depolarization repetitive firing resulted (Fig. 5*A*). The firing was stable and showed little tendency to adapt despite DLH applications lasting for 2 min or more. Only exceptionally did DLH cause the cell to pass through a phase of firing into a quiescent state during continued application of the drug.

TABLE 1. Summary of the responses of lumbar motoneurones to three amino acids (eighteen cats). Cells showing no responses are not included. Depolarizing responses to glutamate include those which showed a post-response hyperpolarization

Response	DLH	Glutamate	Aspartate
Depolarizing	90	58	23
Hyperpolarizing	7	7	1
Biphasic	3	1	0
Inversion of polarity of response during impalement	8	6	2
Total number of cells	108	72	26

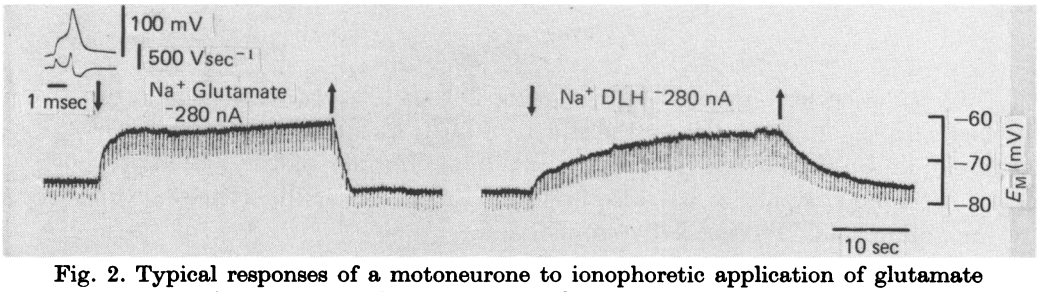

Fig. 2. Typical responses of a motoneurone to ionophoretic application of glutamate and DLH. Both drugs reversibly depolarized the membrane. The glutamate response is characterized by a fast onset and fast recovery with a post-response hyperpolarization. The DLH response is characterized by a slow onset and slow recovery. Neither response was large enough to reach the threshold for firing (refer to the Methods section for the conventions used in this and subsequent Figures).

Generally, glutamate was unable to evoke and maintain repetitive firing. A typical example of a large glutamate response is shown in Fig. 3*A*. The membrane quickly depolarized, and firing resulted when the threshold was reached. The firing soon ended despite the maintained plateau of depolarization. (The small sag in the plateau is a fairly typical feature of large glutamate responses.) The large dose of glutamate used in Fig. 3*A* may have been so excessive as to cause a depolarizing block (Crawford & Curtis, 1964). Fig. 3*B* shows a glutamate response from another cell where the dose was adjusted to be the minimal to evoke firing. Again, the evoked firing was of relatively short duration although the depolarization was maintained. On rare occasions, however, longer repetitive firing could be evoked by glutamate.

Firing evoked by passing a positive current through the recording electrode was generally very persistent. It showed little tendency to adapt even with currents much greater than required to attain the firing threshold.

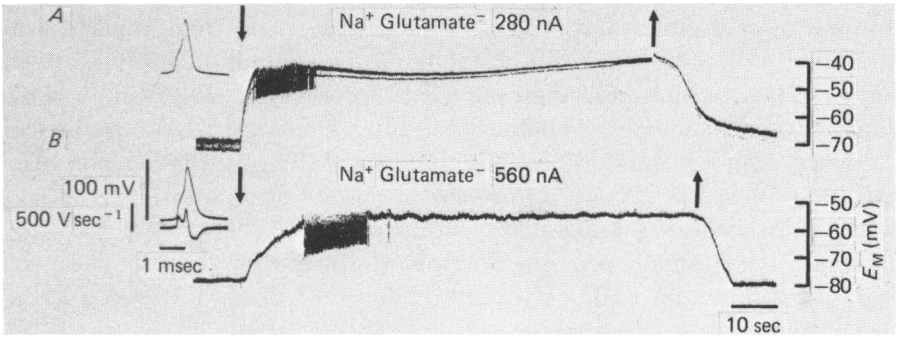

Fig. 3. Large glutamate responses. The thick black bands on the traces indicate periods of repetitive firing. *A*, deep peroneal neurone. Balanced glutamate application evoked a rapid depolarization and a brief burst of repetitive firing. The firing ended quickly despite the maintained depolarization. *B*, tibial neurone. Similar response to that shown in *A*, except that the dose of glutamate was adjusted to be the minimum required to evoke firing. Again, the firing was only of short duration.

2. Atypical responses

Depolarizations were considered to be the typical responses to DLH and glutamate. As can be seen from Table 1, both substances evoked both hyperpolarizing and biphasic responses. Collectively, these may be considered to be atypical responses. Some examples of these are shown in Fig. 4.

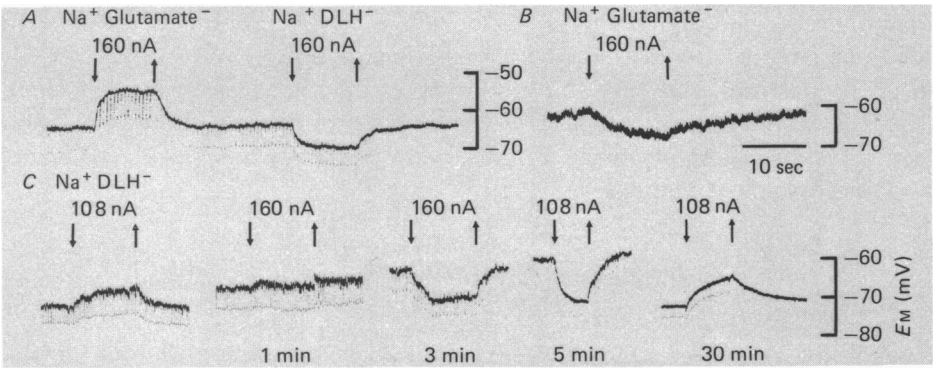

Fig. 4. Atypical responses to DLH and glutamate. *A*, fdl neurone. DLH gave a hyperpolarizing response while glutamate depolarized the membrane in the usual manner. *B*, tibial neurone. Glutamate gave a hyperpolarizing response. *C*, fdl neurone. All traces selected from a single impalement and mounted with respect to the resting membrane potential. Initially DLH evoked a small depolarization. 1 min later there was virtually no response. Later still (3 and 5 min) hyperpolarizing responses were recorded. This change in polarity of the response was accompanied by a depolarization of the membrane. The electrode was then moved to improve the impalement. The DLH response then reverted to a depolarization (30 min).

There was no strict correlation in the occurrence of atypical responses: cells might be hyperpolarized by both amino acids, or by one of the amino acids while the other gave rise to a normal depolarizing response (Fig. 4*A*, *B*). In other cases, responses were seen to change their polarity during the course of the recording from a cell. A dramatic example of this is illustrated in Fig. 4*C*. Just after the impalement, DLH evoked a normal depolarizing response. One minute later the

response had disappeared, and after 3 min it had turned into a hyperpolarization. The sequence was obtained while the membrane potential slowly, but steadily depolarized. The electrode was then moved to improve the impalement. Following this manipulation the membrane potential improved and the DLH response reverted to a depolarization. It should be noted that the plateaux of the depolarizing and hyperpolarizing responses overlap in terms of absolute potential, i.e. the responses do not asymptote towards a common level of potential.

Occasionally, recordings were made from motoneurone axons. These did not usually respond to amino acid application. In four instances, however, DLH evoked hyperpolarizing responses. These are not included in Table 1.

Atypical responses were probably not current artifacts *per se* since ejections of Cl^- from a nearby ionophoretic barrel caused only a DC coupling, or even a small depolarization.

An attempt was made to compare electrophysiological information from cells with atypical responses with that from cells with the more usual depolarizing responses. No consistent theme emerged. In some of the atypical cells the resting membrane potentials were, by normal standards, low (-65 mV or less) as were the input resistance values (< 1 MΩ). There were, however, a number of exceptions where hyperpolarizing responses were recorded from cells which were in 'good' condition.

When atypical responses were evoked, it was often possible to demonstrate that the ionophoretic electrode was very near to the cell membrane. Unbalanced drug applications gave a large DC coupling, and compensation of this was not always achieved by passing a positive current of equal magnitude from one of the other barrels. This would indicate an electrical asymmetry of the ionophoretic barrels with respect to the recording electrode. Further, comparatively low doses of the agonists were required to elicit responses.

B. Conductance changes during amino acid responses

1. DLH responses

Depolarizing responses to DLH were found to be accompanied by a *decrease* in membrane conductance (G_M). An experiment to demonstrate this is shown in Fig. 5*B*. The voltage transients result solely from the potential change across the cell membrane produced by the injected current (see Methods).

Before the application of DLH, G_M was 1·26 μmho. During DLH the transient was of insufficient duration to come to a plateau. By extrapolation G_M was estimated to be 0·65 μmho. After the DLH application both the membrane potential and G_M returned to their control values.

The technique outlined above was used to investigate G_M during DLH responses in forty-two cells. In forty of these there was a decrease, in one no change and in one an increase in G_M during DLH. The decrease in G_M during DLH was extremely stable and persisted throughout responses lasting 2 min and longer. There was no significant change in G_M during Cl^- control applications (using larger currents than for DLH).

Most neurones exhibited anomalous rectification (cf. Nelson & Frank, 1967),

however, the decrease in G_M measured during DLH depolarizations was usually greater than could be accounted for by the passive current–voltage (I–V) properties of the membrane. It was usually possible to bring the membrane potential to the same level as achieved during small DLH responses by passing tonic depolarizing currents through the recording electrode, thereby controlling for the influence of rectification. An example of such an experiment is shown in Fig. 6. In very few cases the values for G_M measured during DLH were either coincident with or very close to the passive G_M–V curve.

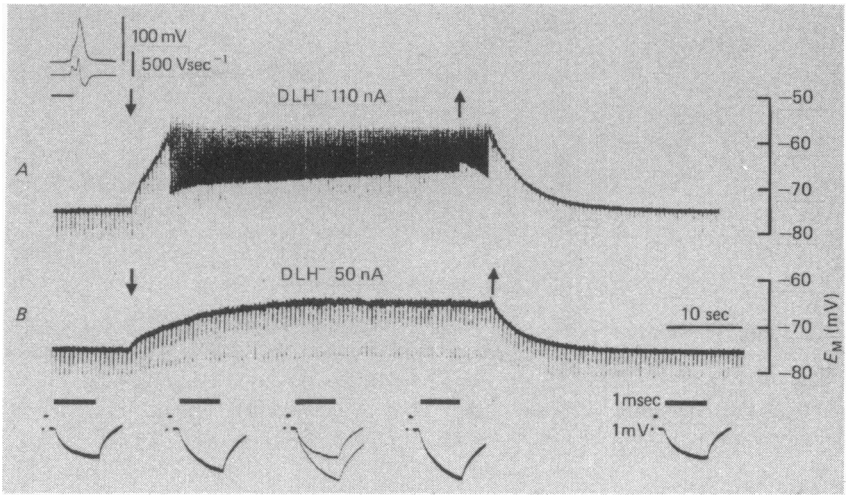

Fig. 5. Depolarizing responses evoked by DLH in an absm neurone (same neurone as in Fig. 8). *A*, an unbalanced application of DLH (−110 nA) evoked stable repetitive firing when the membrane had depolarized by 15 mV. The thick black band on the trace represents the a.h.p.s of the spikes. *B*, a decrease in G_M during a DLH response. A smaller dose of DLH (−50 nA) caused a depolarization of 10 mV, which was not sufficient to evoke repetitive firing. Ten conductance measuring pulses (3 nA, 10 msec) were averaged during the periods shown by bars. A decrease in G_M during DLH is demonstrated by the larger voltage transients recorded during the response. For comparison, the transient recorded before DLH application (smaller) is superimposed on that recorded during DLH in the middle record. Following the response both the membrane potential and conductance returned to their control values.

Measurements of conductance at high levels of depolarization produced by DLH were precluded by the repetitive firing. The conductance changes underlying the a.p. were superimposed on those caused by DLH. It is worth noting, however, that there was often a marked decrease in G_M *just* before DLH induced firing (cf. Fig. 2 in Schwindt & Calvin, 1973).

2. Glutamate responses

Glutamate depolarizations were accompanied by a small decrease, no change or a small increase in G_M (Fig. 7). With large, long lasting applications of glutamate there was often a biphasic change in G_M. An initial decrease in conductance was slowly eroded and subsequently replaced by an increase in conductance. An example of such a glutamate response is shown in Fig. 8. The following points should be emphasized: the conductance was still less than the control level after the firing

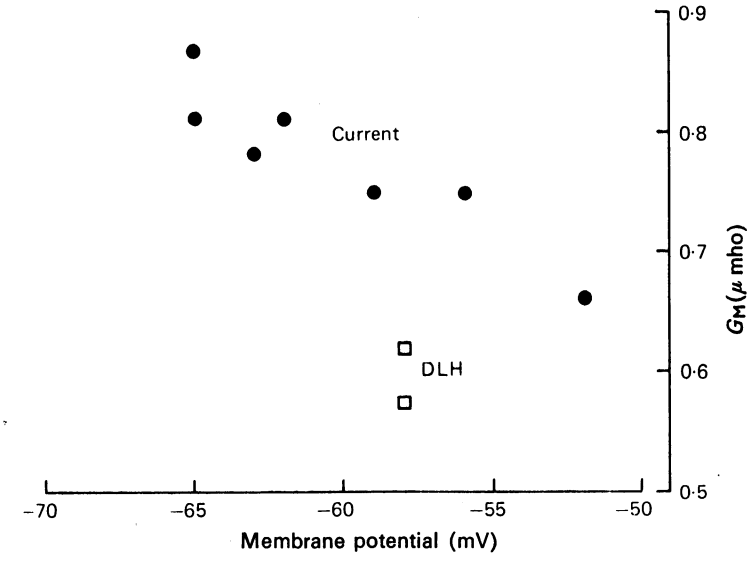

Fig. 6. Conductance decrease during DLH as compared with the G_M/V relation of the passive membrane. Hamstring neurone. The G_M recorded during two DLH responses (□) was less than that produced when the membrane was depolarized by current through the intracellular eletrode (●). During the course of the impalement, the resting membrane potential was between −62 and −65 mV.

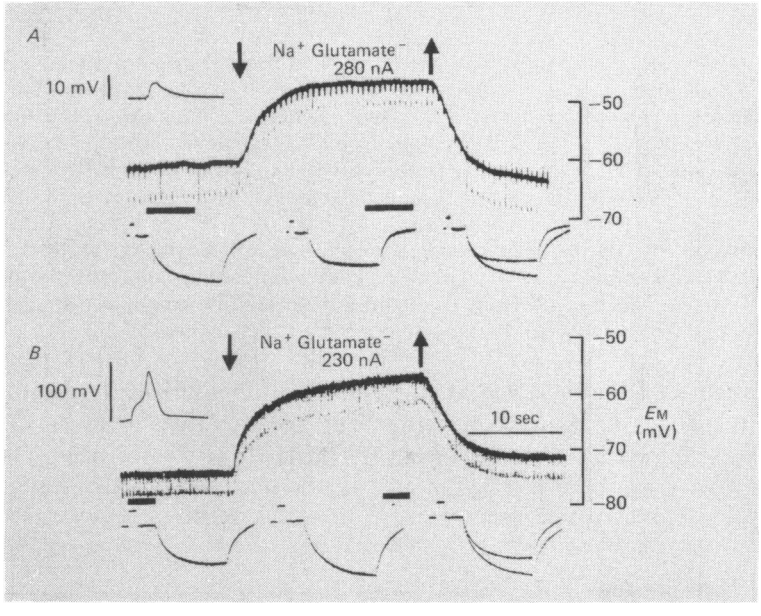

Fig. 7. Changes in G_M during glutamate responses. The responses have been selected for being approximately the same in size and duration. Neither of the responses attained the threshold for firing. Averaged conductance measuring transients: control on the left; during the plateau of the response in the middle; transients superimposed to facilitate comparison on the right. *A*, unidentified motoneurone. A decrease in the size of the transient during the glutamate response indicates an increase in G_M. *B*, deep peroneal neurone. An increase in the size of the transient during the glutamate response indicates a decrease in G_M.

had ended; the change from a low to a high conductance state occurred during the plateau in the response; when the glutamate was turned off, the membrane potential returned more quickly to the control level than did the conductance; there was no desensitization to glutamate despite a long duration application (see also Fig. 3). Similar time dependent changes in conductance were seen with glutamate responses which failed to evoke firing.

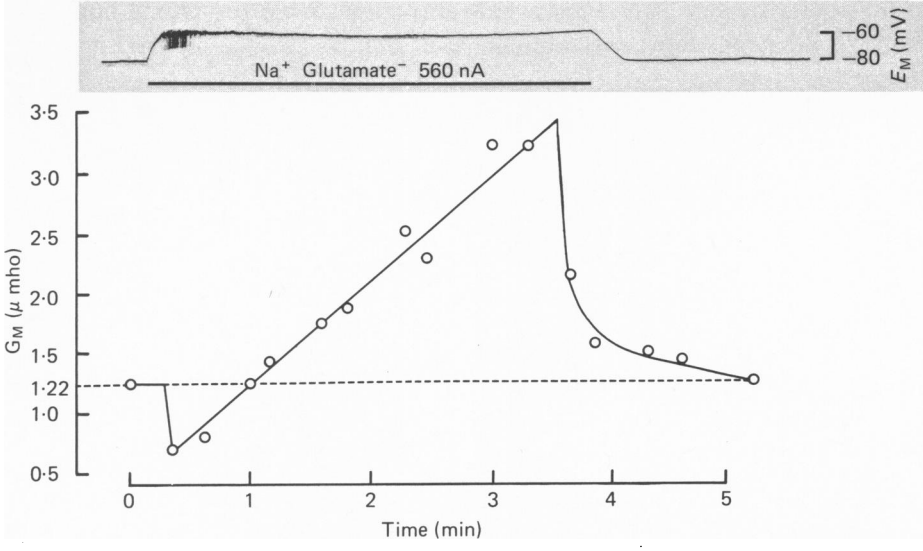

Fig. 8. Changes in G_M during a large glutamate response. Absm neurone. Same cell as in Fig. 5. Conductance values are plotted on the graph vertically below the point in the response at which they were averaged. Line drawn through points by eye. During the first part of the response G_M decreased. Thereafter G_M increased throughout the plateau of the response. When the glutamate was turned off, the membrane potential returned to the control value more quickly than did the conductance.

Often, glutamate could be applied for several minutes without evoking a time dependent increase in G_M, although depolarizing the membrane by up to 20 mV. When compared with the passive G_M–V relationship the over-all effect of glutamate was usually to cause a small, but stable, increase in G_M (see below).

3. Direct comparison of conductance changes induced by DLH and glutamate

Fig. 9 illustrates the changes in G_M underlying a range of DLH and glutamate responses in the same cell. A small dose of DLH was first applied to the neurone and G_M was measured when the response had attained a stable plateau. This procedure was repeated for increasing doses of DLH until the responses were large enough to evoke firing. A control value for G_M was obtained before each application of the drug. A similar set of results for glutamate was then obtained. Finally, a current of + 10 nA was passed through the recording electrode and G_M was measured at the level of membrane potential reached. The graph shows that anomalous rectification was present (G_M decreased when the cell was depolarized) and also that the depolarizations produced by both DLH and glutamate were accompanied by a decrease in G_M. When compared at the same membrane potential the conductance

during glutamate was greater than that of the current depolarized membrane, whereas that during DLH was smaller.

C. *Changes in evoked potentials during DLH and glutamate responses*

1. *Afterhyperpolarizations and i.p.s.p.s*

During DLH responses, a.h.p.s and i.p.s.p.s increased in amplitude in proportion to the depolarization. The increase was stable and persisted throughout long applications of the drug. Examples are shown in Fig. 10.

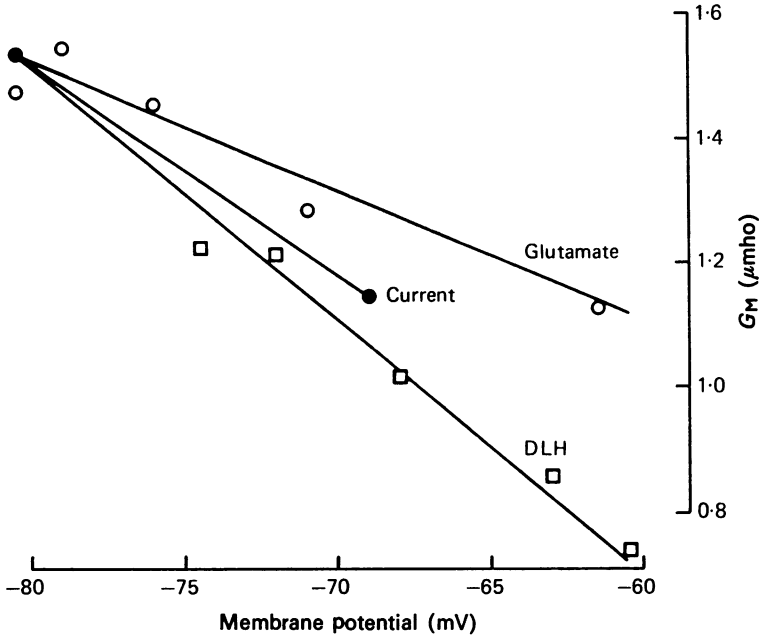

Fig. 9. Comparison of conductance changes during DLH and glutamate induced depolarizations in the same cell. Absm neurone. The amino acids were applied for 20 sec and values for G_M were obtained from ten averaged voltage transients recorded 15 sec after the start of the drug application, when the responses were stable. Conductances are plotted at the absolute levels of membrane potential at which they were recorded (see text for further details). Lines drawn through the points by eye. Both DLH (□) and glutamate (○) depolarizations were accompanied by a decrease in G_M. Depolarization of the membrane by injected current (+10 nA) also produced a decrease in G_M (●), which was greater than that seen with glutamate, but less than that with DLH.

The changes in a.h.p. size during glutamate responses were dependent on the conductance changes induced. A.h.p.s were recorded during the glutamate response shown in Fig. 8. At the beginning of the response, when G_M was less than the control value, the a.h.p.s increased in size. Thereafter, they decreased in parallel with the increase in G_M. At the point in the response where the conductance passes through its control level, the a.h.p.s were still 40% larger than their control. This is to be expected since at this point the membrane was depolarized by 18 mV.

The change in size of i.p.s.p.s during glutamate responses also depended on the G_M during the depolarization. Fig. 10*B* shows a trace from a deep peroneal neurone

where the i.p.s.p. changes during DLH and glutamate responses may be directly compared. During DLH the i.p.s.p. increased to 200%, while during glutamate it increased to 220% of the control. The i.p.s.p.s during glutamate, however, were sampled when the membrane potential was at a more depolarized level than during DLH. With larger glutamate responses there was a transient increase in the size of the i.p.s.p., but this faded as the membrane turned into the high conductance state.

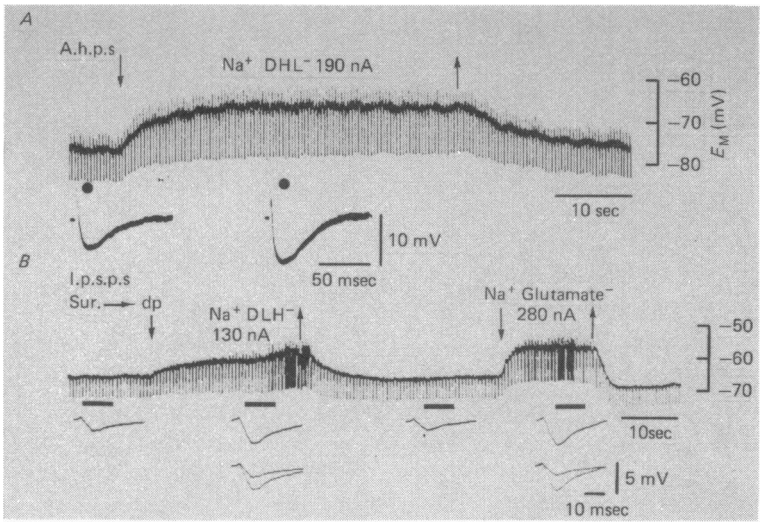

Fig. 10. Changes in evoked potentials during amino acid induced depolarizations. *A*, a.h.p.s during DLH. Hamstring neurone. Antidromic a.p.s evoked by close to threshold stimulation of the ventral root. Renshaw inhibition was virtually absent at this strength. A.h.p.s are shown both as negative deflexions on the membrane potential record and as photographs of three superimposed oscilloscope sweeps taken at the times indicated by the filled circles. During the DLH response of 9 mV there was a stable increase in the a.h.p. to approximately 150% of the control. *B*, comparison of i.p.s.p. changes during small DLH and glutamate responses (just at firing threshold). Dp neurone. Sur. stimulated at $2{\cdot}5 \times T$. The i.p.s.p. increased in size during both drug responses. Bottom row shows i.p.s.p.s superimposed to facilitate comparison.

2. *E.p.s.p.s*

As dicarboxylic amino acids may act as excitatory transmitters in the spinal cord (Curtis & Johnston, 1974) special attention was paid to changes in the configuration of e.p.s.p.s during amino acid induced depolarizations.

To obtain meaningful results these experiments had to be very strictly controlled. Wherever possible monosynaptic e.p.s.p.s were used. These were evoked by stimulation of the appropriate muscle nerve at just over threshold strength to avoid contamination by polysynaptic e.p.s.p.s and i.p.s.p.s. At least ten e.p.s.p.s were averaged both before and during the drug response. During the response the membrane potential approaches the threshold for firing, thus a.p.s often occurred during the averaging periods. Such results were discarded. It was also necessary to ascertain that no 'local responses' occurred during the averaging period. To control for this eventuality, photographs of the individual oscilloscope traces were taken before, during and after the averaging period. From these photographs it was also possible to check that the e.p.s.p.s obtained during the drug response were not contaminated by small asynchronous p.s.p.s. (The occurrence of such small p.s.p.s during drug application was taken to indicate that the drug had diffused and excited nearby interneurones with synaptic connexions to the impaled motoneurone). Finally, extracellular field potentials that may influence the recording of the e.p.s.p.s were checked with the electrode withdrawn to just outside the neurone.

E.p.s.p.s during DLH. During DLH responses the amplitude of the e.p.s.p. *increased* without any change in the initial rising phase. Consequently, e.p.s.p.s obtained during DLH would continue rising to a higher and later peak. The falling phase of the e.p.s.p. during DLH was usually more 'filled out' than the control (Fig. 11), but on rare occasions the membrane potential recovered at a faster rate than the control, so giving the e.p.s.p. a very humped appearance. Marked changes in e.p.s.p. configuration were recorded even when DLH caused a depolarization of only 1–2 mV.

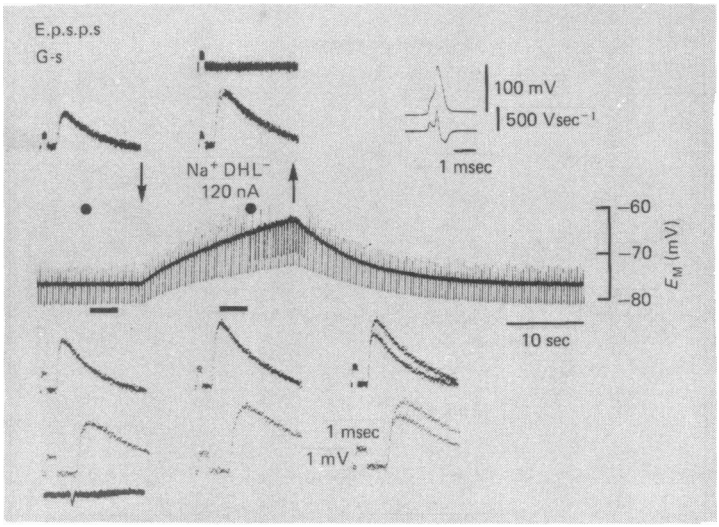

Fig. 11. Changes in the configuration of a monosynaptic e.p.s.p. during a DLH induced depolarization. Same g-s neurone as in Fig. 13. Stimulation of the g-s nerve at $1{\cdot}15 \times T$ (dorsal root volley shown at the bottom left corner of the Figure). Averaged e.p.s.p.s (at two time scales) are mounted below the membrane potential record. The e.p.s.p.s during DLH application were averaged just before they evoked firing. Sample oscilloscope records of three superimposed e.p.s.p.s (taken at the times indicated by the filled circles) are shown above the membrane potential record. The oscilloscope trace above the e.p.s.p. recorded during DLH is a control record to show the absence of extracellular fields. During DLH the monosynaptic e.p.s.p. increased in amplitude. Both the time-to-peak and the half-width increased, but there was no change in the maximum rate of rise.

In Fig. 11 the e.p.s.p.s during DLH were averaged just before they evoked firing (the a.h.p.s of orthodromically evoked spikes are visible at the peak of the response; the other negative going deflexions present throughout the trace are the a.h.p.s of antidromic spikes). In addition to the averaged e.p.s.p.s, photographs from the oscilloscope of three superimposed traces are also shown.

The e.p.s.p. parameters suggested by Rall, Burke, Smith, Nelson & Frank (1967) and by Smith, Wuerker & Frank (1967) were measured for the e.p.s.p.s in Fig. 11 and are given in Table 2. During DLH the e.p.s.p. increased in amplitude by 28%, there being no detectable change in the maximum slope of the rising phase. The time-to-peak and the half-width also increased.

E.p.s.p.s during glutamate application. Changes in e.p.s.p. shape during glutamate responses were largely dependent on the value of G_M at the time of sampling.

Generally, when glutamate caused a decrease in G_M the e.p.s.p.s were increased in size and vice versa. The e.p.s.p.s usually fluctuated much more during glutamate responses than in the control situation. Changes in the e.p.s.p. size with glutamate were often accompanied by changes in the rate of rise which tended to keep the time-to-peak constant. This was especially evident when the size of the e.p.s.p. was reduced (Fig. 12). When e.p.s.p.s were increased in size during glutamate the falling phase was not filled out to the same extent as seen with DLH.

TABLE 2. The change in e.p.s.p. characteristics during the DLH response shown in Fig. 11

	Peak amplitude* (mV)	Maximum slope* (V/sec)	Slope/peak amplitude* ($msec^{-1}$)	Time-to-peak* (msec)	Half-width* (msec)
E.p.s.p.					
Control†	2·88	5·55	1·93	1·11	4·89
During DLH† (and % change over control)	3·69 (+28%)	5·55 (0%)	1·50 (−22%)	1·55 (+40%)	6·11 (+25%)

* These parameters are the same as those used by Rall *et al.* (1967) and Smith *et al.* (1967). *Time-to-peak* is the time taken between the first positive deflexion from the resting membrane potential to the peak of the e.p.s.p. *Half-width* is the duration between points on the rising and falling phases of the e.p.s.p. which are at one half of the peak amplitude.

† Resting membrane potential −77 mV. The average membrane potential at sampling during the response was −67·5 mV.

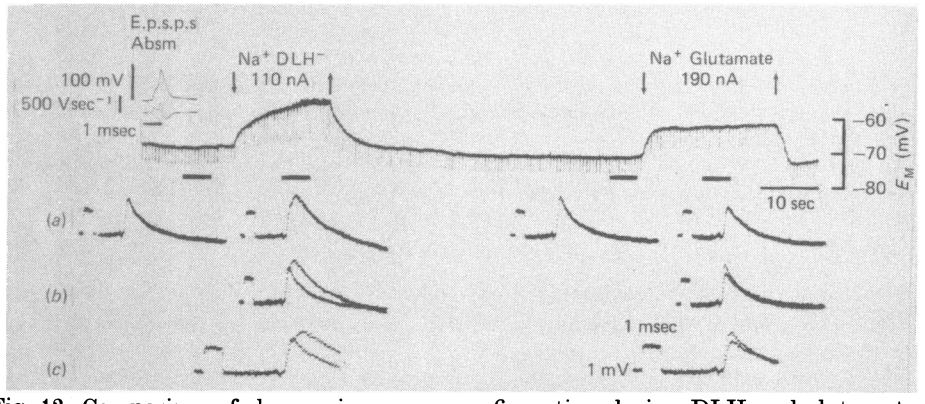

Fig. 12. Comparison of changes in e.p.s.p. configuration during DLH and glutamate responses. A.b.s.m. neurone. Averaged e.p.s.p.s are mounted below the membrane potential record (line *a*). They are also shown superimposed in line *b* and at an expanded time scale in line *c*. During DLH the e.p.s.p. increased in amplitude, the time-to-peak increased and the maximum rate of rise was unchanged. During glutamate the e.p.s.p. decreased in amplitude, the time-to-peak was unchanged and the maximum rate of rise was decreased.

Fig. 12 demonstrates the changes in e.p.s.p.s recorded during DLH and glutamate responses in the same cell. During DLH the e.p.s.p. increased in size and its peak was delayed. During the glutamate response, however, the e.p.s.p. decreased in size (despite sampling at a more negative potential than during DLH) and the time-to-peak was the same as the control.

Fig. 13 is a graphical representation of e.p.s.p. changes during very long DLH

and glutamate responses of similar size. These results and those in Fig. 11 were obtained from the same cell. With DLH the size of the e.p.s.p. increased slowly until, at the plateau of the depolarization, it had attained a stable maximum size. When the ionophoretic current was turned off the e.p.s.p. quickly returned to a value slightly smaller than the control and then recovered fully.

The glutamate response in Fig. 13*B* is qualitatively similar to that in Fig. 8 in that there was a biphasic change in G_M. The e.p.s.p. transiently increased in size during the rising phase of the response. Thereafter it slowly decreased in size while the depolarization was maintained. This reduction in e.p.s.p. was in parallel with the increase in G_M during the plateau. On turning off the glutamate ejecting current the e.p.s.p. recovered to near its control value.

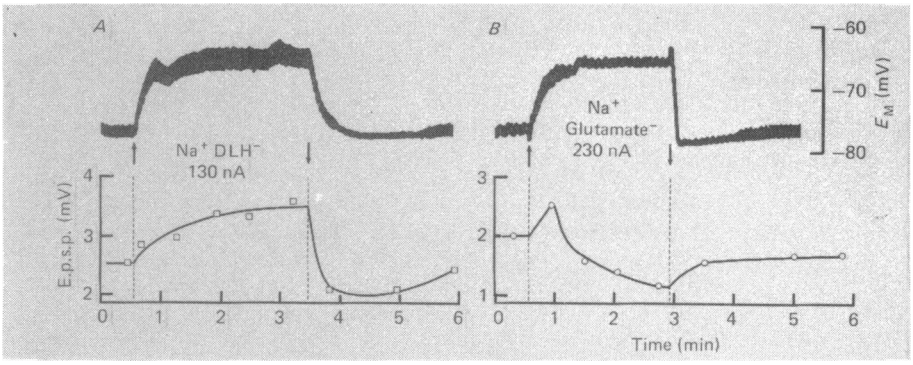

Fig. 13. Changes in e.p.s.p. size during long-lasting DLH and glutamate depolarizations. Same g-s neurone as in Fig. 11. Stimulation of the nerve at $1{\cdot}15 \times T$. E.p.s.p. sizes were measured from the average of ten samples and are plotted below the membrane potential record at the times at which they were taken. *A*, during DLH the e.p.s.p. increased slowly in size to a stable level. *B*, during glutamate the e.p.s.p. transiently increased in size during the rising phase of the response. Thereafter it declined while the depolarization was maintained.

D. Polarization experiments

It is possible that the depolarizing response to DLH is mediated by a decrease in conductance to ions that have an equilibrium potential more negative than the resting potential. Experiments were conducted to investigate how the DLH response changed when the membrane potential was manipulated by intracellular current injection. Two methods were used to inject the current: (1) through the single recording electrode while its resistance was balanced out; (2) by independent impalement of the neurone with two electrodes such that one electrode could be used to record the potential while the other was used to pass polarizing currents (Engberg *et al.* 1972).

1. Single impalement of neurone

Current was injected through the recording electrode and, providing the electrode resistance was accurately compensated, the change in the potential record during current passage was a reliable indication of the absolute membrane potential. However, problems with electrode polarization and rectification usually meant that only results obtained with relatively small currents (< 30 nA) could be relied on.

(We found very little correlation between the rectifying properties of electrodes as investigated in artificial saline and their actual performance in the spinal cord.)

Preliminary experiments with tonic currents showed that the DLH response decreased in size when the membrane was hyperpolarized. Extrapolation indicated a null point at about -95 mV.

Dynamic current–voltage curves. I–V curves were obtained by passing a ramp of negative current through the recording electrode. A train of short duration -3 nA current pulses was superimposed on the ramp. This was used to check that the electrode did not change its resistance during the passage of the current. The absence of electrode polarization was controlled for by passing the same current ramp with the electrode extracellular.

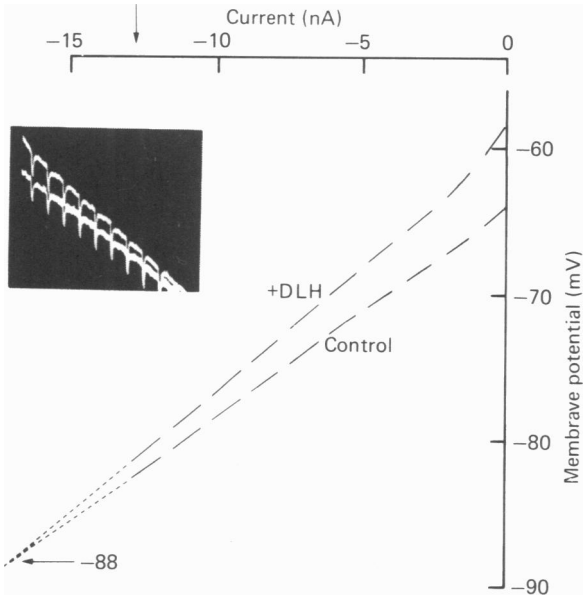

Fig. 14. Dynamic I–V curves recorded for the resting membrane and during an iontophoretic application of DLH. Absm neurone. The curves were obtained by passing a linearly increasing current ramp of 50 nA sec^{-1} through the recording electrode (the arrow on the abscissa marks the limit of the current). The curves, which are plotted on conventional axes, were traced from a photograph taken from a storage oscilloscope (shown in inset). The larger gaps in the curves mark the positions of short duration constant current pulses which have been edited from the tracing. These were used to check the electrical properties of the electrode (see text). The curves have been extrapolated (closely interrupted lines) to a point of intersection at approximately -88 mV.

Examples of I–V curves are shown in Fig. 14. The control curve is almost linear and gives a value for G_M of 0·73 μmho. During the DLH evoked depolarization the I–V curve is both steeper and initially more curved than the control. The slope conductance at the start of the curve is 0·42 μmho, while it is 0·61 μmho for the later, linear part of the curve. Extrapolation of the two curves gives an intersection at -88 mV.

The results from both types of experiments given above suggest that the DLH response is zero in the vicinity of E_K.

2. Double impalement of neurones

The introduction of two electrodes independently into the same cell is especially difficult when one electrode has the configuration of our standard recording-ionophoretic unit. The number of successful double impalements per cat was therefore very small. However, the coupling problems (capacitive and resistive) asso ciated with double-barrelled electrodes were circumvented with this technique.

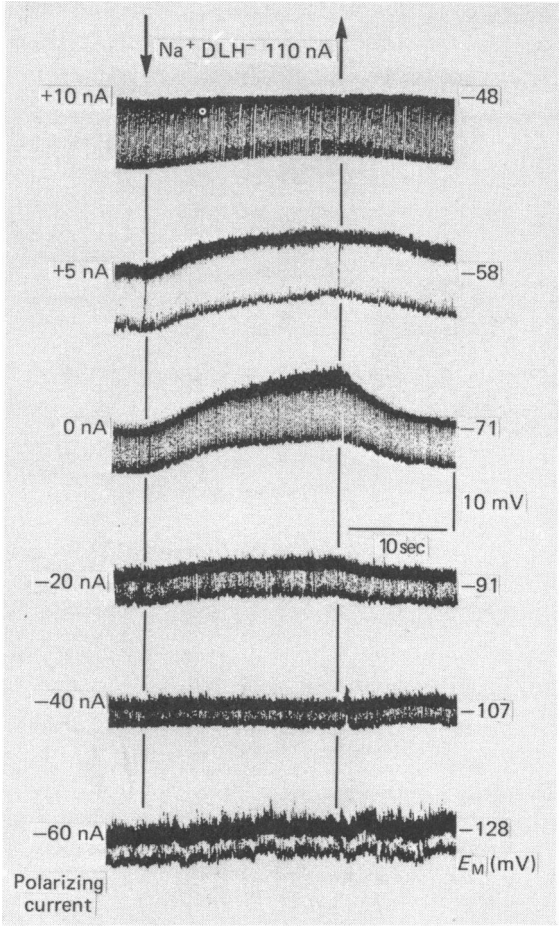

Fig. 15. Changes in DLH response during tonic polarization of the membrane. Double impalement of neurone; potential recorded via one electrode, current injected through the other. The DLH response decreased in size both when the membrane was hyperpolarized and when it was depolarized. The currents used are shown on the left of the traces and the membrane potentials during the passage of these currents on the right.

After double impalement there was occasionally a slight difference in the magnitude of the recorded potentials from the two electrodes; if so, the one with the highest potential was used for recording, the other to polarize.

Two types of experiments were performed: (1) investigation of changes in the DLH response during tonic polarizing currents, and (2) investigation of the changes

in the amplitude of transients produced by large current pulses during DLH responses.

Tonic polarization. After obtaining a control response to DLH the membrane potential was altered by passing constant currents of different magnitude through the second electrode. The DLH response was tested at each level of polarization (Fig. 15). Between each injection of polarizing current the control response was retested (the control response did not recover immediately after long polarizations with -40 and -60 nA). The results show that the DLH depolarization was maximal when the cell was at its resting potential. It decreased in size with excursions of the membrane potential in either a depolarizing or hyperpolarizing direction. There is, therefore, an envelope of membrane potential, between approximately -45 and -100 mV, inside which depolarizing DLH responses could be evoked.

Small constant current pulses were continuously injected (through the recording electrode) to measure slope conductances.

At the most negative levels of membrane potential DLH caused little or no depolarization with no detectable change in G_M. At less negative levels, DLH caused only a small decrease in G_M over the passive conductance–voltage relationship of the membrane.

The results obtained at -48 mV deserve special comment, since at this level the voltage transients sagged markedly (despite their short duration of 10 msec). During DLH this sag increased as may be seen in the modulation of the top trace in Fig. 15.

Pulse polarization. The pulses used in these experiments were large enough to hyperpolarize the membrane to -90 mV or more. The currents required depended on the resting membrane potential of the cell, but were usually between -20 and -80 nA. Measurements from the voltage transient would then give a value for the chord conductance of the membrane (Jack, Noble & Tsien, 1975). Theoretically, if there is a membrane potential level at which the DLH depolarization reverses, this would be apparent from the change in shape of the pulse envelope during DLH. An advantage of this method is that any conductance changes and ionic concentration changes of very slow time course (which might be associated with long duration polarizations) would be avoided.

In Fig. 16 DLH is shown to give 9 mV depolarization at the resting potential, and at the level of the pulses about 4·5 mV. Pulses of -40 nA in the same cell (not illustrated) gave a pulse plateau level of -97 mV and at this level the DLH depolarization was reduced to some 3 mV. Similar results were obtained with other cells; the DLH depolarization was reduced in proportion to the amount of hyperpolarization, but in no case could we obtain a reversal.

A feature of the large voltage transients was that the potential would overshoot and subsequently decay back to a plateau (cf. Ito & Oshima, 1965). The size of the overshoot increased proportionately with the increase in potential. During DLH it was usually possible to detect a decrease in this overshoot.

E. Effects of ionic concentration changes

Chloride. For most of this investigation the recording electrode was filled with a strong solution of KCl. Therefore, there was always a tendency to increase $[Cl]_i$

either by simple diffusion of Cl^- from the electrode, or by injection during the passage of negative currents. This would cause E_{Cl} to move positively. The i.p.s.p. is mediated, at least partially, by an increase in P_{Cl} and thus may be used as a guide to the position of E_{Cl}. As E_{Cl} moves positively, the i.p.s.p. will reverse into a depolarizing potential (Coombs, Eccles & Fatt, 1955*b*). No significant change in the DLH response was observed following the reversal of the i.p.s.p. into a depolarizing potential. On a few occasions the recording electrode was filled with either K acetate or K citrate. The DLH responses were essentially identical to those recorded with KCl filled electrodes. It may therefore be concluded that the response is not primarily mediated *via* a decrease in P_{Cl}.

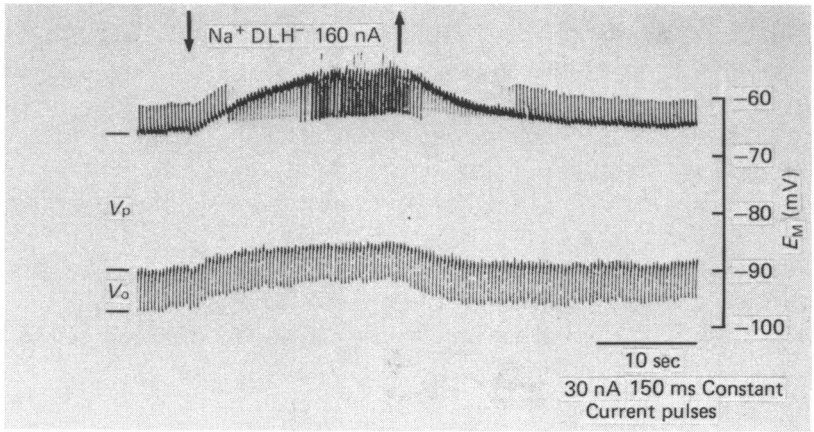

Fig. 16. Changes in large voltage transients during DLH. G-s neurone. Double impalement. Potential was recorded via the central intracellular electrode of the coaxial assembly; constant current pulses (30 nA, 150 msec) were injected through the second intracellular electrode. The membrane potential recording is modulated by the evoked voltage transients, only the overshoots and plateaux of which can be seen. DLH gave a response of 9 mV and evoked a period of repetitive firing. During DLH there was a clear increase in the plateau potential (V_p) and a small decrease in the overshoot potential (V_o). (The upward deflexions at the beginning and end of the upper trace are due to rebound overshoots after each hyperpolarizing pulse. During the DLH depolarization they disappear because they then evoke action potentials).

Potassium. In the experiment shown in Fig. 17 the neurone was especially sensitive to DLH (21 nA was sufficient to evoke a depolarizing response of 9 mV and a period of repetitive firing). During the balanced extracellular application of K^+ Cl^- the DLH induced depolarization gradually diminished, and the duration of repetitive firing decreased. After 6 min the response was only 6·5 mV in size, and the threshold for firing was not attained. When the K^+ ejecting current was turned off, the DLH response recovered quickly (in fact, the DLH responses immediately *after* K^+ were slightly larger than the control). A subsequent application of Na^+ Cl^- had no effect on the DLH response. In a total of eight cells the DLH response was significantly reduced in size during the application of K^+. In four of these cells the penetration was sufficiently stable and durable to allow a comparable control run with Na^+. In each case the depression of the DLH response was greater during K^+ than Na^+.

During long periods of K^+ application there was usually little or no change in

the membrane potential (± 2 mV) or G_M but the a.h.p. was often seen to decrease in size.

It was usually found that the DLH responses *after* K^+ application were larger than the control responses. This is illustrated in the graph of Fig. 17 where dose–response curves for DLH were obtained before, during and after K^+. During K^+ the curve shifted to the right, especially for smaller doses. Six minutes after the K^+ current was turned off, the curve had shifted to the left of the control. Similar results were obtained when the progress of the response to a single dose of DLH was followed throughout the recording, although the increase in response size after K^+ was usually only transient. DLH responses were occasionally seen to *increase* in size during control applications of Na^+.

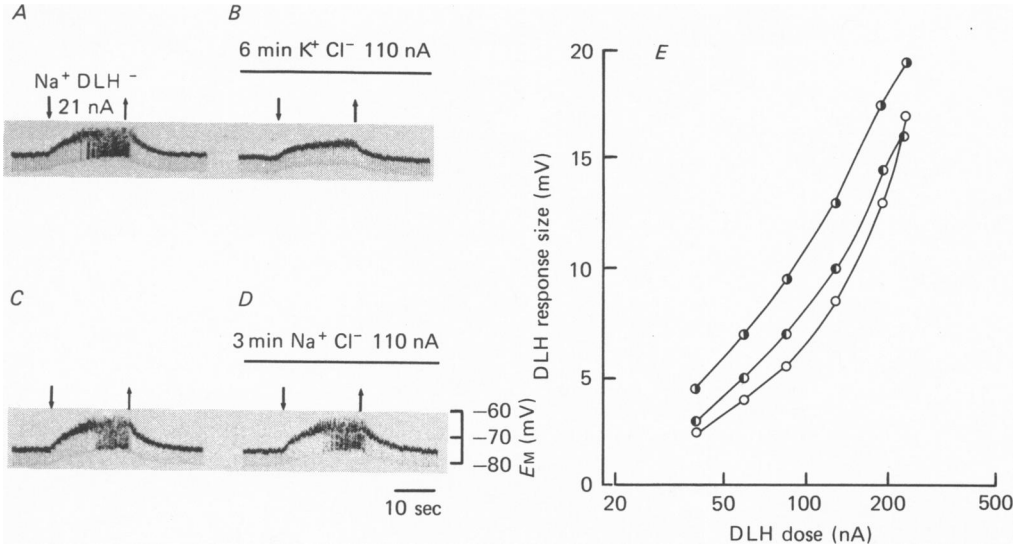

Fig. 17. The effect of K^+ application on a DLH response. A current balanced application of DLH (21 nA) was used throughout the experiment. *A*, control response to DLH (sufficient to evoke repetitive firing) 1·5 min before the start of K^+ application. *B*, 6 min after the start of a current balanced application of K^+ (110 nA) the DLH response had decreased and the threshold for firing was not attained. *C*, DLH response 2 min after the K^+ ejecting current was turned off. *D*, shows there was no significant alteration in the DLH response 3 min after the start of a balanced application of Na^+ (110 nA). Neither K^+ nor Na^+ application had a significant effect on the membrane potential. On the right (*E*) are shown the dose–response curves for DLH before, during and after K^+ application in another cell. ◐, control curve; ○, curve which began 3 min after the start of K^+ ejection (K^+Cl^-, 280 nA); ◑, recovery curve which was begun 5 min after the K^+ ejecting current was turned off. Note that the curve shifts to the right during K^+ application and that the recovery curve is to the left of the control.

In the spinal cord *in vivo* it is difficult to alter significantly the K^+ gradient across the neuronal membrane. We attempted to increase $[K]_o$ at least locally, by extracellular ionophoretic application of K^+. To obtain even a modest increase in $[K]_o$ relatively large ionophoretic currents are necessary. Because a surfeit of extracellular K^+ is very rapidly sequestered (Heinemann & Lux, 1975) the DLH responses had to be studied *during* the ejection of K^+. Problems arise when a number of ionophoretic barrels are passing current simultaneously. It is our experience that an ejected ion of a given polarity is liable to pass directly into a neighbouring barrel of

opposite ejecting polarity to the ion in question ('sucking up'). In the present case DLH^- could be sucked up into the K^+ ejecting barrel. This would give the false impression of a reduced response to DLH. These experiments therefore demanded rigorous controls. Current balanced applications of K^+ were always used (the balancing anion was either Cl^- or $acetate^-$). This prevented coupling artifacts between the ionophoretic and recording electrodes and also minimized the sucking up of DLH. Further, it was necessary to show that any depression of the DLH response during K^+ ejection was greater than that during the application of a similar, or larger, dose of Na^+. The configuration of the ionophoretic electrodes was such that a pair of barrels used for current balanced ejections were situated opposite rather than next to each other. Further, the Na^+ barrel was positioned closer to the DLH than was the K^+ barrel. Therefore, sucking up of DLH would be more likely during the control application of Na^+.

DISCUSSION

An investigation of the mechanism of amino acid induced depolarizations is of special importance for two fields of research.

First, glutamate (as well as some other amino acids and polypeptides) has been proposed to be the excitatory transmitter at a variety of sites in the C.N.S. (for evidence and references see Krnjević, 1970, Johnson; 1972; Curtis & Johnston, 1974; Duggan, 1974; Tebēcis, 1974; Stone, 1976). Of particular relevance in our present study is the possibility that glutamate is the transmitter released by primary afferent fibres (Graham, Shank, Werman & Aprison, 1967; Johnson & Aprison, 1970; Hammerschlag & Weinreich, 1972; Zieglgänsberger & Puil, 1973; Duggan, 1974).

Secondly, in extracellular studies, microionophoresis of DLH (and glutamate) is widely used to excite quiescent neurones in investigations of inhibitory processes. A knowledge of the mechanisms underlying the amino acid induced excitation is important when analysing the superimposed inhibition of firing in such experiments.

It has often been assumed that the depolarization produced by the excitatory amino acids follows interaction of the agonists with a single common receptor (e.g. Curtis *et al.* 1972; Hammerschlag & Weinreich, 1972). This notion is challenged by our results and the following discussion. The most important item is the conductance decrease seen in isolation with DLH but which is also evident a spart of the more complicated glutamate response.

Although homocysteate is not naturally present in the nervous system to any significant extent, our findings illustrate the possibility of different types of synaptic excitation by amino acid transmitters in the C.N.S.

The discussion will be divided into three parts: *A*, concerning directly observable effects by the amino acids on membrane potential, membrane conductance and evoked potentials; *B*, concerning the membrane mechanisms involved, as elucidated by manipulation of membrane potential and ionic concentrations; *C*, concerning implications of the present findings on theories about the e.p.s.p.

A. Characteristics of amino acid responses

1. Response shapes and receptors

In our experiments DLH responses are slow both in onset and recovery whereas glutamate responses are much squarer, and a post-response hyperpolarization is frequently seen (Fig. 2). Previously DLH has been seen to be a more potent, but

slower acting, excitor of neurones than glutamate (Curtis & Watkins, 1963; Curtis, Johnston, Game & McCulloch, 1973). These differences have been accounted for by the fact that DLH has a longer extracellular half-life than glutamate and is therefore able to diffuse farther and interact with progressively more of the neuronal membrane (Curtis & Watkins, 1963; Curtis, Duggan & Johnston, 1970). We propose that the differences in response characteristics may, at least in part, be explained by an interaction of these agonists with kinetically different 'receptors'. This is analogous to the cholinergic system, where muscarinic responses are characteristically slower in onset and of longer duration than nicotinic responses (Purves, 1976). The response to the transmitter candidate Substance P is, like DLH, slow in onset and recovery (Konishi & Otsuka, 1974*a*, *b*; Krnjević & Morris, 1974).

Pharmacological studies indicate that the excitatory amino acid receptor is not a single entity distributed homogeneously throughout the C.N.S. For example, McLennan (1974) suggests that some neurones in the C.N.S. have specific glutamate receptors which are involved in excitatory synaptic action, while other neurones have non-specific excitatory amino acid receptors. Our findings that different conductance changes underlie similarly sized DLH and glutamate responses (see below) indicate that there is more than one excitatory amino acid receptor (see also Freeman, 1976). Alternatively, interaction with a single receptor can lead to changes in more than one conductance channel in proportions that depend on the agonist.

2. Firing

DLH induced firing is persistent with little adaptation (Fig. 5*A*). Glutamate was usually only able to support firing for a short time (Fig. 3). If the responses in Fig. 3 had been recorded extracellularly, the cessation of firing might be interpreted as a desensitization of the membrane to glutamate. The intracellular recording of a maintained depolarization shows that desensitization does not account for the short duration of the firing.

The inability of glutamate to support continuous firing has been observed both following ionophoretic application to single neurones (Curtis *et al.* 1960; Crawford & Curtis, 1964; Zieglgänsberger & Puil, 1973) and application of glutamate containing solutions to nervous tissue (Van Harreveld, 1959; Geller & Woodward, 1974). This phenomenon has been termed 'depolarizing block' (Crawford & Curtis, 1964). Zieglgänsberger & Puil (1973) rarely saw firing during glutamate responses, even though the membrane potential was depolarized beyond the threshold for firing. They suggested that a high membrane resistance was necessary for firing. However, the values they give (3–6 MΩ) are clearly not necessary, since we have found glutamate to cause repetitive firing in cells with input resistances of little more than 1 MΩ, which is well within the normal range for motoneurones. We suggest that for maintained repetitive firing G_M should not increase markedly during the action of glutamate.

Curtis *et al.* (1972) observed a difference in the configuration of extracellularly recorded spikes evoked by glutamate and by DLH. With glutamate (and aspartate) the negative component of the spike was decreased and the positive increased to a greater degree than during excitation at the same frequency by DLH. A partial inactivation of the membrane in the region of the drug application and recording

sites was postulated. DLH responses involved a diffuse area of the membrane, while glutamate gave more intense fluxes over a limited area of membrane. We submit that DLH induced firing is superimposed on a membrane of relatively low conductance. During glutamate application the membrane has a higher conductance, which increases with time (Fig. 8) ultimately preventing action potential generation. Accordingly, with intracellular recording we find that DLH evoked spikes are large, of short duration and stable. Glutamate-evoked spikes slowly decrease in size and increase in duration until they ultimately fail.

3. Conductance changes

DLH responses are accompanied by a stable decrease in G_M which is usually larger than can be accounted for by anomalous rectification.

The conductance changes caused by glutamate are more complicated. Doses of glutamate subthreshold for evoking firing can cause a substantial decrease in G_M (cf. Fig. 7*B*). Although this decrease may be accounted for by anomalous rectification, it contrasts strongly to the marked increase in G_M normally seen during large applications of glutamate (Fig. 8). This conductance increase develops relatively slowly. An equilibrium concentration of glutamate in contact with the somal membrane will probably be achieved quite quickly. We therefore consider this conductance increase to be a time dependent phenomenon, which occurs when the membrane is in contact with relatively high concentrations of glutamate. In any case, glutamate has a dual action on the membrane conductance (in material not presented here we have seen that aspartate has rather similar actions).

So far as we can ascertain, no direct measurements of G_M during DLH responses have been reported previously. There has, however, been the assumption that all excitatory amino acids depolarize by a common mechanism which involves an increase in conductance (Curtis, 1965; Curtis & Johnston, 1974). Glutamate depolarizations in a variety of different neurones have been seen to be accompanied by an increase in conductance (Krnjević & Schwartz, 1967; Zieglgänsberger & Puil, 1973), although in some cases the increase in conductance was only small (Martin, Wickelgren & Beránek, 1970; Hösli, Andrès & Hösli, 1973).

A *decrease* in conductance during amino acid induced depolarizations has not been reported previously for neurones in the mammalian C.N.S. However, Steinberg, Altmann & ten Bruggencate (1974) found that amino acid depolarizations in red nucleus neurones were not always accompanied by an increase in conductance (see also Altmann, ten Bruggencate, Pickelmann & Steinberg, 1976). Zieglgänsberger & Puil (1973) observed no change in conductance during the first 5 mV of glutamate evoked depolarizations. Bernardi, Zieglgänsberger, Herz & Puil (1972) found that in some spinal neurones glutamate evoked a slow depolarization of the membrane without any detectable change in conductance. Larger responses always involved an increase in conductance. The authors suggested that an increase in conductance followed interaction of glutamate with the somal membrane. They further speculated that when there was no measurable change in conductance, glutamate was acting on the dendritic membrane and that the conductance change would not be detected by the intrasomatic electrode (see also Diamond, 1968). It is difficult to test this explanation experimentally in central neurones. From our results it *could* be argued

that glutamate and DLH interact with membrane receptors distant from the site of recording and that they depolarize by a mechanism involving a conductance increase. This conductance increase would not be detected, but the electrotonic spread of the distal depolarization would cause the membrane adjacent to the electrode to depolarize. Anomalous rectification in this part of the membrane would then lead to a decrease in G_M during the response to the amino acids. However, we have shown that the decrease in conductance, at least during DLH, is in *excess* of that which can be accounted for by rectification. The DLH response joins a growing list of other responses in neurones which are mediated by a decrease in conductance (e.g. see Libet, 1970; Engberg & Marshall, 1971; Krnjević, Pumain & Renaud, 1971; Ginsborg, 1973; Gerschenfeld & Paupardin-Tritsch, 1974; Weight, 1974).

4. Changes in i.p.s.p.s, a.p.s and a.h.p.s

I.p.s.p.s. I.p.s.p.s increase in size during responses to DLH and glutamate (Fig. 10). The whole increase can be accounted for by the increase in driving forces on the ions involved in the i.p.s.p. generation as the membrane potential moves farther away from $E_{\text{i.p.s.p.}}$ plus the changes in G_M caused by the drugs. There is no indication of a direct facilitation of the inhibitory transmission. During large glutamate responses the i.p.s.p. increase is only transient. The i.p.s.p. subsequently diminishes as it becomes shunted by the development of the high conductance phase.

A.p.s and a.h.p.s. $E_{\text{a.h.p.}}$ is between -90 and -100 mV (Coombs *et al.* 1955*a*). Therefore, during the depolarization caused by DLH there is an increase in the driving force on the ion(s) involved and the a.h.p. increases in size (Fig. 10).

Krnjević *et al.* (1971) have shown that the muscarinic depolarizing action of ACh on cortical neurones is mediated by a decrease in both the resting g_K and the delayed K^+ current of the a.p. Zieglgänsberger & Reiter (1974) have shown similar effects of ACh in spinal neurones. We found little change in the time course of the antidromic a.p. during DLH – just the decrease of the positive-going phase and the increase in the a.h.p. (Fig. 10) that could be expected during a depolarizing response. Therefore, the decrease in G_M during DLH is not mediated through the ionic channels involved in the a.p., but involves only resting permeabilities.

During glutamate, the changes of the a.h.p. varied as discussed in Results. Thus the dual action on G_M by glutamate complicated the situation and its effect on the specific K^+ channels of the a.h.p. cannot be judged from the present data.

5. Changes in e.p.s.p.s

In our study, it was generally found that the size of the e.p.s.p.s was inversely related to G_M during the drug responses. Thus, e.p.s.p.s increased stably during DLH, despite the depolarization (Figs. 11–13). With glutamate the e.p.s.p. increased over the control when G_M was decreased, and would subsequently fall in size as the conductance rose (Fig. 13). There were also discrete changes in the configuration of the e.p.s.p.s.

DLH. In Fig. 11 the e.p.s.p. recorded during DLH is greater in terms of size, half-width and time-to-peak than the control. The rate of rise ($V\ sec^{-1}$) of the initial phase is, however, the same as the control. There was the expected decrease in G_M during DLH (see below).

The amplitude of the e.p.s.p. is a function of the time course and magnitude of the synaptic current and the input impedance at the synaptic site (Rinzel & Rall, 1974). The e.p.s.p. half-width is very sensitive to changes in G_M (the time course of the passive potential decay being determined by τ_M), whereas the rise time and peak amplitude are resistant to large changes in G_M (Edwards, Redman & Walmsley, 1976*a*). Further, the duration of the synaptic current is not affected by changes in G_M (Jack, Miller, Porter & Redman, 1971). There are two possible explanations for the change in shape of the e.p.s.p. during DLH. First, DLH causes a change in the synaptic current. A longer tail of current is necessary to account for the delayed peak of the potential. This explanation is only tenable for a single synapse or a distributed synaptic input which is electrotonically close to the recording electrode (e.g. exclusively somatic synapses). The second explanation concerns the case of distributed synaptic input. Although care was taken to stimulate only the Ia fibres in the muscle nerve, the activated synapses will be located diffusely over the motoneuronal membrane. The electrically stimulated e.p.s.p. is made up of discrete 'unitary miniature' e.p.s.p.s (Burke, 1967; but see Edwards *et al.* 1976*a*, *b*). With intrasomatic recording an e.p.s.p. on a distant dendrite should rise more slowly and be smaller than an e.p.s.p. produced by a similar conductance change on the soma (Rall, 1962; Rall *et al.* 1967; Jack & Redman, 1971). According to Burke (1967), the faster somatic miniature e.p.s.p.s contribute to the rising phase, while those from distant sources will be summed in nearer the peak and the first part of the falling phase of the compound e.p.s.p. During DLH τ_M increases and the electrotonic length decreases ($L \propto 1/\sqrt{R_M}$, Jack *et al.* 1971). Therefore distant synapses move electrotonically closer to the soma, and their potentials are summed in earlier in the compound e.p.s.p. This would qualitatively explain the higher peak of the e.p.s.p. recorded during DLH.

The change in time course of the e.p.s.p. during DLH is very similar to that seen during the injection of small depolarizing currents into the neurone (see Fig. 5 in Edwards *et al.* 1976*c*; cf. also Werman & Carlen, 1976).

When the half-width and time-to-peak were normalized by τ_M (*ad modum* Rall *et al.* 1967) there was only a slight difference in the values for the control and the DLH e.p.s.p. This indicates that the change in e.p.s.p. configuration during DLH can be accounted for solely by the decrease in G_M during the response. When our results are inserted in Fig. 5 of Rall *et al.* (1967), the changes in half-width and time-to-peak are approximately those which would be expected for an increase in τ_M (their figure was for τ_M increasing from 5 to 7 msec while our measured values were from 5·9 to 7·65 msec).

It is concluded that the changes in the e.p.s.p. seen during DLH are probably accounted for by the decrease in G_M during the response with the associated changes in the electrotonic properties of the membrane.

Glutamate. When e.p.s.p.s increased in size with glutamate they continued to a higher, later peak than the control, the initial phases being identical (as with DLH). The falling phase, however, was usually faster than the control, so tending to keep the half width duration constant. This is in contrast to e.p.s.p.s recorded during DLH. The accelerated falling phase of the e.p.s.p. with glutamate is similar to that seen during the injection of small tonic hyperpolarizing currents (see Fig. 5 in Edwards *et al.* 1976*c*). The accelerated decay may be accounted for by a delayed conductance increase during the e.p.s.p. in the presence of glutamate.

E.p.s.p.s recorded during glutamate responses where G_M was increased were smaller than their control (e.g. Fig. 12). For depressed e.p.s.p.s the time to peak usually remained constant and the maximum slope (V sec^{-1}) of the rising phase was therefore reduced. Zieglgänsberger & Puil (1973) found that during glutamate responses, the e.p.s.p. time-to-peak was shorter and the maximum slope faster than the control. We cannot reconcile our results with these authors, except to stress that e.p.s.p.s during glutamate were often found to be very variable in size.

An action of the amino acids on presynaptic terminals cannot be ruled out. Such an action *could* of course contribute to the changes of e.p.s.p. recorded.

6. Atypical responses

We have no tenable explanation for the rare hyperpolarizing responses seen with both DLH and glutamate. The latter is known to be without effect when applied intracellularly (Coombs *et al.* 1955*b*). Inadvertent injection of glutamate does not, therefore, explain the atypical responses recorded when the ionophoretic electrodes were clearly very close to the cell membrane.

The possibility exists that atypical responses are caused by the liberation of an inhibitory transmitter, either following interaction of the excitatory amino acid with presynaptic endings, or following depolarization of nearby inhibitory interneurones as proposed by Zieglgänsberger & Puil (1973). That the responses result from the liberation of a glycine or GABA-like transmitter is precluded for the following reasons: the hyperpolarizations were usually accompanied by a net decrease in G_M (when allowance was made for the passive rectification of the membrane); hyperpolarizing responses could still be recorded after evoked i.p.s.p.s had been inverted into depolarizing responses following injection of Cl^- ions from the recording electrode. Further, these responses were not accompanied by a noticeable increase in synaptic activity, which might be expected to result from indirect actions via interneurones in the neighbourhood. However, the possibility that amino acids cause the release of a catecholamine transmitter is not precluded. Such hyperpolarizing responses would be expected to be accompanied by a decrease in G_M (Engberg & Marshall, 1971).

B. Mechanisms involved in the amino acid responses

The mechanism of action of excitatory amino acids on mammalian neurones is not as clear cut as in the lower phyla. At the crayfish neuromuscular junction, for example, the majority of the glutamate current is carried by Na^+ with a small contribution from Ca^{2+}, the reversal potential of the response being +39 mV (Onodera & Takeuchi, 1976).

Depolarizing amino acid responses in mammalian spinal neurones are resistant to applications of tetrodotoxin and have been reported to be unaffected by increases in the intracellular concentration of either K^+ or Cl^- (Curtis *et al.* 1972; Zieglgänsberger & Puil, 1972). Reversal potentials for the responses (or the level of potential achieved during a maximum response) are consistently more negative than zero, and usually lie between −20 and −30 mV (*N*-methyl-DL-aspartate: Curtis *et al.* 1972; glutamate: Curtis, 1965; Bernadi *et al.* 1972; Zieglgänsberger & Puil, 1973).

Although glutamate is a weak chelator of Ca^{2+}, this is unlikely to explain its

excitatory action (but see Krnjević, 1974). The depolarization produced by much stronger chelators is weaker and of a longer latency than that of glutamate (Curtis, Perrin & Watkins, 1960; Krnjević & Phillis, 1963; but see Engberg, Flatman & Lambert, 1976). It has been shown by Tan (1975) that acidic amino acids have the ability to mobilize membrane bound Ca^{2+}. This may act as an intermediary step in the excitation produced by these agents.

To the best of our knowledge, no report has appeared where a decrease in permeability to one or more of the major environmental ions has been suggested as a mechanism for amino acid induced depolarization.

1. Potential dependency of the DLH response

The decrease in G_M during DLH responses makes it likely that most, if not all, of the depolarization is caused by a decrease in the membrane permeability for ions with an equilibrium potential negative to the resting membrane potential. The I–V relationships obtained with intracellular current injections support this notion in that the DLH depolarization decreases when the membrane is hyperpolarized. The extrapolation in Fig. 14 indicates an equilibrium potential of -88 mV, the experiments with tonic currents indicate a null point between -90 and -100 mV and from the data obtained in the pulse experiments (partly illustrated in Fig. 16), a null point at -110 mV can be calculated. These values are within the range of those given for the equilibrium potentials for the action of ACh on cortical neurones (Krnjević *et al.* 1971) and spinal neurones (Zieglgänsberger & Reiter, 1974). At both sites ACh is thought to act by a decrease in K^+ conductance. It seems likely that the same mechanism accounts for the depolarizing action of DLH on spinal motoneurones. If this is the case one might expect the DLH depolarization to reverse when the membrane potential is shifted to levels beyond about -100 mV.

We have not been able to show an actual reversal, neither in this series of experiments nor in later ones (to be published), where the membrane has been polarized well beyond E_K. A possible explanation for this is that hyperpolarization changes the characteristics of the membrane, decreasing the relative availability of K^+ channels that can be closed by DLH. Alternatively, DLH might act only on outward diffusion of K^+. If so, DLH would have very little, if any, effect on G_M at membrane potentials more negative than E_K. In any case, slope conductances (as measured with small pulses) change very little with DLH at high membrane potentials (see Fig. 15). Further, G_M generally increases strongly with hyperpolarization which may have a shunting effect on the DLH response.

When the membrane is depolarized by injected current, an event mediated *via* a decrease in K^+ conductance would be expected to increase in size (cf. Weight & Votava, 1970). The DLH response, in fact, became smaller and, with sufficient depolarization, virtually disappeared (Fig. 15). The potential dependency of the DLH response is a bell-shaped relationship with the maximum sized responses being recorded at the resting potential (Fig. 15). During large tonic depolarizations, there are also changes in the configuration of the small conductance measuring pulses. These changes are so far poorly understood. The voltage transients rise to a peak and then sag back to a plateau somewhat smaller than the peak. Although DLH caused little or no change in the membrane potential, the degree of sag during the

pulses was markedly increased. This sag tended to voltage clamp the membrane. The shape of the pulses was reminiscent of those that would be obtained by current injection into an RC circuit with a parallel inductive element (cf. Hodgkin & Huxley, 1952; Fig. 2.10; Jack *et al.* 1975). The sag is possibly due to a change in K^+ channel activation. The behaviour of the DLH response during depolarization indicates that, in the absence of complications caused by firing, there will be a limitation on the maximum size of the response.

In summary, DLH decreases the conductance of ionic channels whose availability is potential dependent. It is likely that other potential dependent conductance changes (e.g. delayed and anomalous rectification) are able to shunt the conductance decrease caused by DLH.

2. Effects of ionic concentration changes

Potassium. If, as discussed above, the DLH response is mediated through a decrease in g_K, then alterations of E_K would be expected to influence the size of the depolarization. In *in situ* experiments in the C.N.S. it is difficult to change the K^+ concentration on either side of the membrane. It is not possible to increase significantly the already high $[K^+]_i$ by ionophoresis (Coombs *et al.* 1955*b*; Krnjević & Schwartz, 1967). The achievable increase in $[K^+]_o$ is also small. Krnjević & Schwartz (1967), using the data of Curtis, Perrin & Watkins (1960), calculate that the maximum increase in $[K^+]_o$ in any substantial volume of tissue will be in the region of 1–2 mM. From the data of Lux & Neher (1973) a *local* increase in $[K^+]_o$ of about 6 mM at equilibrium might be expected at a distance of *ca.* 40 μm from the point of release with an ejecting current of +250 nA. The consequence for E_K for the neurone as a whole is, however, still open to conjecture. Nevertheless, in very carefully controlled experiments such as illustrated in Fig. 17, the DLH response decreased in size during K^+ application, and this is consistent with there being a decrease in g_K during the response.

Chloride. A reduction in g_{Cl} could account for a depolarization accompanied by a decrease in conductance. This might make a very small contribution to the DLH response, but a major role for this mechanism is ruled out since the response is relatively insensitive to Cl^- injection. Furthermore, the 'null point' for the DLH response is more negative than the normal E_{Cl} for motoneurones. The possibility is not ruled out that DLH activates an outwardly directed Cl^- pump. However, it has been shown by Lux (1971) that passive membrane properties remain unaltered following inhibition of active Cl^- extrusion. It is therefore unlikely that stimulation of a Cl^- pump by DLH would result in the observed depolarization with decrease in G_M.

3. Uptake mechanism

The notion that amino acid depolarizing responses are a consequence of the active uptake of the agonist has been investigated and dismissed (Curtis *et al.* 1970; Balcar & Johnston, 1973). We would, however, like to advance the hypothesis that the high conductance state observed during long applications of glutamate is a manifestation of its uptake.

Two kinetically distinct mechanisms are responsible for glutamate sequestration

(Logan & Snyder, 1972): a low affinity mechanism, which is found generally throughout the nervous system; a high affinity mechanism which is associated with more specialized subcellular particles and nerve terminals. The high affinity system is not specific for glutamate, but transports a range of structurally similar compounds (Balcar & Johnston, 1972*b*; Hammerschlag & Weinreich, 1972). The energy for the high affinity uptake is provided by the large Na^+ gradient across the neuronal membrane.

Glutamate-induced depolarizations are insensitive to tetrodotoxin (Zieglgänsberger & Puil, 1972) as is glutamate uptake (Balcar & Johnston, 1972*a*). The Na^+ entry involved with these processes is therefore through a different channel(s) from that associated with firing, the motoneuronal action potential being susceptible to tetrodotoxin (Curtis *et al.* 1972; Blankenship, 1968).

During the glutamate response shown in Fig. 8 the conductance increased from a level somewhat lower than the control value to nearly three times the control value while the potential remained virtually clamped at the plateau (-58 mV) of the response. If the slow increase in conductance during glutamate application was due solely to an increase in P_{Na}, then the membrane would be expected to depolarize further towards E_{Na}. Therefore, in order to keep the potential constant at the plateau of the response, there must be an outward current of equal magnitude to the Na^+ current. The identity of the ionic species which carries this current is not known. It is possible that the inward Na^+ current is balanced by the entry of negatively charged glutamate ions (Krnjević, 1970). Zieglgänsberger & Puil (1973) found that both i.p.s.p.s and a.h.p.s may be temporarily reversed into depolarizing potentials following large glutamate responses. This indicates that there had been an efflux of K^+ and possibly an influx of Cl^- during the responses.

From the foregoing, it is attractive to postulate that the high conductance state is a manifestation of a glutamate uptake mechanism in which an increased membrane permeability provides the necessary movement of counter ions (be it Na, K or Cl). Because large doses of glutamate applied for a long time are necessary to evoke the high conductance state, it could be further conjectured that ionic movements associated with the low affinity uptake mechanism are responsible. Our hypothesis may be readily tested by investigating the conductance changes underlying the responses to various glutamate analogues (see Balcar & Johnston, 1972*a*, *b*). It would be predicted that analogues which are poor substrates for the L-glutamate uptake system would depolarize by a mechanism involving a decrease in G_M. Conversely, analogues transported in preference to L-glutamate would be expected to promote an increase in G_M more readily than glutamate.

Although glia actively accumulate glutamate (Henn & Hamberger, 1971), they do not depolarize nor do they show a conductance increase when glutamate is applied (Krnjević & Schwartz, 1967, and our unpublished observations). Glial cells are often impaled just outside the motoneuronal membrane; they have a high membrane potential, surprisingly combined with a very high G_M. It is probable that the glutamate transporting mechanism is different for neurones and for glia (see also Hösli & Hösli, 1976).

DLH did not cause an increase in G_M in our experiments. However, in using a racemic mixture of homocysteic acid, it is likely that we have been predominantly studying the effects of the D isomer, since this is more potent as an excitor of spinal

neurones than the L isomer (Curtis & Watkins, 1963). In rat brain slices, D-homocysteate is not taken up, whereas the L isomer is taken up by two kinetically distinct transport systems (Cox & Watkins, 1976). In other experiments we have shown that, in terms of potency, response shape and conductance changes, D-homocysteate is similar to DLH, whereas L-homocysteate resembles L-glutamate (Lambert *et al.* 1978).

C. Implications for theories on the mechanism of the e.p.s.p.

The question now arises as to whether our findings with the putative transmitter glutamate and the non-physiological compound DLH can help to elucidate the mechanism of generation of the Ia e.p.s.p. Until recently, transmission at these synapses has been considered a central corollary of that at the skeletal neuromuscular junction (Eccles, 1964). Transmission would be chemical and a post-synaptic increase in conductance to Na^+, K^+ and possibly Ca^{2+} would be implicated in the depolarization. However, acceptance of this hypothesis is no longer universal, and some doubts have been expressed (e.g. Edwards *et al.* 1976*c*; Werman & Carlsen, 1976). The principal objections to the 'classical' theory of the generation of the Ia e.p.s.p. may be summarized as follows.

(*a*) It has proved to be very difficult to reverse the initial part of the Ia e.p.s.p. Such difficulties have not been encountered in reversing polysynaptic e.p.s.p.s and the latter part of the Ia e.p.s.p. (Edwards *et al.* 1976*c*; Werman & Carlen, 1976). Nevertheless, a complete reversal of the Ia e.p.s.p. *has* been achieved (Eccles, 1964; Marshall & Engberg, 1973). This will be discussed in a forthcoming paper by Engberg & Marshall.

(*b*) The magnitude of the Ia e.p.s.p. does not increase linearly on hyperpolarization as does the polysynaptic e.p.s.p.

(*c*) The required conductance increase during the initial part of the e.p.s.p. has proved difficult to detect (Smith *et al.* 1967). Yet, as Barrett & Crill (1974) have calculated that the conductance increase accompanying 'quantal' e.p.s.p. generation could be in excess of $80–100 \times 10^{-10}$ mho, the conductance increase accompanying an e.p.s.p. involving many boutons should be detectable (see Smith *et al.* 1967).

(*d*) No effective antagonist of the e.p.s.p. has been discovered to date.

A possible explanation of the failure to demonstrate a reversal of the first part of the Ia e.p.s.p. and an increase in G_M during the e.p.s.p. may appear on reference to our findings on the decrease in G_M accompanying the action of DLH and glutamate. Were the mechanism of the Ia e.p.s.p. to involve a similar conductance change, the difficulty in demonstrating a conductance increase is easily understood. Moreover, if an ionic mechanism were involved which was mediated by a conductance increase to Na^+ (and possibly Ca^{2+}) and a *decrease* to K^+, the reversal potential for the Ia e.p.s.p. may well be outside the individual range of the equilibrium potentials for the participant ions (Brown, Muller & Murray, 1971; Calvin, 1969). Such a mechanism would have certain advantages over the 'classical' mechanism since there would be little, if any, reduction of the dendritic length constant and the synaptic current would act on the initial segment with minimal attenuation.

We are unable as yet to support or reject definitively glutamate's candidacy as the transmitter at the Ia synapse. Certainly, its inability to maintain motoneuronal

firing when ionophoretically applied casts some doubt on its role. However, it is possible that glutamate's extrajunctional actions could mask a more specific junctional action. Further, a repetitive liberation of short lasting glutamate 'pulses' (as could be expected in the natural function of the synapse) might be more effective in producing action potentials.

We have hitherto not tested other putative transmitters such as Substance P on the motoneurone, although we have embarked on a study of the influence of other naturally occurring small chain peptides on spinal neurones. Using the independent double impalement technique, we are also investigating changes in the configuration of the Ia e.p.s.p. in response to large polarizing currents.

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