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CORTICAL PYRAMIDAL TRACT INTERNEURONES AND THEIR SENSITIVITY TO L-GLUTAMIC ACID

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SUMMARY

1. Pyramidal tract interneurones, defined as neurones which are activated synaptically as a result of pyramidal tract stimulation, have been identified in the rat cerebral cortex. The number of evoked spikes depended upon stimulus strength, and stimulation in a specific thalamic nucleus produced a burst of activity lasting for up to 1 sec.

2. These cells are readily excited by a brief (50 msec) pulse of glutamate applied by micro-iontophoresis. Other, unidentified cells are not so responsive.

3. Synaptically evoked spikes resulting from pyramidal tract stimulation can be blocked by the iontophoretic and I.P. administration of substances shown to antagonize glutamate excitation of cells.

4. The results support suggestions that glutamic acid is a neurotransmitter in the cerebral cortex. The evidence presented further indicates that glutamic acid could be the transmitter released by the pyramidal tract.

INTRODUCTION

In spite of the surge of interest in recent years in the processes of synaptic transmission in the central nervous system, relatively little attention has been paid to the possible transmitter liberated by fibres of the pyramidal tract.

The pyramidal tract stains poorly for acetylcholinesterase (Koelle, 1954) and possesses almost negligible choline acetylase activity (Feldberg & Vogt, 1948). The tract is probably not, therefore, cholinergic. The lack of fluorescence in the pyramids seen by appropriate histochemical techniques similarly suggests that the amines noradrenaline, dopamine and 5-hydroxytryptamine are unlikely to be transmitters here (Carlsson, Falck & Hillarp, 1962; Dahlström & Fuxe, 1964).

Among the remaining substances suspected as being neurotransmitters at present are certain amino-acids. When applied by microiontophoresis, acidic amino-acids such as L-glutamic acid and aspartic acid have potent

excitatory actions on cells in many areas of the central nervous system. Neutral amino-acids such as γ-aminobutyric acid are, by contrast, potent neuronal depressants (Crawford & Curtis, 1964; Curtis & Watkins, 1965; Krnjević, 1970).

L-glutamic acid (Glu) is present in the cerebral cortex in larger concentrations than in any other area of the central nervous system (Berl & Waelsch, 1958) and, although this fact is not a uniquely significant one in view of the varied metabolic processes in which Glu participates, the additional observations that Glu exists in high concentrations in synaptosomes (Kuhar & Snyder, 1969) possibly in synaptic vesicles (Kuriyama, Roberts & Kakefuda, 1968), and that it can be released from synaptosomes by electrical stimulation (Bradford, 1970) lend strong support to the possibility of a transmitter role for glutamate.

Further support may be derived from the findings that only certain forms of neural stimulation which excite cortical cells increase the amount of Glu 'released' at the cortical surface (Jasper & Koyama, 1968, 1969). Also, a high affinity uptake process for Glu into cortical homogenates has recently been described (Logan & Snyder, 1971), suggesting that specific membrane transport mechanisms exist for Glu.

Until recently, however, no association could be made between Glu and any neural pathway in which it might be involved as a transmitter, due to the lack of suitable antagonist substances. The announcements by several groups of workers of an antagonism of amino-acid excitation by some amino-acid derivatives (Haldeman, Huffman, Marshall & McLennan, 1972; Curtis, Duggan, Felix, Johnston, Tebécis & Watkins, 1972) prompted the present investigation to determine whether these substances had any effect on synaptic activity evoked from the pyramidal tract.

METHODS

Male rats weighing 250–300 g were used exclusively. The animals were anaesthetized with urethane (25% solution in physiological saline) 5 ml./kg, which, according to several authors, leaves activity in the brain hardly distinguishable from that of unanaesthetized (encéphale isolé) preparations (Holmes & Houchin, 1966; Cross & Dyer, 1971; Bradley & Dray, 1972).

The preparation of animals, and the methods used for stimulation and identification of the pyramidal tract, recording and micro-iontophoresis have been described previously (Stone, 1972*a*, *b*). Two modifications of these techniques were also employed in these experiments.

First, the spike recording from a cell, after passing through a pulse-shaper unit was passed into an instantaneous rate-meter (Ecko) and from this a direct continuous recording of the cell firing rate was obtained on a Servoscribe pen recorder.

Secondly, since some difficulty was experienced in recording from the rather small interneurones of the rat's cortex with multibarrel iontophoretic electrodes (over-all tip diameter 4–8 μm), these were combined with conventional single micropipette

electrodes having tip diameters not greater than 1 μm and containing 1 M potassium acetate. These recording pipettes were affixed to the multibarrel pipettes by means of an insulating fixative varnish (Voltalac) or Araldite ® (CIBA Ltd), which was applied to suitable points on the electrode assembly after apposition of the tips under microscopic control. No fixative was applied to those parts of the assembly which were to be inserted into the brain. The tip of the single electrode was arranged to project 2–10 μm beyond the multibarrel pipette orifice (Fig. 1). This allowed satisfactory recording of small cells, and simultaneous iontophoresis.

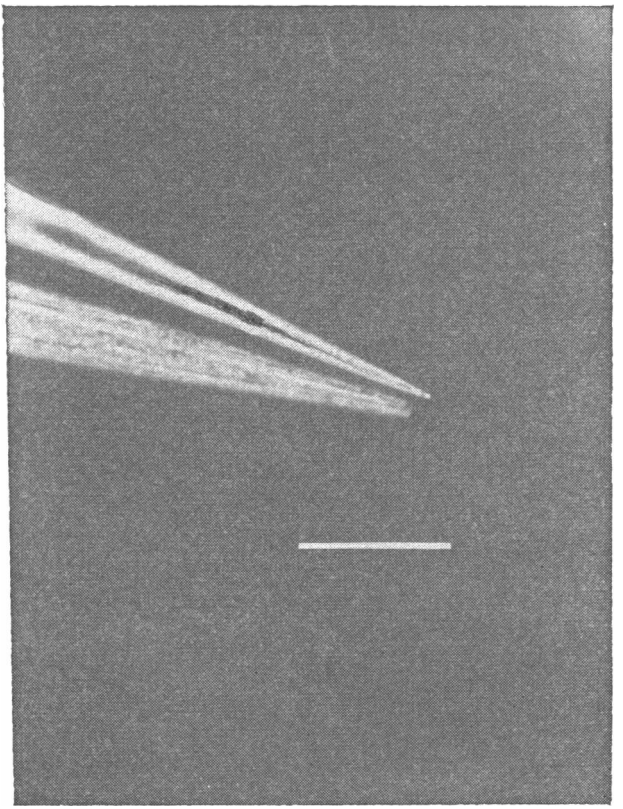

Fig. 1. A photograph of the tip of the electrode system used. A single micropipette recording electrode, tip approximately 1 μm diameter, is shown affixed to and projecting approximately 6 μm beyond a multibarrel pipette, tip approximately 5 μm diameter. Scale: 50 μm.

For micro-iontophoresis, the following solutions were used: sodium L-glutamate (B.D.H.), 200 mM, pH 8·0; acetylcholine chloride (Koch-Light), 200 mM, pH 5·0; L-glutamic acid diethyl ester hydrochloride (Sigma), 200 mM, pH 5·0; L-methionine DL-sulphoximine (Sigma), 200 mM, pH 5·0.

Glutamic acid diethyl ester was also injected intraperitoneally in doses of 300 or 600 mg/kg. These doses were chosen as being similar to the doses used by Desi, Farkas, Sos & Baogh (1967) to produce behavioural changes in rats.

All the cells studied in these experiments were spontaneously active.

RESULTS

Cortical interneurones activated by the pyramidal tract

Most of the evidence to be presented comes from a study of interneurones in the cerebral cortex which were activated by the pyramidal tract. The characteristics of these cells have been described in a report by Stefanis (1969). Fig. 2*A* illustrated records of the activity of a cell which was not a pyramidal tract cell (Stone, 1972*b*) but which appeared to be an interneurone excited by pyramidal tract stimulation. The

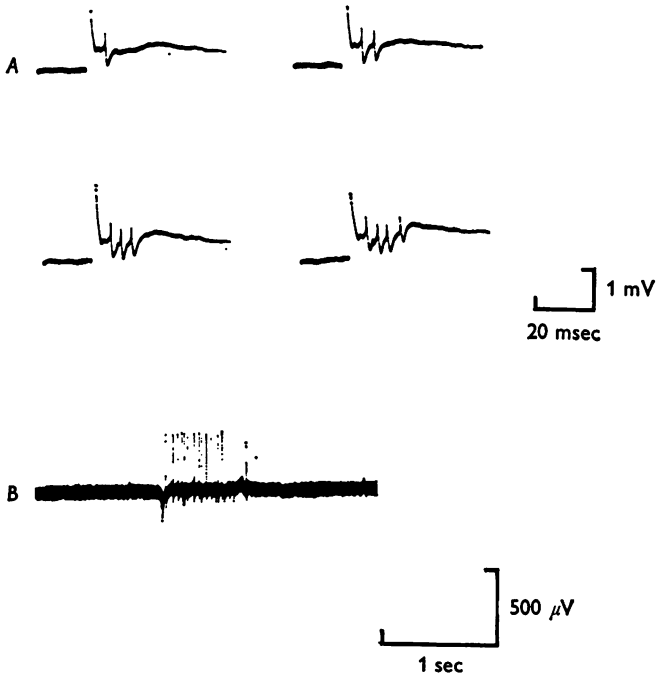

Fig. 2. Evoked activity of an interneurone in the cerebral cortex. The cell illustrated has an initial latency of about 6 msec in *A*.

A, the number of evoked spikes increases from 1 to 4 as the pyramidal tract stimulating current is increased from 20 μA through 40, 80 and 120 μA.

B, a burst of activity of the same cell as *A*, produced by stimulation of nucleus ventralis posterolateralis of the thalamus. Negativity is upwards.

number of spikes elicited in such cells could usually be varied from one up to three or four by increasing the strength of pyramid stimulation. This behaviour, and three other factors, confirmed the essential similarity between these cells and those studied by Stefanis.

First, the cells would respond to peripheral or specific thalamic stimulation (nucleus ventralis posterolateralis) (Stone, 1972*a*) with a train of spikes which lasted for up to one second (Fig. 2*B*).

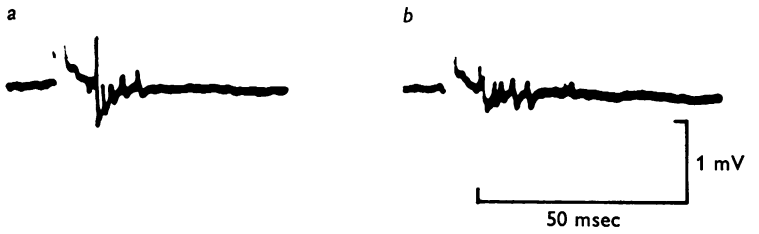

Fig. 3. *a*, A pyramidal tract cell antidromic spike is seen followed by a short burst of small spikes recorded from an interneurone. In *b* the stimulus current has been reduced so that the antidromic spike is no longer produced. The interneurone activity remains, however, indicating that the pyramidal tract cell in *a* was not primarily responsible for its activation.

Secondly, some of these cells appeared to be located in close proximity to pyramidal tract cells. An example is shown in Fig. 3*a*, in which a pyramidal tract cell antidromic spike is followed by a short train of smaller spikes which may be presumed to be recorded from an interneurone: pyramidal tract cells never show repetitive activity to that extent. The pyramidal tract cell recorded in this case, however, was not the cell primarily responsible for activating the interneurone since, as shown in Fig. 3*b*, the interneuronal activity could still be elicited when the pyramid stimulating current was reduced below the threshold necessary to activate the pyramidal tract cell seen in Fig. 3*a*.

Thirdly, the cells were situated relatively deeply in the cortex, their distribution coinciding with that of the larger pyramidal tract cells. The mean depth of fifteen of these interneurones was $1{\cdot}25 \pm 0{\cdot}26$ mm (S.D.).

Most of the results reported below were from interneurones, the first recorded spike of which appeared to be due to monosynaptic activation from the pyramidal tract. The criteria for identifying such spikes were, first, that the first spike would follow stimulation of the pyramids at frequencies of not more than 50–100 Hz, and secondly there was some variation of latency with successive stimuli even when delivering a stimulus of 1 mA intensity. Whilst being much greater than the almost undetectable variation of latency for antidromically activated pyramidal tract cells (not more than 0·1 msec), the latency variation of interneurones was quite small, never in these experiments exceeding 0·5 msec for the first monosynaptic spike.

Finally, identified pyramidal tract cells have never been observed to produce more than a single synaptically induced spike in the present

series of experiments. Interneurones, as mentioned above, can readily be made to produce three or four spikes.

Glutamate sensitivity of interneurones

Many cortical neurones which are spontaneously active will respond to the iontophoresis of L-glutamate with an increase of firing rate (Krnjević & Phillis, 1963; Krnjević, 1964), although pyramidal tract cells have a relatively high threshold. The response of a cell to the iontophoresis of glutamate is shown in Fig. 4*a*. The cell did not appear to be either a

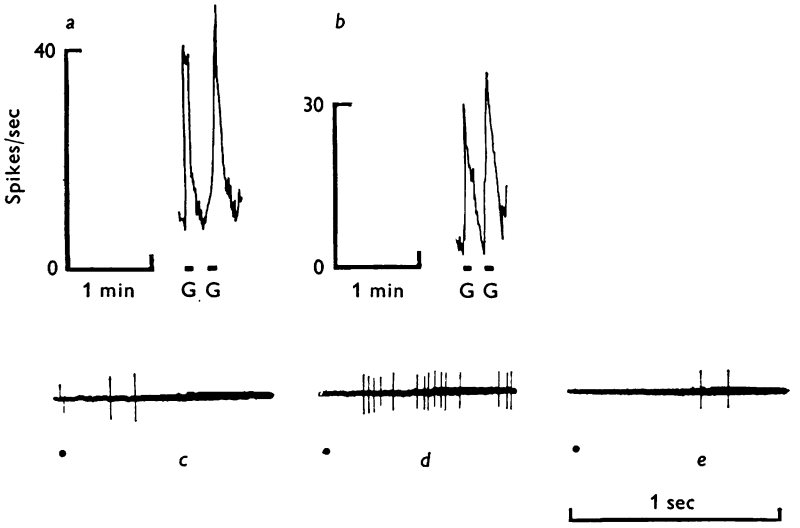

Fig. 4*a* and *b*. Rate-meter records of the firing of a pyramidal tract interneurone (*b*) and a cell not responding to pyramidal tract stimulation (*a*). Glutamate 40 nA (G) was applied during the periods indicated by the bars below the records. The responses are similar.

c and *d*. Photographic records of 1 sec sweep duration, following the application of a brief pulse of glutamate lasting 50 msec (indicated by artifact and dot below record). Only the interneurone is readily excited (*d*). *e*, a control 50 msec pulse of chloride as in *c* and *d* has no effect on the cell. The spikes seen in *c* and *e* are indicative of the spontaneous activity of the cell.

pyramidal tract cell or a pyramidally activated interneurone. The action of glutamate is quite rapid, both in onset and termination, and the cell firing rate is increased some 300%. In Fig. 4*c*, however, a brief pulse of glutamate lasting 50 msec (the shortest iontophoretic pulse duration available with our equipment) was applied as indicated by the dot below the photographic spike record. The cell does not appear to be able to respond to so brief an application of glutamate.

Fig. 4*b* and 4*d* show analogous records of an interneurone activated with short latency from the pyramidal tract. Whilst the response to a 5 sec pulse of glutamate is similar to that seen in Fig. 4*a*, though even more rapid in onset, this cell readily responds to the 50 msec pulse of glutamate. This exquisite sensitivity to glutamate has been seen on each of ten pyramidal tract interneurones so tested and on no other non-pyramidal tract cell.

The final record in Fig. 4 shows the lack of effect produced by a 50 msec pulse of chloride ions from the sodium chloride containing barrel of the micropipette. The spikes seen in Fig. 4*c* and 4*e* are indicative of the ongoing spontaneous activity of the cells and did not appear to be produced by the glutamate or inward current pulses.

Blockade of glutamate excitation

Experiments were also performed to check on the ability of the reported glutamate antagonists to block glutamate excitation. These tests were of necessity performed on pyramidal tract cells so that the specificity of action could be determined by comparing excitation by glutamate and by acetylcholine (Stone, 1972*a*).

Fig. 5 shows a typical excitation by glutamate applied iontophoretically, to be susceptible to blockade by L-glutamic acid diethyl ester hydrochloride (GDEE) whilst excitation by acetylcholine is only slightly reduced. In this case the antagonist is also being applied iontophoretically. L-methionine-DL-sulphoximine proved similarly capable of preventing glutamate excitation whilst leaving acetylcholine responses relatively unaffected. This antagonist did, nevertheless, cause a greater reduction of the acetylcholine response than did GDEE, sometimes causing a reduction of 50%. GDEE has therefore been used in most of these experiments. It is important to note that GDEE does not appear to have the ability to antagonize glutamate on all cortical neurones. Indeed Curtis *et al.* (1972) have previously noted the poorer antagonism seen in the cortex compared to other areas of the central nervous system. In the present experiments clear antagonism was seen on forty-two of the 102 cells tested. Antagonism was complete on ten of these. On eight cells glutamate responses were apparently potentiated by GDEE after a latency of 1–2 min.

Fig. 5 confirms that excitation by glutamate of pyramidal tract interneurones can also be blocked by GDEE. It is unfortunately necessary to assume that this action is as specific on these cells as it appears to be on pyramidal tract cells, since there is no suspected transmitter substance which regularly excites non-pyramidal tract cells, as acetylcholine excites pyramidal tract cells, with which the action of GDEE can be compared.

Since GDEE was injected parenterally (intraperitoneally) in some experiments, it was also necessary to ascertain the specificity of GDEE in blocking glutamate when acting by this route. A dose of 300 mg/kg was found, as illustrated in Fig. 6, to antagonize glutamate excitation of

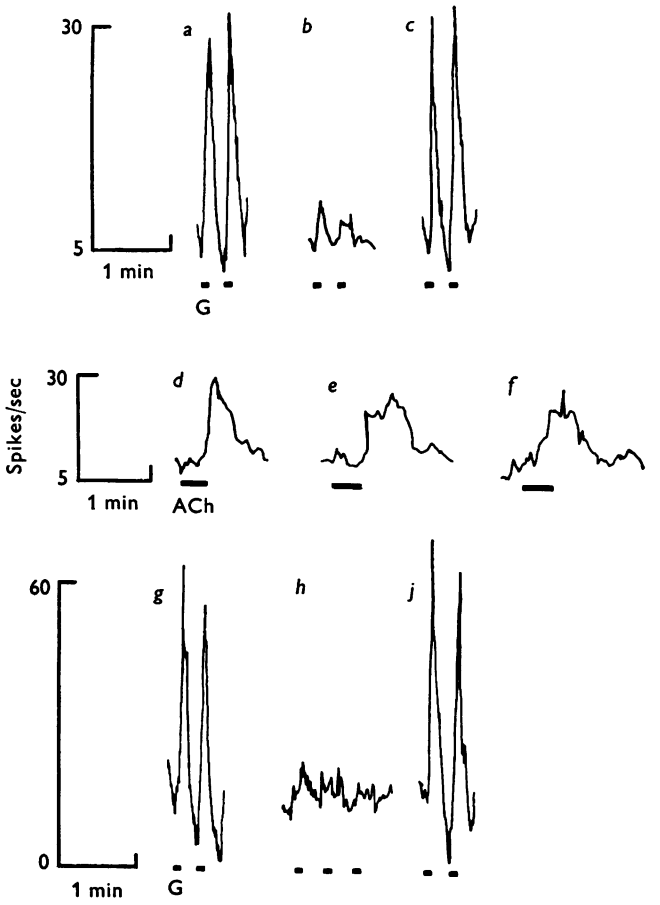

Fig. 5. Rate-meter records of firing rate of a pyramidal tract cell (*a–f*) and a pyramidal tract interneurone (*g–j*).

(*a–c*) and (*g–j*), Responses to glutamic acid 60 nA (G) and (*d–f*) responses to acetylcholine 60 nA (ACh), (*a*, *d* and *g*) before (*b*, *e* and *h*) 5 min after beginning and (*c*, *f* and *j*) 5 min after ending the iontophoresis of glutamic acid diethyl ester hydrochloride, 80 nA.

pyramidal tract cells and interneurones whilst having little effect on the acetylcholine excitation of pyramidal tract cells. This dose was chosen since it was similar to the doses found by other investigators to cause EEG activation and learning deficits in rats (Dési *et al.* 1967). The maximum

degree of blockade was usually seen about 15–20 min after the injection of the antagonist. Definite antagonism was always apparent, however, within 5 min of the injection.

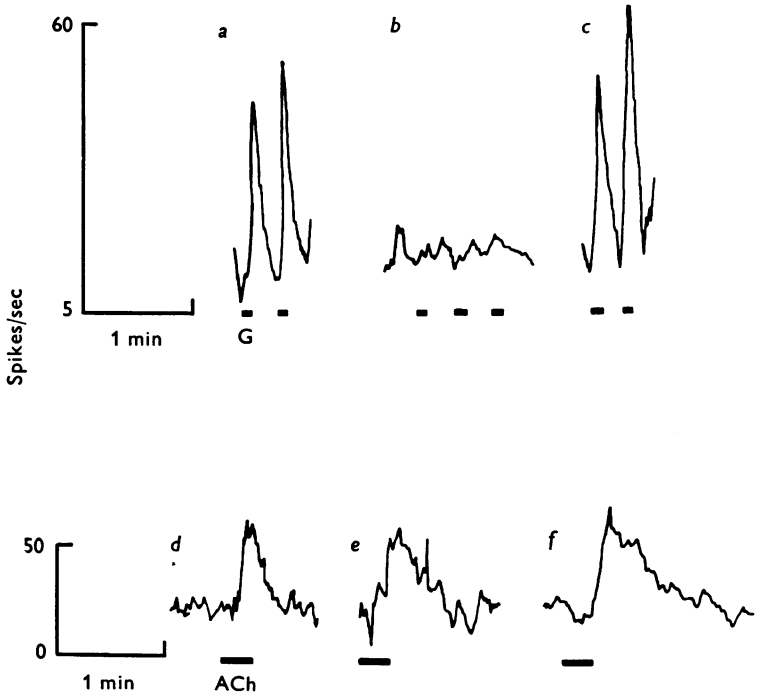

Fig. 6. Rate-meter records of firing of a pyramidal tract cell.
a–c, Responses to glutamate 60 nA (G) and *d–f*, responses to acetylcholine 60 nA (ACh), (*a* and *d*) before (*b* and *e*) 15 min after and (*c* and *f*), 60 min after the I.P. injection of glutamic acid diethyl ester hydrochloride 300 mg/kg.

Blockade of synaptically evoked excitation

Following the isolation of a cell activated monosynaptically from the pyramids as described above, attempts were made to block the evoked activity of the cell by the glutamate antagonists. On twenty-one of twenty-eight cells tested in this way, the synaptically induced spike or spikes have been blocked within 5 min of the I.P. injection of GDEE. Two examples of this result are shown in Fig. 7. In Fig. 7*a* an apparently monosynaptically activated single spike is seen. This was blocked by the intraperitoneal injection of GDEE as shown in Fig. 7*b*.

A possibly clearer example is seen in Fig. 7*c–e*. Here a pyramidal tract cell antidromic spike (latency 2·0 msec) is followed by a synaptically induced spike of the same cell, monosynaptically produced according to

the above criteria. A comparison of Fig. 7*c* and Fig. 7*e* (the latter on a faster time scale) shows clearly the latency variation of the second spike (latency 3·48 msec in *c*, and 3·85 msec in *e*). GDEE blocked the synaptically induced spike (Fig. 7*d*) without affecting the antidromic response. It should be noted here that the small size of some of the spikes studied is due to the fact that no attempt was made to approach these cells very closely. Whilst this would be necessary for the iontophoretic application of agents, it serves no purpose when substances are introduced systemically, but greatly increases the risk of cell damage.

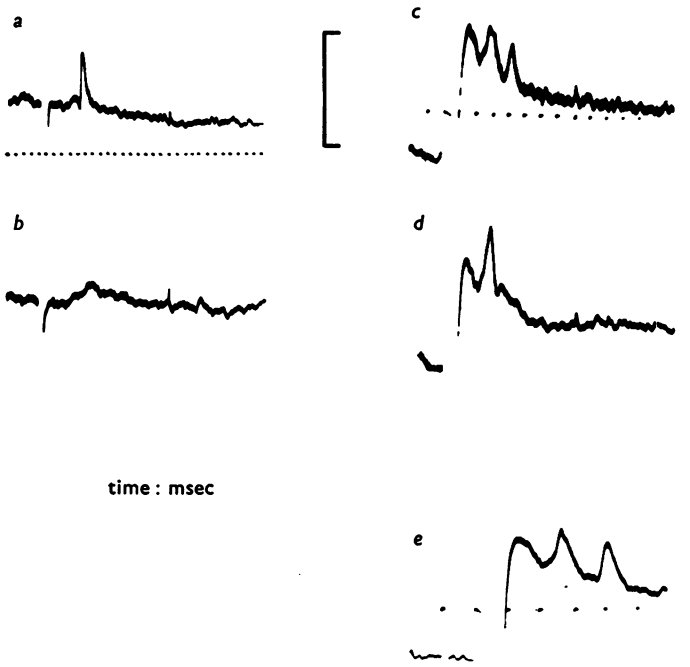

Fig. 7. *a*, A largely negative spike recorded from a cortical neurone activated monosynaptically from the pyramidal tract is shown.

b, The cell is no longer excited 4 min after the I.P. administration of glutamic acid diethyl ester 300 mg/kg.

c and *e*, A pyramidal tract cell antidromic spike latency 2·0 msec is followed by a monosynaptically activated spike of the same cell, latency 3·48 msec in (*c*) 3·85 msec in (*e*).

d, The monosynaptically activated spike is blocked 2 min after the I.P. injection of glutamic acid diethyl ester 300 mg/kg. Time: msec voltage calibration: 400 μV.

Attempts to block pyramidally evoked synaptic activity by iontophoretic application of glutamate antagonists proved less successful. Antagonism has been demonstrable by this method on five of twenty

interneurones recorded to date. However, this by no means invalidates the proposal that GDEE is an antagonist of pyramidally induced activity as seen after parenteral administration, as discussed later.

DISCUSSION

The interneurones described in this report have many properties similar to those studied by Stefanis (1969). It is most likely therefore that a similar population of neurones has been studied here, indicating the existence of pyramidally activated interneurones in the rat cortex analogous to those in the cat (Stefanis, 1969).

It is probable that these cells are inhibitory interneurones mediating recurrent inhibition of pyramidal tract cells (Stefanis, 1969). Such inhibition has been discussed on many previous occasions (Armstrong, 1965; Brooks & Asanuma, 1965; Phillips, 1959; Stefanis & Jasper, 1964).

The relatively deep cortical location of these cells and the potent action of specific afferent volleys upon them also suggests that they may be stellate cells upon which specific thalamocortical afferents probably synapse (Colonnier & Rossignol, 1969).

In agreement with the reports of Curtis *et al.* (1972) Haldeman *et al.* (1972) and Haldeman & McLennan (1972) the present results show that glutamic acid diethyl ester is able to antagonize the responses of some neurones to iontophoretically applied glutamate. This antagonism appears to be quite specific, there being only slight interference with acetylcholine excitation of pyramidal tract cells, and no apparent effect on spike size or duration. However, Curtis *et al.* (1972) have pointed out that excitatory responses to some closely related amino acids are also reduced by GDEE.

The potentiation of glutamate excitation by GDEE is probably analogous to the same phenomenon observed occasionally by Haldeman & McLennan (1972) during the iontophoresis of GDEE and glutamic acid dimethyl ester. It is unlikely that this potentiation is due to a reduction in the rate of removal of glutamate from its site of action, since Balcar & Johnston (1972) have shown that GDEE does not inhibit the uptake mechanisms for glutamate (which are probably responsible for its removal and effective inactivation) in brain slices. An alternative possibility is that proposed by Haldeman & McLennan (1972) that, since GDEE probably acts on the same receptors as glutamate to cause blockade, and since it is very closely related chemically to glutamate, it may occasionally produce a stimulation of the receptors causing excitation. It is also possible that some hydrolysis of the ester could occur with the production of glutamate itself.

It is not clear why there should be a greater reduction of acetylcholine

sensitivity following the iontophoretic ejection of GDEE than after I.P. administration. It is possible that the distribution of receptor sites for the two putative transmitters may be very different, and this would affect the relative antagonist potency of GDEE as seen by the different methods of administration. An alternative possibility is that little of the injected GDEE passes the blood–brain barrier, so that the effective concentration of GDEE at cortical cells would be much less than after iontophoretic ejection. This suggestion would also explain the much slower onset of antagonism after I.P. injection. The demonstration that iontophoretically applied glutamate may be antagonized by I.P. GDEE shows that the ester can cross the blood-brain barrier to some extent.

Perhaps the most significant finding presented here concerns the exquisite sensitivity to L-glutamate of interneurones activated with short latency from the pyramidal tract. This outstanding sensitivity of some cortical units to pulses of L-glutamate has in fact been noted previously in the cat cerebral cortex (Krnjević, 1964) but no attempt was made at that time to identify the responsive cells. It is tempting to suggest that this phenomenon is indicative of the presence of specific subsynaptic receptors for glutamate, and the more usual, longer latency responses may not be related to synaptic activity. Such a distinction would remove the oft quoted objection to the possibility that glutamate may be a transmitter, that it appears to be a universal excitant, depolarizing almost all the mammalian central neurones tested. That this assertion is in any case fallacious has been pointed out by Johnson (1972), and several reports exist of differential sensitivity of neurones to glutamic acid (McLennan, Huffman & Marshall, 1968; Morgan, Vrvobá & Wolstencroft, 1972).

It is unlikely that the different glutamate sensitivities noted here were due to neurone size, since several cells apparently unaffected by pyramidal tract stimulation have been found not to respond to brief glutamate pulses. Since these were not pyramidal tract cells their size was probably comparable to the pyramidal tract interneurones studied here. Intracellular marking experiments are needed to clarify this point, although the experiments would be better carried out on cells of the cat's cerebral cortex, which are slightly larger than those of rat.

The blockade of synaptically evoked activity by GDEE raises the possibility that glutamate may be involved in the synaptic transmission process, possibly at pyramidal tract axon terminals (Krnjević, 1970). Certainly the blockade of evoked activity following intraperitoneal injection has a similar latency of onset as the blockade of iontophoretically applied glutamate. Although blockade of the activation of only five cells resulted from the iontophoresis of GDEE, this could be due to the existence of collaterally activated synapses at some distance from the ion-

tophoretic electrode. Following the systemic administration of a substance, that substance will become fairly evenly distributed throughout any given tissue, and, in the case of brain, this means that all synapses will be potentially affected by the injected agent. It will not matter, in this case, if a neural input to the cell under study makes synaptic contact at some distance from the recording electrode. If the same substance is applied by micro-iontophoresis it will reach only those synapses situated relatively close to the iontophoretic micropipette. The somewhat poorer success obtained in blocking synaptically evoked activity by the microiontophoresis of GDEE compared with the I.P. injection, therefore, suggests that synapses activated by pyramidal tract collaterals exist at some distance from the iontophoretic electrode. By interpolation from the results in Fig. 6 of Herz, Zieglgänsberger & Färber (1969), it might be expected that the iontophoretic ejection of glutamate with a current of 100 nA would reach sites up to 100 μm distant from the micropipette. If GDEE can travel through brain tissue in a manner comparable to glutamate, this would suggest that the collaterally activated synapses discussed above occurred at least 100 μm from the site of recording, presumed to be the cell body. This would indicate a probably dendritic location for these synapses.

The demonstration of this blockade, considered with the presence of high concentrations of glutamate in the cortex (Berl & Waelsch, 1958), its presence in synaptosomes (Kuhar & Snyder, 1969), and release therefrom by electrical stimulation (Bradford, 1970), the existence of a high affinity uptake system for glutamate (Logan & Snyder, 1971) and the cortical release of glutamate produced by stimulation of only certain corticopetal pathways (Jasper & Koyama, 1968; 1969) support the idea that glutamate may be a neurotransmitter in the cerebral cortex (Krnjević, 1970).

REFERENCES

ARMSTRONG, D. M. (1965). Synaptic excitation and inhibition of Betz cells by antidromic pyramidal volleys. *J. Physiol.* **178**, 37–38*P*.

BALCAR, V. J. & JOHNSTON, G. A. R. (1972). Glutamate uptake by brain slices and its relation to the depolarisation of neurones by acidic amino acids. *J. Neurobiol.* **3**, 295–302.

BERL, S. & WAELSCH, H. (1958). Determination of glutamic acid, glutamine, glutathione and Gaba and their distribution in brain tissue. *J. Neurochem.* **3**, 161–169.

BRADFORD, H. F. (1970). Metabolic response of synaptosomes to electrical stimulation: release of amino acids. *Brain Res.* **19**, 239–247.

BRADLEY, P. B. & DRAY, A. (1972). The effect of different anaesthetics on responses of brain stem neurones to iontophoretically applied transmitter substances. *Br. J. Pharmac.* **45**, 169–170*P*.

BROOKS, V. B. & ASANUMA, H. (1965). Recurrent cortical effects following stimulation of medullary pyramid. *Archs ital. Biol.* **103**, 247–278.

CARLSSON, A., FALCK, B. & HILLARP, N.-A. (1962). Cellular localization of brain monoamines. *Acta physiol. scand.* **56**, suppl. 196.

COLONNIER, M. & ROSSIGNOL, S. (1969). Heterogeneity of the cerebral cortex. In *Basic Mechanisms of the Epilepsies*, ed. JASPER, H. H., WARD, A. A. & POPE, A. London: Churchill.

CRAWFORD, J. M. & CURTIS, D. R. (1964). The excitation and depression of mammalian cortical neurones by amino acids. *Br. J. Pharmac.* **23**, 313–329.

CROSS, B. A. & DYER, R. G. (1971). Unit activity in rat diencephalic islands – the effect of anaesthetics. *J. Physiol.* **212**, 467–482.

CURTIS, D. R., DUGGAN, A. W., FELIX, D., JOHNSTON, G. A. R., TEBÉCIS, A. K. & WATKINS, J. C. (1972). Excitation of mammalian central neurones by acidic amino acids. *Brain Res.* **41**, 283–301.

CURTIS, D. R. & WATKINS, J. C. (1965). The pharmacology of amino acids related to gamma-aminobutyric acid. *Pharmac. Rev.* **17**, 347–391.

DAHLSTRÖM, A. & FUXE, K. (1964). Evidence for the existence of monoamine-containing neurones in the CNS. I. Demonstration of monoamines in the cell bodies of brain stem neurones. *Acta physiol. scand.* **62**, suppl. 232.

DESI, I., FARKAS, I., SOS, J. & BAOGH, A. (1967). Neurophysiological effects of glutamic acid ethylester. *Acta physiol. hung.* **32**, 323–335.

FELDBERG, W. & VOGT, M. (1948). Acetylcholine synthesis in different regions of the C.N.S. *J. Physiol.* **107**, 372–381.

HALDEMAN, S., HUFFMAN, R. D., MARSHALL, K. C. & MCLENNAN, H. (1972). The antagonism of the glutamate-induced and synaptic excitation of thalamic neurones. *Brain Res.* **39**, 419–425.

HALDEMAN, S. & MCLENNAN, H. (1972). The antagonistic action of glutamic acid diethyl ester towards amino-acid-induced and synaptic excitations of central neurones. *Brain Res.* **45**, 393–400.

HERZ, A., ZIEGLGÄNSBERGER, W. & FÄRBER, G. (1969). Micro-electrophoretic studies concerning the spread of glutamic acid and GABA in brain. *Expl Brain Res.* **9**, 221–235.

HOLMES, O. & HOUCHIN, J. (1966). Units in the cerebral cortex of anaesthetized rat and the correlation between their discharges. *J. Physiol.* **187**, 651–671.

JASPER, H. H. & KOYAMA, I. (1968). Amino acids released from the cerebral cortex surface in cats following stimulation of mesial thalamus and mid brain reticular formation. *Electroenceph. clin. Neurophysiol.* **24**, 292.

JASPER, H. H. & KOYAMA, I. (1969). Rate of release of amino acids from the cerebral cortex in the cat as affected by brain stem and thalamic stimulation. *Can. J. Physiol. Pharmacol.* **47**, 889–905.

JOHNSON, J. L. (1972). Glutamic acid as a synaptic transmitter in the nervous system. A review. *Brain Res.* **37**, 1–19.

KOELLE, G. B. (1954). The histochemical localization of cholinesterases in the central nervous system of the rat. *J. comp. Neurol.* **100**, 211–236.

KRNJEVIĆ, K. (1964). Micro-iontophoretic studies on cortical neurons. *Int. Rev. Neurobiol.* **7**, 41–98.

KRNJEVIĆ, K. (1970). Glutamate and γ-aminobutyric acid in brain. *Nature, Lond.* **228**, 119–124.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963). Iontophoretic studies of neurones in the mammalian cerebral cortex. *J. Physiol.* **165**, 274–304.

KUHAR, M. J. & SNYDER, S. H. (1969). Localization of ^{3}H-glutamic acid (^{3}H-GLU) and ^{3}H-glycine (^{3}H-GLY) in synaptosomes of rat cerebral cortex. *Fedn Proc.* **28**, 578.

KURIYAMA, K., ROBERTS, E. & KAKEFUDA, T. (1968). Association of the γ-aminobutyric acid system with a synaptic fraction from mouse brain. *Brain Res.* **8**, 132–152.

LOGAN, W. J. & SNYDER, S. H. (1971). Unique high affinity uptake systems for glycine, glutamate and aspartic acids in central nervous tissue of the rat. *Nature, Lond.* **234**, 297–299.

MCLENNAN, H., HUFFMAN, R. D. & MARSHALL, K. C. (1968). Patterns of excitation of thalamic neurones by amino acids and acetylcholine. *Nature, Lond.* **219**, 387–388.

MORGAN, R., VRBOVÁ, G. & WOLSTENCROFT, J. H. (1972). Correlation between the retinal input to lateral geniculate neurones and their relative response to glutamate and aspartate. *J. Physiol.* **224**, 41–42*P*.

PHILLIPS, C. G. (1959). Action of antidromic pyramidal volleys on Betz cells in cat. *Q. Jl exp. Physiol.* **44**, 1–25.

STEFANIS, C. (1969). Interneuronal mechanisms in the cortex. In *The Interneurone*, ed. BRAZIER, M. A. B. Los Angeles: University of California Press.

STEFANIS, C. N. & JASPER, H. H. (1964). Recurrent collateral inhibition in pyramidal tract neurones. *J. Neurophysiol.* **27**, 855–877.

STONE, T. W. (1972*a*). Cholinergic mechanisms in the rat somatosensory cerebral cortex. *J. Physiol.* **225**, 485–499.

STONE, T. W. (1972*b*). Cortical responses to pyramidal tract stimulation in the rat. *Expl Neurol.* **35**, 492–502.