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Actions of Glutamic Acid on Spinal Neurones

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Summary. Spinal neurones from the 6th and 7th lumbar segments of cats were recorded intracellularly. Glutamic acid (GLUT) was applied extracellularly by means of the microelectrophoretic technique from another parallel electrode and caused, in almost all cells, a depolarization of the cell in association with conductance change. In some cases, initial depolarization occurred with no detectable conductance change.

Motoneurones could not be brought to continous firing except in two cases. High "doses" of GLUT (up to 2000 nA) caused maximal depolarization up to —30 mV (range —32 mV to —24 mV). The soma conductance was increased at this time by about 75% (range 60—90%).

The spikes and both EPSPs and IPSPs were attenuated by shunting. The peak latency of the EPSP was shortened. Spikes evoked by different EPSPs were found to exhibit different sensitivity to GLUT. Ocassionally, the IPSPs were initially increased following depolarization. The IPSP was sometimes reversed after the termination of GLUT application. The after-hyperpolarization following the antidromic spike of some motoneurones was converted into an after-depolarization for 1—2 min.

The present data show that the following criteria for an excitatory transmitter can be met by GLUT; (1) strong depolarizing action presumably associated with Na^+ influx, (2) an associated conductance change and (3) an equilibrium potential. It could not be proven that the EPSP reversal point and the equilibrium potential for GLUT are the same.

Key words: Glutamic acid — Spinal neurones — Intracellular recording — Neurotransmitter — Microelectrophoresis

Introduction

Since the first description of the excitatory effect of glutamic acid on nerve cells (Hayashi, 1954) much data have accumulated about the action of this amino acid on neurones in various structures of the CNS of both mammals and invertebrates (see McLennan, 1970; Phillis, 1970). It is presently quite clear that glutamic acid is a transmitter in invertebrates (Robbins, 1959; van Harreveld and Mendelson, 1959; Baker, 1964; Takeuchi and Takeuchi, 1964; Gerschenfeld and Lasansky, 1964; Kerkut *et al.*, 1965; Kravitz *et al.*, 1965; Otsuka *et al.*, 1967).

Intracellular studies with simultaneous extracellular application of glutamate

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done on neurones in the mammalian CNS revealed a strong depolarizing action (Curtis *et al.*, 1960; Krnjević, 1964; Bernardi *et al.*, 1972). However, the amino acid has been rejected as a transmitter because the reversal potential for its action is different from that of the EPSP (Curtis, 1965). Presently, there are several reasons why a transmitter role in the mammalian CNS is once again putative for glutamic acid. It has been detected in the CNS in large amounts (Waelsch, 1957; Singh and Malhotra, 1962) and has also been located within nerve terminals (Ryall, 1964) in association with synaptic vesicles (Kuriyama *et al.*, 1968). In detailed histochemical studies, Aprison *et al.* (1965) showed that glutamic acid is present in a substantial quantity in dorsal root fibers of the dorsal column. The fast action of this amino acid, and the rapid reversion of the effect, probably due to an efficient uptake system (Logan and Snyder, 1971), give further credence to the belief that this substance is an excitatory transmitter in the mammalian CNS (see reviews by Curtis and Johnston, 1970; Krnjević, 1970; Johnson, 1972).

The primary purpose of this investigation was to study the action of glutamic acid on dorsal horn neurones, likely candidates for glutaminergic transmission (Graham *et al.*, 1967), and to provide more electrophysiological data about the action of the amino acid on neuronal membranes. Also, motoneurones were often encountered and studied but their reaction to glutamate was not unlike that of other spinal neurones. A brief preliminary report of this work has been published previously (Bernardi *et al.*, 1972).

Methods

The experiments were performed in 49 adult cats of either sex (body weight 2—4 kg). Surgery was performed during pentobarbital anaesthesia (35 mg/kg, intraperitoneally). The animals were anaemically decerebrated immobilized with gallamine and artificially respired. Blood pressure was monitored continually. A bilateral pneumothorax was made routinely. An electric blanket and heat radiators were used to maintain rectal temperature between 36.5° and 38° C. Recordings began more than 5 hours after injection of the anaesthetic.

The spinal cord was exposed by laminectomies at the lumbar level (L3—S2) and covered with a pool of paraffin kept at body temperature. The dura mater was dissected and pinned back to the muscles. A tear in the pia mater at the recording site was carefully made and the spinal cord was penetrated at the entry zone of the dorsal roots. For dorsal root, ventral root and peripheral nerve stimulation, bipolar Ag-AgCl-electrodes were used. Stimulation voltage varied between 0.2—5.0 V (duration 0.1—0.25 msec).

The electrode assembly consisted of a single and a double barrelled micropipette fixed together with epoxy. The tip of the intracellular electrode (tip diameter 0.5—1.5 μm, 5 to 30 MΩ) protruded beyond the tip of the application electrode (tip diameter 2—3 μm, 1 to 2 MΩ) by 70—110 μm. Since non-polymerized material might cause changes in the nervous tissue, care was taken that the epoxy did not touch the tissue. Hence, only the upper parts of the electrode shanks were fixed together leaving the last 2—3 mm free to the tip. Dental wax between the two shoulders of the electrodes strengthened the electrode assembly. The intracellular micropipette was filled with a 10 : 1 mixture of K-citrate (1.6 M) and KCl (1 M). The extracellular micropipettes were filled with monosodium L-glutamate (3 M, pH 8.3) and NaCl (2M, pH 6—7). In general, retaining currents of 10 nA were used to reduce leakage. Intracellular recordings and current injection were performed using conventional technique. Coupling resistance was about 1—2 kΩ. Recordings were stored on tape for later analysis.

In all iontophoretic studies, current effects seriously interfere with drug effects. Thus, in most cases (except in those otherwise mentioned), *current compensation* was employed; a current of the same magnitude but of opposite polarity was applied simultaneously through an adjacent barrel filled with NaCl (see also Salmoiraghi and Stefanis, 1967). Current com-

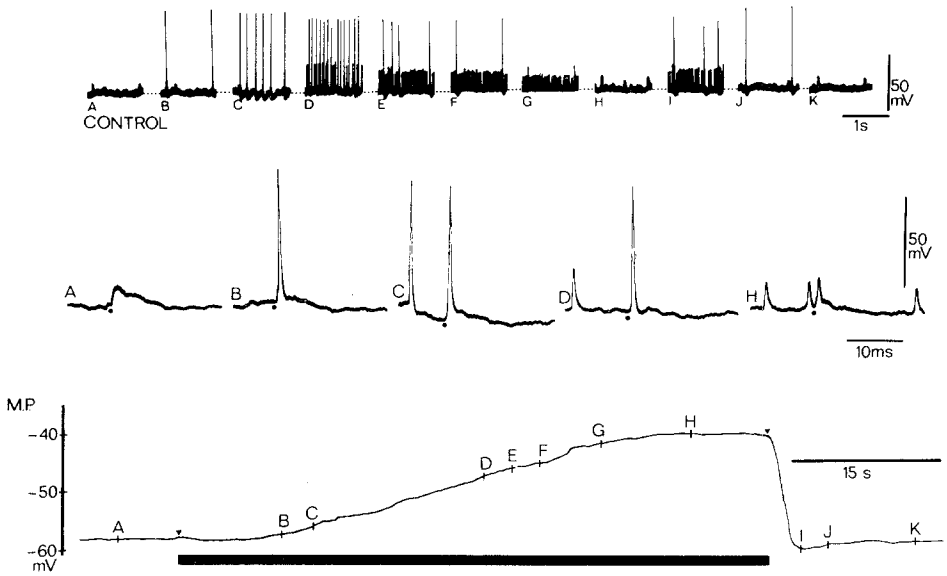

Fig. 1. *Intracellular recording from a dorsal horn neurone* (cutaneo-sensitive). 70 nA of GLUT was applied. Intertip distance, 90 μm. A dissected dorsal rootlet of L_7 was stimulated (1/s). *Upper row:* A, control; B—H, recordings taken at different levels of depolarization caused by GLUT; I—K, reversion of the effect. *Middle row:* corresponding recordings taken with faster oscilloscope sweeps. *Lower row:* penwriter recording of the membrane potential. Letters refer to the recordings taken at respective points. Bar indicates period of application. Resting potential was —58 mV. Phoretic current was compensated (see Methods). Note that in D—F, the full spike following the stimulus persisted longer than the other spikes (see also Fig. 7)

pensation was assumed to be complete when the baseline showed no detectable jump when the phoretic current was switched on.

In some experiments, the cutaneous receptive fields of dorsal horn neurones were studied using a fine hair brush and an arterial clamp.

Results

The results were obtained from 88 spinal neurones recorded intracellularly from the 6th and 7th lumbar segments. 24 neurones were dorsal horn neurones (10 cutaneo-sensitive; 3 identified as ascending tract neurones by dorso-lateral funiculus stimulation at C_3). 28 neurones were unidentified ventral horn neurones which could not be fired by antidromic stimulation of the ventral root. The remainder were motoneurones. While probing for nerve cells, a large number of fibers were impaled. They were identified by a fast rise time of their action potentials, the lack of membrane swing and the depth in the spinal cord. They were not affected by glutamate (GLUT) even when large amounts were applied for long periods.

Almost all neurones in this investigation were depolarized by GLUT application. The pattern of response to extracellularly-applied GLUT is well known from extracellular studies (Curtis *et al.*, 1960; McLennan, 1970). Figure 1 shows an intracellular recording from a cutaneo-sensitive dorsal horn cell. Stimulation of a dissected dorsal rootlet of L7 evoked an EPSP, followed by a barely detectable

IPSP/EPSP sequence (*late response*). The time course of depolarization caused by extracellular application of GLUT can be seen from the penwriter recording. The relatively great distance (intertip distance of about 85 μm) between the application electrode and the impaled cell and the fact that the phoretic current was compensated (see below) may account for the comparatively slow onset (7 sec) and slow termination (3 sec) of the effect. When GLUT was applied, the first spike occurred after about 10 sec on the first EPSP following the stimulus (B). A few seconds later, spikes also occurred on the EPSPs of the late response (C). After 25 sec (D), the late response spikes did not always reach full amplitude but were present only as A-spikes. From D to F, there is a gradual decreasing of A-spike height whereas the amplitude of the spike of the first EPSP is only slightly decreased according to the level of depolarization. In G, the B-part of the spike on the first EPSP failed. The spike on the first EPSP is also gradually reduced from G to H; the amplitude of all signals is reduced maximally in H ("depolarization block", see Curtis *et al.*, 1960; Crawford and Curtis, 1964). The recovery was a "mirror-image" of the above effects; (1) the reduced A-spikes grew in amplitude, (2) a full AB-spike occurred on the EPSP immediately following the first stimulus and (3) the spikes of the late response regained their initial amplitude. Between I and J, the late response spikes are absent and only the first EPSP fired a spike. Full recovery was reached in K (see also Fig. 7).

In many cases, cells did not discharge although the firing level was surpassed during GLUT application. This was almost invariably the case for motoneurones. Such neurones were 5—10 mV more depolarized than the firing level. Only in two instances (total number of motoneurones encountered was 36), were spikes fired in addition to that on top of the EPSP which closely followed the stimulus. It might be assumed that the difference in reaction to GLUT between motoneurones and other neurones (most probably smaller cells) is due to differences in membrane resistance. Most of the cells which were spontaneously active or those which could be fired by GLUT, had a higher membrane resistance than motoneurones (3 to 6 MΩ and 1—2 MΩ respectively).

Figure 2 shows the changes in antidromic spike evoked from a motoneurone by GLUT application. A continuous plot of the percentage changes in membrane conductance are shown together with the corresponding changes of membrane potential. At left, 3 pulses obtained at A, B and D are shown. The marked diminution in test pulse size indicates that the membrane resistance is reduced considerably by GLUT application. The depolarization can be seen to start with no detectable decrease in test pulse amplitude. The pulses diminished thereafter and a maximum reduction of about 70% can be seen in D. The neurone was depolarized to a membrane potential of about —30 mV but never discharged a spike except the antidromic one. The strong depolarization remained for about 15 sec after termination of drug application while the pulse height steadily increased. Such data indicate that the soma conductance returns to its initial level more rapidly than the depolarization which may be sustained by dendritic depolarization. As in 90% of other cases, repolarization of the membrane potential began when the soma conductance was about 50—60% of its initial value. The upper part of Fig. 2 shows the typical changes which an antidromic spike undergoes during GLUT iontophoresis. The spike amplitude is reduced, and the AB-

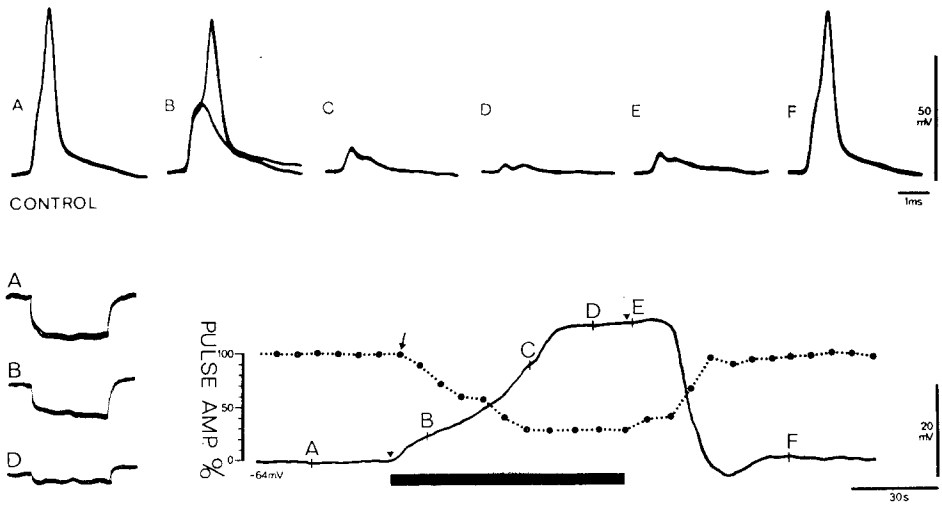

Fig. 2. *Intracellular recording from a biceps-semitendinosus motoneurone.* 200 nA of GLUT was applied. Intertip distance, 85 μm. An antidromic spike is evoked (two traces superimposed). A, control; B—D, recordings taken at different levels of depolarization caused by GLUT; E—F, during recovery. Note that a depolarization has already occurred before the point (arrow) where the conductance begins to change. *Right bottom:* penwriter recording of the membrane potential; letters refer to the recordings above. Bar indicates period of application. Dotted line shows simultaneous measurements of conductance changes (%). The amplitude of 4 consecutive current pulses were averaged. *Left bottom:* hyperpolarizing test pulses (duration 20 msec). A, B, D taken before the corresponding antidromic spikes. Resting potential, —64 mV; initial membrane resistance, 1.2 MΩ. Stimulus frequency, 1/s

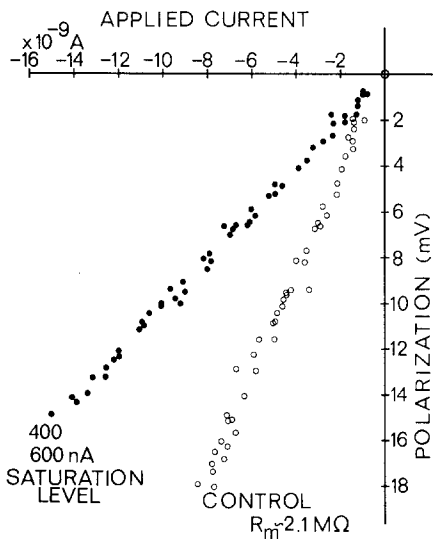

Fig. 3. *Membrane resistance changes of an interneurone during GLUT application.* Intertip distance, 90 μm. Duration of hyperpolarizing test pulses, 20 msec. Initial membrane resistance, 2.1 MΩ. "Saturation level" was obtained by applying 600 nA of GLUT but the membrane resistance could not be brought beyond the value already reached after 400 nA of GLUT

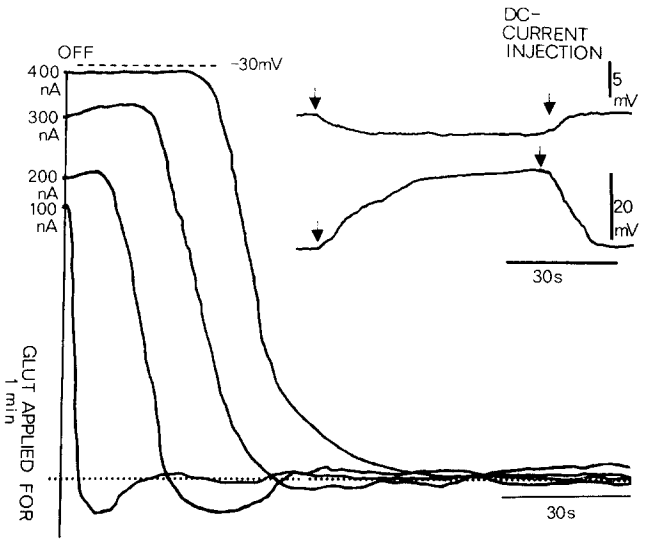

Fig. 4. A and B. A) *Recovery of the membrane potential after different doses of GLUT.* GLUT was applied in all cases for 1 min. Intertip distance, 110 μm. After 200 and 300 nA, the maximal depolarization was reached after "off". The depolarization caused by 400 nA was the maximal level which could be evoked in this neurone (—31 mV). Note that after 100, 200, and 300 nA, the membrane potential went temporarily in the hyperpolarizing direction. Dotted line shows initial resting potential at —59 mV. B) DC-current injection into the same cell. The membrane potential is displaced up to about 0 mV. 300 nA of GLUT was applied. Arrows indicate the start and termination of the applications

break barely detectable in Fig. 2A, became progressively apparent (between Fig. 2A and B) with intermittent failure of the B-spike. This indicates that invasion of the soma-dendritic membrane is made difficult by the increased soma conductance. From B to C, a progressive reduction of the A-spike takes place, and in D, only a small depolarizing transient is present. Recovery from the effect can be seen from E and F. The spike in F still shows an AB-break which is more pronounced than that of the "control". A constant finding was that these alterations persisted for a few minutes following maximal depolarization. In 12 cases, cells clearly hyperpolarized (up to 4—7 mV) at the onset of GLUT application (compensated). This was particularly obvious when intertip distances were long and when low "doses" were used. Recovery from this hyperpolarization, which was associated with an increase of spike amplitude, was quite slow. Hence, current effects may be excluded.

Resistance measurements were taken from 29 neurones upon maximal depolarization with high "doses" of GLUT (Fig. 3). At 400 nA, a *saturation level* is reached. That is, a further increase in the amount of GLUT (to 600 nA) did not cause a greater increase in conductance even when it was applied for a relatively long period (about 5 min). The membrane potential during this maximal conductance change was around —30 mV. It is clear from tests using supramaximal "doses" of GLUT (up to 2000 nA) that this value represents the maximum depolarization which can be caused by GLUT under such experimental conditions and is, presumably, close to the equilibrium potential for the action of this amino

acid. The value was between —32 and —24 mV (see also Figs. 2, 4 and 8). The reversal potential for GLUT action was measured in 5 cells. Depolarizing DC-currents were applied and the membrane potential was displaced to values around zero mV. The action of GLUT became, under these conditions, hyperpolarizing (see right part of Fig. 4). Because of instabilities in the recording system, a more exact measurement of the reversal potential could not be achieved but it is clearly within the range of maximal depolarization caused by high "doses" of GLUT.

Where the "dose" of GLUT was too low to cause maximal depolarization, the membrane potential tended to move in a depolarizing direction for a few seconds after termination of application. This phenomenon can be seen from Fig. 4. Four different amounts of GLUT were applied. 400 nA of GLUT applied for 1 min caused a depolarization to —32 mV. A maximal depolarizing effect after application of 200 and 300 nA was not observed until after the cessation of the phoretic currents. When 100 nA of GLUT was applied, the reversion of the effect started very soon after termination of the phoretic current. During recovery, the membrane potential was very often observed to be a temporary hyperpolarization (see also part B of Fig. 5). This was particularly obvious when small amounts were applied. It is obvious from the DC-recordings in all Figs. presented here that the onset of the GLUT action and the cessation have a different time course. This deviation from the expected time-course might be due to a fast uptake mechanism present for GLUT.

Depolarizing and hyperpolarizing DC-currents were applied intracellularly in 7 neurones in order to study their effects on the time course of recovery from different amounts of GLUT. In 4 of these neurones, recovery was accelerated by about 40% with hyperpolarizing current (10—15 nA). In contrast, depolarizing currents (10—20 nA) were found to have no effect on the time course of recovery from GLUT action.

In addition to the action potential, the EPSP and the IPSP were altered markedly during GLUT application. Figure 5 shows a polysynaptic IPSP evoked by dorsal rootlet stimulation and an EPSP evoked by sural nerve stimulation. The amplitude of both the IPSP and the EPSP was diminished by GLUT. However, it is evident that the IPSP can be reduced in amplitude much more readily than the EPSP. The peak latency of the IPSP is not changed detectably but the peak latency for the EPSP is shortened considerably by GLUT. If a cell initially showed a high membrane resistance and could be made to repetitively fire by GLUT application, the peak amplitude of the IPSP often increased when the cell was depolarized (Fig. 5 part B). Afterwards, the IPSP was also progressively reduced and then almost vanished during maximal depolarization. In 8 cells, the IPSP was reversed after termination of GLUT application. In these cases, the cells were strongly hyperpolarized (up to 10 mV). The membrane conductance reached initial values after about 2—4 sec.

Not only postsynaptic transients are influenced differently by GLUT-induced conductance changes. It is clear that spikes, evoked by different EPSPs are also altered (Fig. 6 and Fig. 1). Both Figures show recordings obtained from cutaneo-sensitive dorsal horn neurones. The neurone shown in Fig. 6 was identified as a cell giving origin to an axon ascending in the dorso-lateral funiculus. The cutaneo-

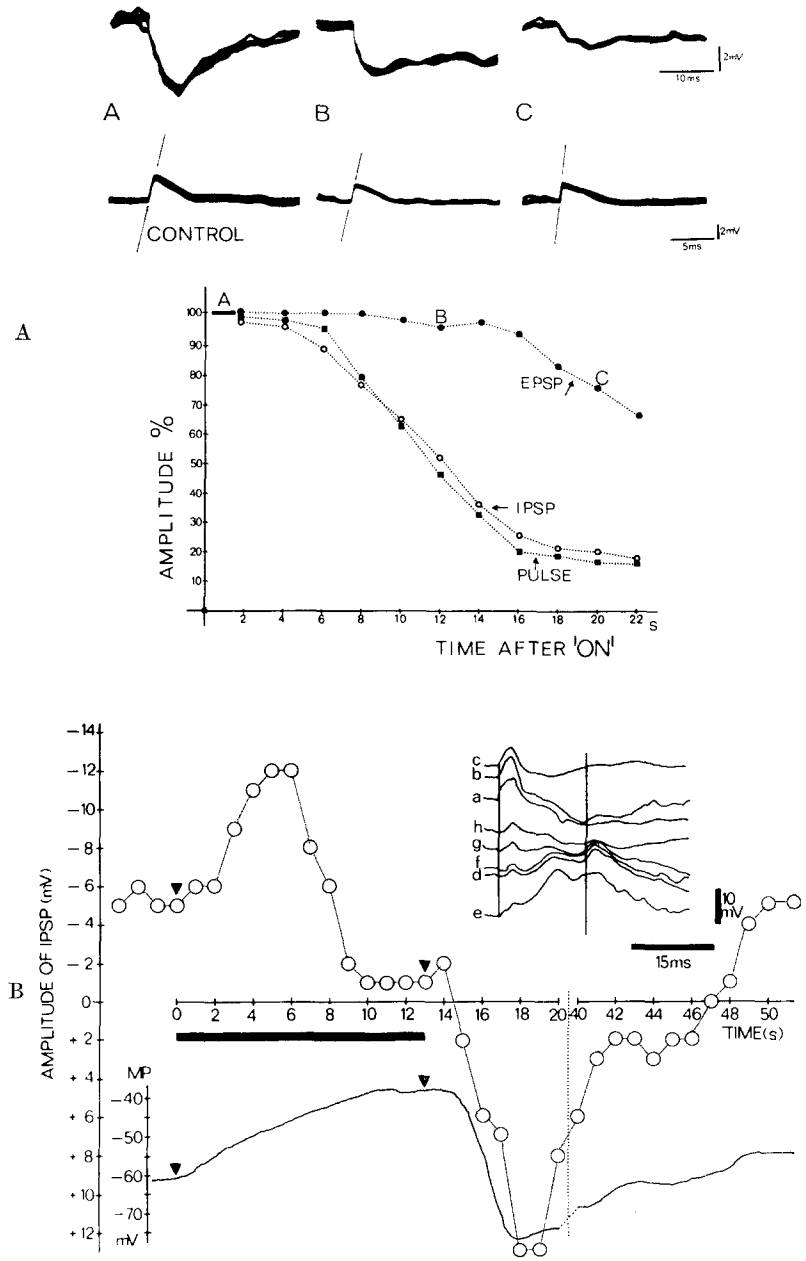

Fig. 5. A and B. A) *Changes of an IPSP and an EPSP in an interneurone* (6 traces superimposed). IPSP was evoked by dorsal rootlet stimulation and the EPSP by sural nerve stimulation. 300 nA of GLUT was applied. Intertip distance, 100 μm. Columns B and C give the changes at different times after the control. A, control (resting potential —59 mV); B, after 12 s; C, after 20 s (membrane potential —40 mV). Graph shows different sensitivity to GLUT of the EPSP (closed symbols) and the IPSP (open symbols). Peak amplitudes are measured. Squares are the conductance changes. Note that the peak latency of the EPSP is changed. B) *Changes* of an IPSP evoked in a dorsal horn cell. Insert shows a reversal of the IPSP following GLUT application. a—c, during GLUT application; d—h, recovery

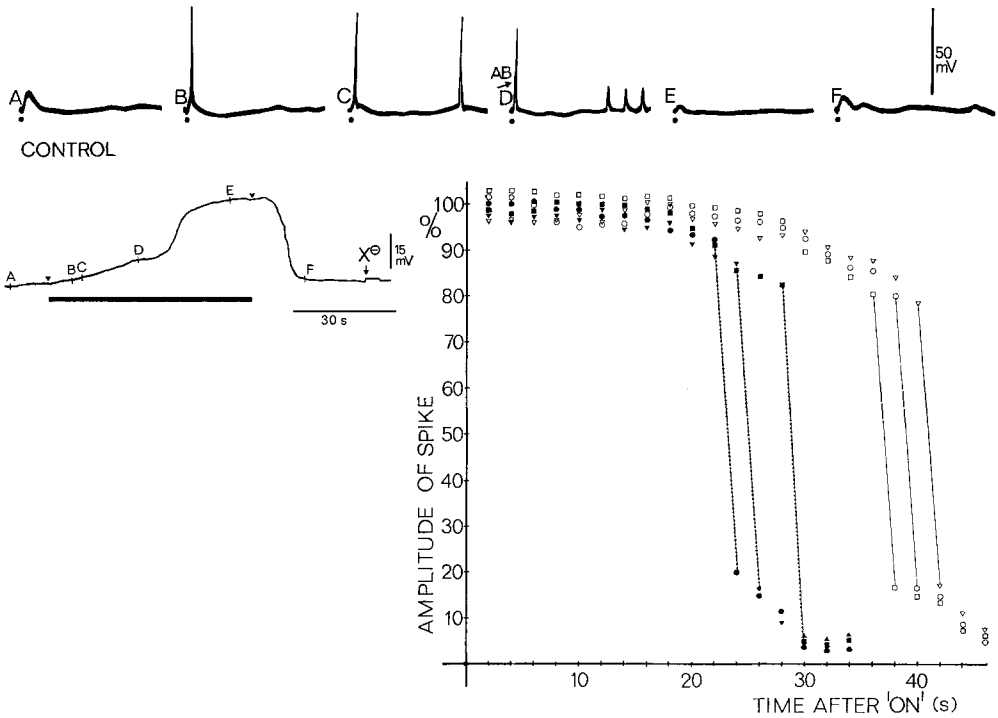

Fig. 6. *Intracellular recording from a dorsal horn cell* (cutaneo-sensitive). 200 nA of GLUT was applied. Intertip distance, 100 μm. A dissected dorsal rootlet of L_7 is stimulated (1/s). A, control; B—E, recordings taken at different levels of depolarization caused by GLUT; F, 15 s after "off". Penwriter recording shows the membrane potential. Letters refer to the recording above. At X^-, negative current (200 nA) was applied to test for coupling resistance. Graph shows the different disappearance time of the B-spike: open symbols, spike right after the stimulus; closed symbols, spikes on the late response EPSPs

receptive field was found on the lateral side of the ipsilateral hind paw. It was associated with an eccentrically located inhibitory receptive field. Pressure applied onto the inhibitory receptive field caused a hyperpolarization of the neuron neurone by 10—12 mV and reduced the amplitude of an injected current pulse by about 70%. Thus, the inhibition was associated with a conductance change of the soma membrane.

It was evident during GLUT application that the spike on the early EPSP also persisted longer (D) in this neurone than did the spikes evoked on the late response EPSPs. The graph shows the various times when the two types of spikes exhibited loss of the B-spike. Initially, both types of spikes were reduced concomitantly by the depolarization which was associated with a conductance change. The B-spikes of the late response spikes, however, failed earlier. Time lags of about 8—15 sec were observed in these cases. A similar discrepancy in sensitivity to GLUT of spikes evoked by different stimuli was present in about 60% of tests performed on cells which could be made to fire continually by GLUT. In 20 motoneurones, the decaying phase of the antidromic spike was altered, markedly, following depolarization by GLUT. In fact, this alteration persisted, in some cases,

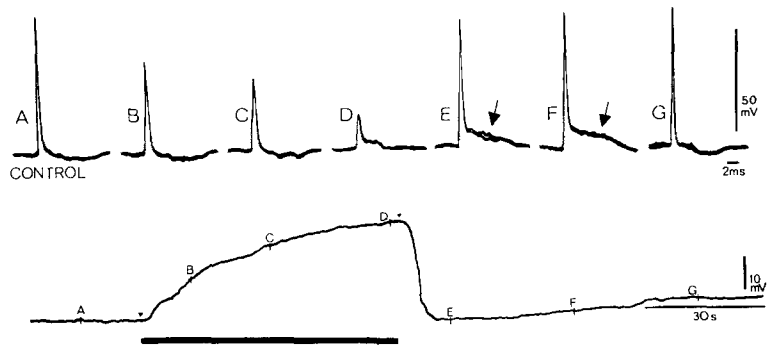

Fig. 7. *Intracellular recording from a motoneurone where the after-hyperpolarization is temporarily converted into an after-depolarization* (two traces superimposed). 300 nA of GLUT was applied as indicated by bar. Intertip distance, 100 μm. Antidromic stimulation, 1/s. A, control; B—D, recordings taken at different levels of depolarization; E—F, recordings taken during recovery from the effect. Penwriter recording of the membrane potential. Letters refer to recordings shown above

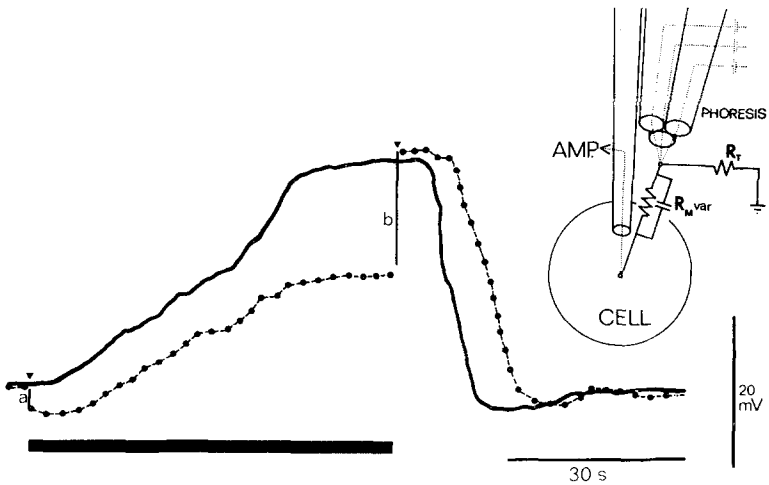

Fig. 8. *Recordings of the changes in the membrane potential of a motoneurone after GLUT application by different modes.* 300 nA of GLUT was applied. Intertip distance, 80 μm. *Solid line:* the phoretic current was compensated for by a current of same magnitude but of opposite polarity passed through an adjacent NaCl-filled barrel. *Dotted line:* uncompensated phoretic current. (a) and (b) show the coupling of the phoretic current at "on" and "off" respectively. *Insert:* Schematic drawing illustrating the recording and application arrangement. R_m represents the membrane resistance which is altered by GLUT application. R_t is the resistance of the nervous tissue which is assumed to remain fairly constant during application. Note that the cell is slightly more depolarized and recovery takes longer after application by the uncompensated mode

for periods up to 90 sec. The conversion of an *after-hyperpolarization* (mixture of after-hyperpolarization and recurrent IPSP) into an *after-depolarization* can be seen in Fig. 7.

As mentioned previously (see Methods), all GLUT applications were performed with compensation for the phoretic current. The reason for this was, that

in this type of experiment, current effects can seriously interfere with recording of the membrane potential. Since GLUT was applied as an anion, the baseline "jumped" in a hyperpolarizing direction when the phoretic current was switched on.

Figure 8 shows the results obtained from the two modes of application, *i.e.*, with and without compensation. The dotted baseline shows an initial downward movement (a) of about 4 mV when the phoretic current was switched on. This is followed (delay about 10 sec) by a continuously increasing depolarization. Upon termination of the phoretic current, the DC recording shows a much greater "jump" upward (b) than that which was present initially. The cell was slightly more depolarized without compensation and the effect persisted longer (see Discussion). An explanation for the phenomenon that the amount of electrical coupling at the onset (a) and termination (b) of the phoretic current are different is that the membrane resistance (R_M) which represents the main resistance between the application and recording electrodes, is decreased during GLUT application (see insert of Fig. 8). The polarization caused by the phoretic current is then less attenuated and more readily picked up by the recording electrode. In such cases, the recorded membrane potential would be an algebraic result of the depolarizing action of GLUT and the hyperpolarizing current. Coupling in a hyperpolarizing direction frequently masked the increase of depolarization caused by low "doses" (100—200 nA) of GLUT. The spike was attenuated during such times and its rising phase showed a more pronounced AB break. A few cells were very sensitive to phoretic current but were not clearly affected by GLUT. Such cells were hyperpolarized by comparatively low anionic phoretic currents. The spikes were increased in amplitude whereas the IPSPs were reduced or even reversed. One explanation for such extreme sensitivity to phoretic current and the failure to observe a depolarization is that the application electrode had penetrated or, at least touched, the cell membrane.

Discussion

In the present studies, the effects caused by extracellular GLUT application were quite reproducible; nearly all spinal neurones encountered could be depolarized. This depolarization was, in each case, associated with a measureable increase in soma membrane conductance when the cell was depolarized by about 5 mV ($\pm$ 2 mV). However, there was one outstanding difference between motoneurones and other spinal neurones in their reaction to GLUT application. With few exceptions, motoneurones could not be brought to continuous firing whereas other cells generally fired at high rates during the depolarization caused by GLUT. A fairly constant finding was that neurones which could be brought to continuous firing by GLUT application had, initially, a higher membrane resistance than motoneurones. The reason why motoneurones would not fire continually (except possibly γ-motoneurones) may be that, although the membrane potential had surpassed the firing level, the associated conductance change had meanwhile caused a pronounced decrease in the membrane resistance. Hence, no spike except the antidromic one could be evoked. An outstanding feature of many of these neurones which could be brought to repetitive firing by GLUT application

was that the spike which occurred on top of the EPSP during the late response (see Figs. 1 and 8) was more sensitive to GLUT application than spikes evoked by the EPSP closely following the stimulus. Initially, both types of spikes showed almost the same height and rise-time but the spikes on the late response EPSPs displayed a more pronounced AB break sooner and their B-spikes failed earlier. The progressive appearance of an AB-break is a sign for an advancing block of invasion of the soma-dendritic membrane. One reason for this block is that the increased conductance of the soma membrane hinders the transition of depolarization from the initial segment to the soma-dendritic membrane, and finally blocks by shunting and Na-inactivation, the generation of a full action potential ("depolarization block" Curtis *et al.*, 1960; Crawford and Curtis, 1964). While the mechanism underlying the "depolarization block" is well understood that underlying the differential sensitivity of differently evoked spikes is not clear. It might be due, at least in part, to the altered time constant of the membrane. Usually, the EPSPs of the late response had much slower rise times than the EPSPs closely following the stimulus and are, therefore, much more attenuated. This might make them insufficient to evoke spikes after the membrane time constant has been shortened to a critical value. A selective action of GLUT on different excitatory synaptic sites cannot be excluded. However, this can be only determined by a more detailed analysis of the influence of the amino acid on a variety of differently evoked postsynaptic potentials.

In a number of tests, GLUT application altered distinct parts of the action potential for a long period. As can be seen from Fig. 7, the membrane resistance had already reached its initial value and the spike was of almost full amplitude but the after-hyperpolarization still remained inverted. One can assume that the cell has changed its Na^+ and Cl^- content during maximal depolarization caused by GLUT applications and remains in a state of ionic disturbance for a considerable time. It is known from the work of Ito and Oshima (1964) that intracellular injection of Na^+ converts the after-hyperpolarization and the recurrent IPSP into depolarizing responses. It is clear from Fig. 5B that IPSPs could also be reversed by GLUT application. Therefore, one might assume that large amounts of Na^+ are taken up by the cell during GLUT application and that some time is needed to relieve the high internal concentration. Further evidence for this explanation can be derived from the recent experiments of McIlwain *et al.* (1969), Pull *et al.* (1970) and Okamoto and Quastel (1970). They showed that Na^+ is taken up in considerable amounts following depolarization by GLUT. The reason why the membrane potential is always hyperpolarized in the above case might be that an electrogenic Na^+-pump is stimulated by the high amount of Na^+ present in the cell (Koike *et al.*, 1972). The slight hyperpolarization present in a few experiments during application of low doses of GLUT cannot be explained by the same mechanism because it is not preceded by depolarization. Although there exists no direct evidence, one might assume that GLUT activated nearby inhibitory neurones which exert a hyperpolarizing action on the cell under study (see Zieglgänsberger and Herz, 1971). In experiments where the phoretic current was not compensated, such initial hyperpolarization always occurred. In contrast to the hyperpolarization described above, these hyperpolarizations occurred stepwise and were followed by a slow depolarizing action. The observation that the membrane

potential makes a larger depolarizing "jump" than it initially did in the opposite direction is due to the fact that over the course of the experiment, the membrane resistance is changed and coupling artefacts are gradually increased. This phenomenon might partly account for "desensitization" phenomena sometimes seen in such investigations. The membrane was not as strongly depolarized during application with compensation, as it was when the GLUT current was uncompensated; this observation may be explained by the fact that current compensation reduces the amount of substance reaching the site of action because a substantial fraction of the ejected ions is taken up again by the compensating current (G. Sothmann, personal communication).

One of the main objections against GLUT being an excitatory transmitter in the mammalian CNS is that the reversal potential for the action of GLUT and that for the EPSP are not the same (Curtis, 1965 but see Curtis and Johnston 1970; see also Coombs *et al.*, 1955). This objection is weakened by a re-investigation (Smith *et al.*, 1967) which demonstrates that a reversal of the EPSP in motoneurones is hard to attain, or that in the majority of cases, a true reversal does not occur. Current injection into the soma region of the cell would not uniformly depolarize all of the soma-dendritic membrane but would only affect those synapses which are located in the vicinity of the current source.

Some histological (Conradi, 1969) and electrophysiological (Smith *et al.*, 1967) studies have demonstrated that excitatory synaptic sites are widely distributed over the cellular surface and that the dendritic tree of large spinal cells extends far beyond 300 μm (Scheibel and Scheibel, 1969). The latter value was found to be the maximal distance where clear excitation could be evoked by diffusing GLUT (Herz *et al.*, 1969). Also, remote dendritic membrane areas and other synapses can strongly influence the membrane potential measured at the soma, the probable site of impalement (Lux *et al.*, 1970). One might assume from these data that electrophoresed GLUT does not reach all dendritic branches which contribute to transient membrane potential changes. It is likely that the "saturation potentials" measured in the present experiments are at a more hyperpolarized level than the true glutamate equilibrium potential. It may well be the case that the soma and proximal dendritic membrane are strongly depolarized whereas the more distal portions are not affected. The inward current at the soma will then cause an electrical source at the dendrites delivering current to a sink at the soma. When the membrane potential is displaced at the true glutamate equilibrium level with current injection, cells are then hyperpolarized by GLUT application. Hence, the true equilibrium potential is quite likely to be at a more depolarized level than that found in the present investigation.

A synopsis of data described shows that some electrophysiological criteria for an excitatory transmitter are met by GLUT; (1) a strong depolarizing action (presumably associated with a Na^+ influx), (2) a marked conductance change and (3) an equilibrium potential. The rapid recovery of cells from maximal "doses" of GLUT provides indirect support of evidence for a fast inactivation system, an indispensable feature of chemical neurotransmission. Although an exact measurement of the equilibrium potential of an excitatory transmitter in the mammalian CNS is seriously hampered by experimental limitations, the value obtained appears to be close to (if the above considerations are made) the assumed reversal potential

of the EPSP. If it can be proven with improved techniques that the EPSPs are not mediated by GLUT then this amino acid might be considered a good candidate for a potent modulator of neurotransmission.

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While this paper was in press, a paper by Curtis *et al.* (Brain Res. **41**, pp. 283—301, 1972) was published confirming some of our results.

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