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THE EXCITATION AND DEPRESSION OF MAMMALIAN CORTICAL NEURONES BY AMINO ACIDS

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Amino acids related to L-glutamic and γ-amino-*n*-butyric acid have been administered electrophoretically, and by pressure ejection, into the extraneuronal environment of single neurones in the pericruciate cortex of cats anaesthetized with allobarbitone or allobarbitone-urethane. Acidic amino acids related to glutamic acid, particularly *N*-methyl-D-aspartic acid, excited cortical neurones. Neutral amino acids related to γ-amino-*n*-butyric acid, particularly 3-amino-1-propanesulphonic acid, depressed cortical neurones. Some of the depressants blocked the antidromic invasion of Betz cells by pyramidal volleys. There are no essential differences between the sensitivities of cortical and spinal neurones towards locally administered amino acids. A transmitter function of such amino acids within the mammalian central nervous system is considered unlikely.

When ejected electrophoretically from glass micropipettes, many acidic amino acids related to glutamic acid excite neurones in the feline spinal cord (Curtis, Phillis & Watkins, 1960 ; Curtis & Watkins, 1960b, 1963), brain stem (Curtis & Koisumi, 1961), lateral geniculate nucleus (Curtis & Davis, 1962), thalamus (Curtis & Andersen, 1962 ; Andersen & Curtis, 1964a), retina (Noell, 1960), cerebral cortex (Krnjević & Phillis, 1963a), cerebellar cortex (Krnjević & Phillis, 1963a ; Crawford Curtis, Voorhoeve & Wilson, 1963) and hippocampal cortex (Andersen, Crawford & Curtis, unpublished). In contrast, neutral amino acids related to γ-amino-*n*-butyric acid (GABA) are depressants (Curtis, Phillis & Watkins, 1959 ; Curtis & Watkins, 1960a, b, 1961 ; Noell, 1960 ; Krnjević & Phillis, 1963a). The similarity in the actions of these particular amino acids upon spinal interneurones, Renshaw cells, motoneurones, and geniculate and thalamic neurones has led to the use of the term " unspecific " when describing the excitant and depressant effects. Thus it has been considered that the actions of these two classes of amino acids are not closely related to the normal processes of synaptic transmission, although it is conceivable that some of these compounds may have a nonsynaptic regulatory function within the nervous system.

Recent experiments in which amino acids were ejected near single cortical neurones (Krnjević & Phillis, 1963a) have shown that in general the amino acid sensitivity of these cells is very similar to that of spinal interneurones. However, certain of the differences which have been reported raise doubts as to the non-

specificity of amino acid action. Whereas D-glutamic acid was approximately half to two-thirds as potent as the L-isomer as an excitant of spinal (Curtis & Watkins, 1963) and thalamic neurones (Andersen & Curtis, 1964a), cortical neurones are stated to be very much more sensitive to the L- than to the D-isomer (Krnjević & Phillis, 1961). Furthermore, *N*-methyl-D-aspartic acid, a powerful excitant of thalamic, geniculate and spinal neurones (Curtis & Watkins, 1963) has been reported to desensitize cortical neurones to subsequent administration of L-glutamic acid (Krnjević & Phillis, 1963a), a phenomenon which had not been observed elsewhere in the nervous system in the absence of a depression of nerve cell excitability following excessive depolarization (Curtis & Watkins, unpublished). Of the neutral amino acids, Krnjević & Phillis (1961, 1963a) reported that taurine and 3-amino-1-propanesulphonic acid were inactive when ejected near cortical neurones ; however, both compounds, particularly the latter, were depressants of spinal interneurones (Curtis & Watkins, 1960b, 1961).

It was felt that some of these conflicting findings may have arisen from differences in the technique of testing neuronal excitants and depressants, and in the interpretation of the observed results. Consequently the amino acid sensitivity of neurones in the pericruciate region of cats anaesthetized with allobarbitone has been reinvestigated, and the results compared with the sensitivity of spinal interneurones. It is concluded that there are no essential differences between the behaviour of these types of cells towards electrophoretically administered amino acids.

These results were presented briefly at the August (1963) meeting of the Australian Physiological Society.

METHODS

All experiments were performed upon cats anaesthetized with allobarbitone (Dial, Ciba Ltd.) or Dial Compound (Ciba Ltd.) which contained allobarbitone (100 mg/ml.) and urethane (400 mg/ml.). The usual dose was 35 to 50 mg/kg of allobarbitone injected intraperitoneally. The pyramids were exposed by partial removal of the basi-occiput, and the animal's head was then mounted in a rigid frame which could be tilted in two directions so that the pericruciate cortex could be placed approximately in a horizontal plane. The cat was suspended by a pelvic and mid-thoracic clamp with the abdomen and thorax held clear of the base of the animal frame. The preparation was maintained at $37 \pm 1°$ C by two heating pads, one of which was automatically controlled.

The pericruciate cortex was exposed by removal of the overlying bone and the posterior wall of the frontal sinus. The usual exposure was confined to one side of the skull, and extended for 1 to 1.2 cm laterally from the mid-line and 0.7 to 0.8 cm caudal to the coronal suture. After opening the dura the cortex was covered with several layers of polyethylene or cellophane sheeting, apart from a small opening left at the site of recording. During microscopic examination of the cortex the pia mater was gently removed with fine forceps from a suitable avascular area, and throughout the experiment mammalian Ringer solution (at 38° C and saturated with 95% oxygen and 5% carbon dioxide) irrigated the exposed tissue. In order to stabilize the cortex a small transparent pressure plate was applied to the site of recording without affecting the blood flow in underlying vessels (see Phillips, 1956).

Experiments were also carried out upon spinal interneurones in cats anaesthetized with pentobarbitone sodium (see Curtis & Watkins, 1960b).

The microelectrophoretic method of controlling the efflux of substances from the four drug-containing barrels of five-barrel micropipettes has been described in previous publications (Curtis & Eccles, 1958 ; Curtis & Watkins, 1960b, 1963). The overall tip diameter of the

micropipettes was 4 to 8 μ, and filling was carried out by centrifugation. Thus amino acids could be tested within a short time of preparation of the solution. This was particularly necessary when alkali (sodium hydroxide) had been added to produce a *p*H in excess of 8.5, since the *p*H fell during storage of pipettes which contained such solutions. This was presumably due to absorption of carbon dioxide from the atmosphere and to interaction between the alkali and Pyrex glass. When storage was necessary, filled micropipettes were placed in an atmosphere of nitrogen (3 to 4° C).

The concentrations of the amino acid solutions which were used are given in the Tables of this paper. When it was necessary to alter the *p*H in order to produce adequate ionization of these compounds (see Curtis & Watkins, 1960b), hydrogen ion concentrations were measured by means of a glass electrode system.

Extracellular spike responses were recorded by means of the central barrel of the five-barrel micropipette, which contained 4 to 5 M-sodium chloride solution. This was connected to a cathode follower by a small silver-silver chloride junction, and after suitable amplification the spikes were displayed on two double-beam oscilloscopes. Photographic records could be made from one of these oscilloscopes, whilst the other was used to monitor a firing frequency indicating system (Andersen & Curtis, 1964a). Essentially this consisted of a pulse generator which was triggered by the amplified spikes. The triggering level could be altered manually to discriminate between large and smaller spikes; the output pulses were observed simultaneously with the spikes and were counted by a rate meter. Thus the firing frequency of a cell was displayed continuously on a paper recorder. The use of this system ensured that for each test of an amino acid the ejecting current should be maintained until a maximum excitant or depressant effect was observed. Because of the comparatively slow response time of this recording system the plots of firing frequency (see Figs. 1 and 2) did not permit an accurate assessment of the latencies of onset and offset of amino acid-induced firing. Filmed records were necessary for this purpose.

The electrophoretic currents were measured with an accuracy of ± 0.5 nA, and the terms "cationic" and "anionic" are used in this paper to describe currents which eject cations and anions respectively. One barrel of most micropipettes contained a 0.2 M solution of DL-homocysteic acid (*p*H 8–8.5). When ejected as an anion this amino acid is a powerful excitant of neurones (Curtis & Watkins, 1963) and enables ready detection of otherwise quiescent cells. Care had to be taken however that excessive amounts of this amino acid were not ejected and, in general, anionic currents of only 5 to 10 nA were allowed to flow intermittently when cells were being looked for within the cortex.

In several experiments hydrostatic pressure was used to eject amino acid solutions from the individual barrels of micropipettes which had comparatively large orifices (2.5–3.5 μ internal diameter). Silver wires within the sealed system allowed an appropriate retaining voltage to be applied to each barrel. This method of ejection was found to be unreliable, presumably because of plugging of the pipette orifices by tissue components. In some cases pressures of less than 100 mg Hg were adequate to eject sufficient DL-homocysteic acid to excite neurones, in other cases pressures as high as 350 mm Hg were ineffective, although the same cells could be readily excited when the amino acid was ejected electrophoretically as an anion from the same pipette. Observations upon the effect of a test substance ejected by pressure have been included only when either DL-homocysteic or L-glutamic acid excited neurones when ejected with pressures of less than 200 mm Hg from other barrels of the same micropipette assembly. This requirement does not however completely exclude the possibility that substances in adjacent barrels were not ejected by even higher pressures.

The amino acids used were all of analytical purity, and were either provided by Dr. J. C. Watkins (Watkins, 1962) or purchased commercially (California Corporation for Biochemical Research; British Drug Houses, Ltd.). One sample of D(−)-glutamic acid was supplied by Dr K. Krnjević, and when tested upon spinal interneurones was indistinguishable from the sample used in the present investigation.

In view of the difference between the present findings upon the potencies of D(−)- and L(+)-glutamic acid, and those of Krnjević & Phillis (1963a), the optical rotation of the samples

of these substances was kindly determined by Dr A. M. Sargeson upon 2% solutions in 5 M-hydrochloric acid, with the results

$$[\alpha]_D^{20}\ \text{L}(+)\text{-glutamic acid} = +29.5°$$
$$[\alpha]_D^{20}\ \text{D}(-)\text{-glutamic acid} = -30°$$

RESULTS

This investigation was confined to neurones in the pre- and postcruciate region, many of which were firing spontaneously, either in a random fashion or as spindles (Dempsey & Morison, 1943; see also Verzeano & Calma, 1954). The majority of the cells were identified as Betz cells by the responses evoked by single or tetanic stimulation of the pyramids (Phillips, 1956, 1959, 1961); these cells were found at depths of between 0.8 and 1.3 mm below the cortical surface. The relative sensitivities of pyramidal cells and of the more superficially located neurones towards excitant and depressant amino acids did not appear to be different. Thus the general term " cortical neurone " will be used throughout this report to indicate a cell which was located within 2 mm of the cortical surface, and from which spike responses could be produced by the local ejection of an excitant amino acid. The distribution of these neurones was similar to that found in other studies (see Amassian, 1961). It was usually possible to move the micropipette until the extracellular spike responses of one neurone were of the order of 250 to 500 μV (negative-positive), and so could be clearly distinguished from the smaller spikes of more distant cells. Under such circumstances the effects of the flow of current associated with electrophoretic ejection were minimal (see Curtis & Koizumi, 1961) and could readily be distinguished from amino acid actions by testing the effect of current flow through the micropipette containing sodium chloride. In some instances the spike response of a particular neurone could be identified over a distance of 50 to 100 μ, and under these conditions there was little difference between the comparative potencies of the amino acids determined at several different levels of spike size.

One difficulty common to many neurones was the change in spike shape which was observed during activation by an amino acid. Such changes, which are completely reversible, were originally observed when excitant amino acids were ejected near spinal interneurones (Curtis *et al.*, 1960), particularly with L-glutamic acid. It was proposed that the observed " depolarization block," in which the initially negative-positive spike potentials were converted either to predominantly positive spikes, or merely decreased in size before failing entirely, was associated with the change in membrane conductance and the depolarization produced by the excitant (Curtis *et al.*, 1960). In general, these alterations in spike shape of cortical neurones occurred at lower firing frequencies (20 to 60 spikes/sec) than has been observed with spinal interneurones, thalamic and geniculate neurones. As cortical neurones could be fired synaptically (or antidromically in the case of Betz cells) at higher rates than this, it was considered that the comparative ease with which depolarizing substances inactivated the spike generating mechanism might be related to disturbances in cerebral cortical circulation consequent upon the surgical procedures. Corpuscular clumping was usually evident in the smaller cortical veins, and it is possible that all the cortical neurones studied were slightly depolarized as a result

of tissue hypoxia. Deterioration in the cortical circulation and heightened sensitivity to excitant amino acids were most evident towards the end of experiments which lasted 12 to 18 hr.

Action of acidic amino acids

The difficulties which are associated with the assessment of the relative potencies of electrophoretically administered excitant amino acids have been discussed by Curtis & Watkins (1963). The most satisfactory technique is to obtain records from a large number of neurones, and to compare the maximal effects of different doses of amino acids against a reference compound (in this investigation L-glutamic acid). Reasonably consistent results were obtained for any one compound when tested upon four or five cells in each of two or three preparations. Fresh solutions, in various combinations and in fresh micropipettes, were used for each preparation. The relative potencies of amino acids were determined from the ratio of ejecting currents which produced equal firing frequencies, and therefore express the ratios of concentrations required for equal effects, a method generally accepted when the activities of agents interacting with a common receptor are compared. This method is preferable to a comparison between the effects produced by equal extraneuronal concentrations (see Krnjević & Phillis, 1963a, p. 282) as, in the latter instance, differences in response, and therefore in potency ratios, depend upon the portions of the dose/response curves which are being studied.

The range and mean potencies are given in Table 1, together with the number of separate tests. Furthermore, to facilitate comparison, the potency of these amino acids as excitants of spinal interneurones is also included (from Table 1, Curtis & Watkins, 1963). The time parameters of the excitation of cortical neurones by the various amino acids were very similar to those found for spinal neurones. The excitant action of all acidic amino acids was readily reversible, and recovery occurred even after long periods of "depolarization block" had been produced by the administration of excessive amounts of amino acid.

N-Methyl-D-aspartic acid was the most potent excitant amino acid of those tested, and had the longest duration of action after the electrophoretic current was termi-

TABLE 1

EXCITANT AMINO ACID POTENCIES

* Data from Curtis & Watkins (1963). † With solutions at *p*H 3·1, delayed excitation was observed (see text). ‡ Data from Curtis & Watkins (1960b). Sat. = saturated.

Amino acid	Concentration within micropipette (M)	*p*H of solution	Potency upon cortical neurones			Potency upon spinal interneurones
			Mean	Range	Tests	
N-Methyl-D-aspartic acid	0·1	8·3–8·6	6·5	2–16	24	5–12*
DL-Homocysteic acid	0·2	8·0–8·5	4	2·5–6	23	2–6*
L-Glutamic acid	2	8·0–8·2	1·0	Standard		1*
L-Aspartic acid	2	8·2–8·5	1	1–1·2	5	1*
D-Glutamic acid	2	8·0–8·3	0·7	0·5–0·8	16	0·5–0·7*
D-Aspartic acid	2	8·2–8·4	0·8	0·7–1·0	5	0·5–0·7
L-Asparagine	Sat.	3·1–4·5	Inert†		17	Inert ‡
D-Asparagine	Sat.	3 and 5	Inert		9	

nated (Curtis & Watkins, 1963). In some experiments diffusion of this substance from a 0.1 M solution was sufficient to fire neurones. When ejected with currents of 3 to 10 nA this amino acid produced a graually increasing rate of firing over 15 to 30 sec (Fig. 1), and the rate declined over a period of 2 to 10 sec after the ejection had ceased. When larger amounts of *N*-methyl-D-aspartic acid were used (currents of 12 to 30 nA) the cell increased its rate of firing more rapidly until a " depolarization block " was observed, the spike then being indistinguishable from the background noise. Simultaneously the spontaneous and antidromic spikes of the neurone were also blocked. Several seconds after the current which ejected the amino acid had been terminated, spikes often reappeared transiently for about 1 sec, presumably as portions of membrane, which had been rendered incapable of generating spikes by the amino acid, recovered and the level of depolarization remained above the threshold for spike generation.

The high potency of this compound probably explains the finding of Krnjević & Phillis (1963a) that *N*-methyl-D- and *N*-methyl-DL-aspartic acid caused a prolonged

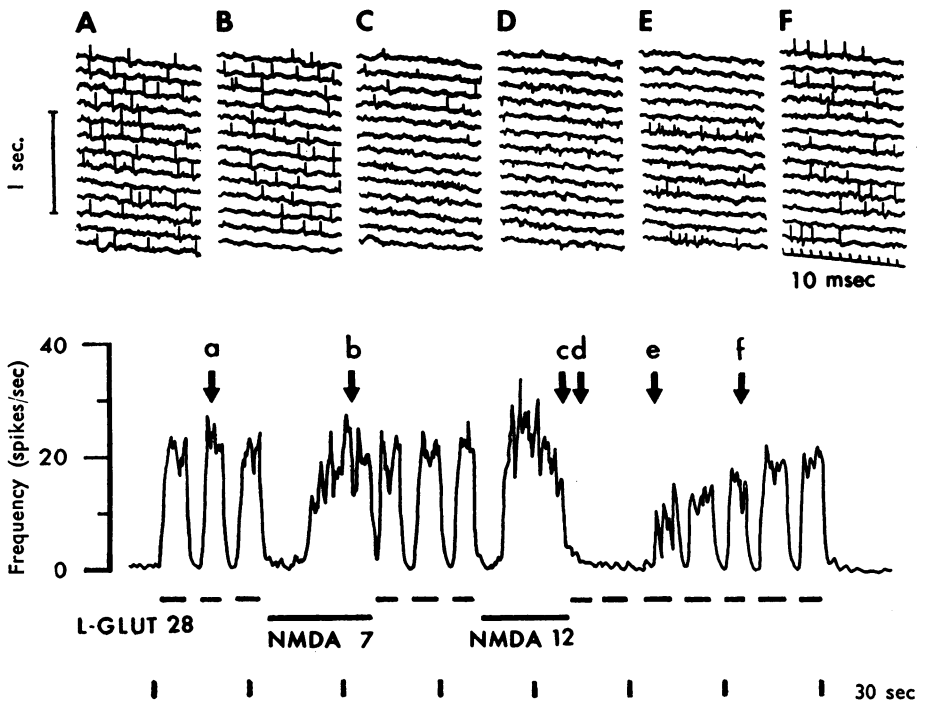

Fig. 1. Effect of *N*-methyl-D-aspartic acid (NMDA, retaining current 4nA) upon the firing of a neurone located in the postcruciate area by L-glutamic acid (L-GLUT, 28nA). In the lower half of the figure successive ejections of each of these compounds are indicated by horizontal bars (upper bars, L-glutamate ; lower bars NMDA), with the frequency of firing (spikes/sec) plotted against time. Sections (A-F) of the concurrent filmed records of the extracellular spike potential (negative upwards) are placed above this frequency record, each being representative of the spikes at times indicated by the arrows and the letters a-f (see text). Time, 10 msec for filmed records ; 1 sec for rate of vertical film movement ; 30 sec for paper recorder.

reduction in the sensitivity of cortical neurones to L-glutamic acid. Many tests of this interaction were carried out, but the finding could be repeated only if sufficient *N*-methyl-D-aspartic acid had been administered to produce a block of neuronal spikes. The results from one such experiment are shown in Fig. 1. Initially three successive ejections of L-glutamic acid (28 nA) gave reasonably consistent firing rates of about 20 spikes/sec. Following this, the ejection of *N*-methyl-D-aspartic acid (7 nA) produced a firing frequency of 15 to 20 spikes/sec, with no change in the shape of the spike potential (compare A and B), and the firing produced by the following dose of L-glutamic acid (within 3 sec of the end of the ejection of *N*-methyl-D-aspartic acid) was unaffected. However, when a current of 12 nA was used to eject *N*-methyl-D-aspartic acid a maximum frequency of approximately 30 spikes/sec was attained, the spike was altered in shape, and the cellular responses were eventually blocked (C). Subsequently, two doses of L-glutamic acid produced only small positive spikes (D) but, 35 sec after the cessation of the *N*-methyl-D-aspartic acid ejection, L-glutamic acid again fired the cells with negative-positive spikes. Initially these spikes were small (compare E with A) and full recovery of spike size (F) was observed after 1 min.

Although in most cases the onset of complete block by *N*-methyl-D-aspartic acid was comparatively slow, with several cells and particularly with electrophoretic currents greater than 20 nA the onset was abrupt and the spikes suddenly vanished with little or no preliminary alteration in shape. Following the administration of sufficient *N*-methyl-D-aspartic acid to suppress cellular responses the usual sequence of recovery was return of antidromic invasion, restoration of spike amplitude and shape, and finally re-establishment of the sensitivity to L-glutamic acid.

Similar observations have been made upon the apparent reduction in sensitivity of neurones to L-glutamic acid by prior administration of DL-homocysteic acid and *N*-*n*-propyl-D-aspartic acid, and also to test doses of DL-homocysteic acid by *N*-methyl-D-aspartic and L-glutamic acids. Furthermore, experiments upon spinal interneurones have failed to demonstrate any depression of the firing induced by either L-glutamic or DL-homocysteic acid by a preceding dose of *N*-methyl-D-aspartic acid. In fact, as occasionally observed with cortical cells, the effectiveness of L-glutamic or DL-homocysteic acid was actually increased when these were ejected shortly after the administration of *N*-methyl-D-aspartic acid was terminated. When sufficient of the latter was ejected to produce a depolarization block of spinal interneurones the effects of subsequent doses of other excitant amino acids were reduced, as found with cortical neurones.

Several cortical cells were tested with graded doses of *N*-methyl-D-aspartic acid and it was found that there need be a difference of only 10 to 20% between currents (concentrations) which produced neither "depolarization block" nor a reduction in the sensitivity and those which did produce block and reduction in the sensitivity. It was thus concluded that the diminution in amino acid sensitivity was primarily a consequence of alterations in membrane conductance and potential, rather than of receptor desensitization due to a prolonged occupation of amino acid receptors by the *N*-alkyl excitant amino acid. This phenomenon is possibly related to the frequently observed reduction in the spontaneous discharge rate which follows

induction by amino acids of high frequency firing of spinal, thalamic and cortical neurones.

Action of depressant amino acids

Neutral amino acids related to GABA reduce the sensitivity of neurones in the cortex to depolarization by acidic amino acids, and to excitation by other means. The potency of these substances can be most readily assessed in terms of the

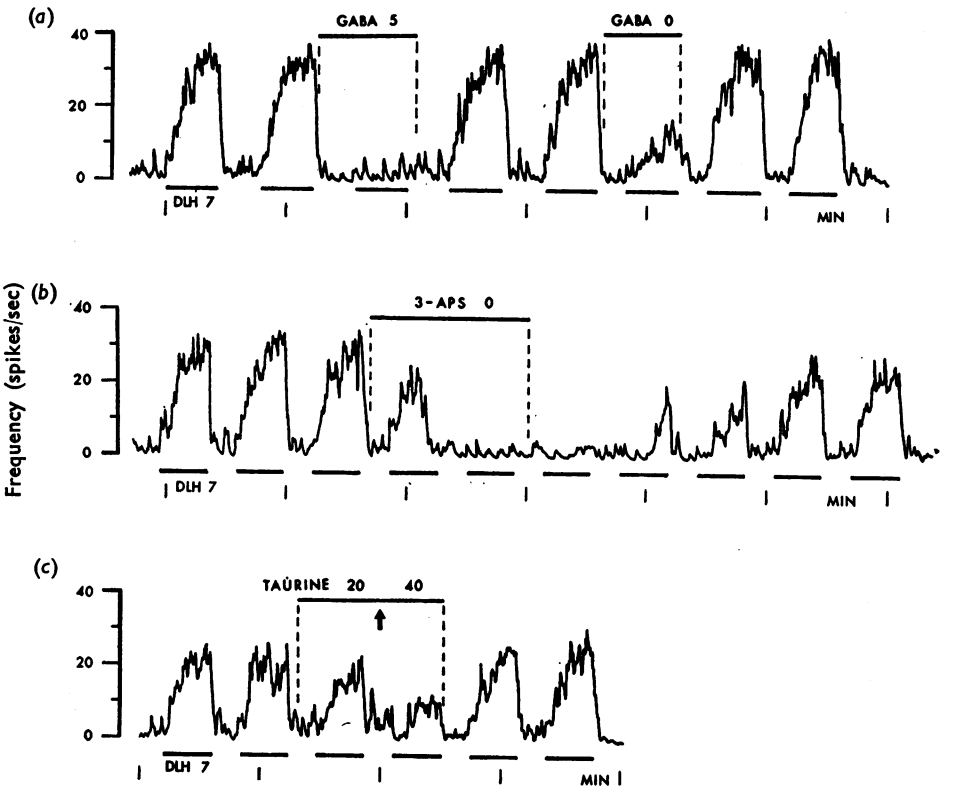

Fig. 2. (*a*) The effect of γ-amino-*n*-butyric acid (GABA) upon the frequency of the firing of a cortical neurone induced by repeated doses of DL-homocysteic acid (DLH, currents of 7nA, lower signal trace). GABA was retained by an anionic current of 20 nA and was ejected during the first interval (upper bar) by a cationic current of 5 nA. During the second interval the retaining current was terminated, allowing free diffusion of this substance from the micropipette which contained a molar solution (*p*H 2·9). Note the short duration of depressant action.
(*b*) Firing frequency of the same cortical unit as in Fig. 2*a*, showing the effect of the removal of the retaining current (anionic, 4nA) from a barrel which contained 3-amino-1-propane-sulphonic acid (3APS, 0·1M, *p*H 9·4). The lower horizontal bars again indicate repeated ejections of DLH, using currents of 7nA. The depressant action of the neutral sulphonic acid lasted for over 2 min after the retaining current was reapplied.
(*c*) The effect of taurine upon the DLH-induced firing (lower bars, 7nA) of the same cortical cell as (*a*) and (*b*). During the period of the upper horizontal bar taurine was ejected as an anion initially with a current of 20 nA which was doubled at the arrow. Time in minutes below each frequency plot.

electrophoretic current needed to produce a specified reduction in the firing rate of a cell excited by an acidic amino acid. In this series of experiments DL-homocysteic acid was used as the control excitant substance, and was ejected either continuously or at fixed intervals and for constant times (see Fig. 2, *a* to *c*). Table 2 lists the neutral amino acids used in this study, together with the concentrations and *p*Hs of the solutions. Potencies are expressed relative to that of GABA (– – –), the number of symbols indicating greater (– – – –), less (– –) and very weak (–) activity.

TABLE 2

DEPRESSANT AMINO ACID POTENCIES

The potency of cortical neurones is relative to γ-amino-*n*-butyric acid (– – –) ; – – – –, greater; – –, less ; – very weak. *Late excitation with low *p*H solutions, see text.

Amino acid	Concentration within micropipette (M)	*p*H of solution	Potency upon cortical neurones
γ-Amino-*n*-butyric acid	1, 2	2·9–7·2	– – –
γ-Amino-β-hydroxybutyric acid	2	3·1	– –
γ-Hydroxybutyric acid	1	7	0
3-Amino-1-propanesulphonic acid	0·2	9–10·6	– – – –
Taurine	0·8	8·5–10	–
Guanidino-acetic acid	0·025	3–3·1	–
β-Guanidino-propionic acid	1	3	– –
ε-Aminocaproic acid	0·5	3·1–6·8	– *
ω-Aminocaprylic acid	0·5–1	3·0–7·7	0 *

*γ-Amino-*n*-butyric acid (GABA).* The depression of excitability, and of spontaneous firing, produced by this compound had a short latency of both onset and offset, and could be demonstrated by diffusion of GABA from a 2 M solution (Fig. 2, A) or when the amino acid was ejected electrophoretically from solutions within the range of *p*H 2.9 to 7.2 ($pK_1 = 4.23$). It is thus extremely unlikely that there is any difference between the action of GABA cations and zwitterions, as has been proposed by Kuno (1960, 1961), Muneoka (1961) and Kuno & Muneoka (1961, 1962); (see also Curtis *et al.*, 1959 ; Curtis & Watkins, 1960a, b ; Krnjević & Phillis, 1963a).

γ-Amino-β-hydroxybutyric acid was approximately half as potent as GABA as a depressant of the firing of cortical neurones induced by DL-homocysteic acid, whereas γ-aminobutyrylcholine was a very weak depressant. The available sample of this latter compound contained GABA, and it is possible that this accounted for the depressant action which was observed.

γ-Hydroxybutyric acid. Although not an amino acid, this compound was of interest because of recent reports which indicate that systemic administration of sodium γ-hydroxybutyrate results in the depression of activity in animals and humans (Laborit, Jouany, Gerard & Fabiani, 1960 ; Laborit, Kind & Regil, 1961 ; Drakontides, Schneider & Funderburk, 1962) and in a reduction of spinal reflexes (Basil, Blair & Holmes, 1964). This compound was ejected electrophoretically as an anion, and in several tests (currents as high as 100 nA) failed to modify either

the spontaneous activity of cortical neurones, or the spikes evoked by an excitant amino acid. (Similar results were observed when this acid was administered near spinal interneurones.)

3-Amino-1-propanesulphonic acid. This compound was sufficiently potent to block the firing of many cortical neurones induced by DL-homocysteic acid merely by diffusion from solutions of 0.1 M. Indeed, considerable difficulty was experienced in controlling the diffusional efflux of this amino acid from Pyrex micropipettes, due no doubt to the high electro-osmotic mobility of this substance in aqueous solution. Under suitable conditions (0.1 to 0.2 M solution, *p*H 9 to 10, pipette orifice less than 2 μ internal diameter) this amino acid could be ejected either as an anion or as a cation, although the diffusional efflux was controlled to a large extent by a small anionic current. When ejected as a cation, 3-amino-1-propanesulphonic acid proved to be a powerful depressant of all cortical neurones upon which it was tried, and was more potent than GABA. This is illustrated in Fig. 2, B where the diffusion of 3-amino-1-propanesulphonic acid from an 0.1 M solution depressed the firing of a cortical neurone induced by DL-homocysteic acid to a greater extent than did GABA which diffused from a pipette of approximately the same internal diameter containing a 2 M solution. Furthermore, the depressant action of 3-amino-1-propanesulphonic acid was of longer duration than that of GABA. Similar results were obtained using spinal interneurones. In earlier experiments upon spinal neurones the anomalous behaviour of 3-amino-1-propanesulphonic acid under these conditions led to the use of solutions of *p*H 2.8 for ejecting neutral amino acids near interneurones (Curtis & Watkins, 1961) and in such circumstances it was also greater and more prolonged than that of GABA.

As found previously in spinal cord (Curtis & Watkins, unpublished), if micropipettes had been stored for more than a few hours, even in nitrogen and with small sealed caps upon the open upper ends of the barrels, or if concentrated solutions of *p*H lower than 8.5 were contained within pipettes which exceeded approximately 2 μ in diameter, it proved impossible to control the diffusional efflux of 3-amino-1-propanesulphonic acid. In most experiments, despite the intermittent ejection of DL-homocysteic acid from an adjacent barrel, very few neurones were located when these micropipettes were passed through cerebral or spinal tissue.

Taurine. This compound was readily administered as an anion from solutions of *p*H above 8.5. When ejected with currents of 20 to 80 nA, taurine caused depression or abolition of the response to DL-homocysteic acid of all cells which were tested (see Fig. 2, C), and although weaker than either GABA or 3-amino-1-propanesulphonic acid, taurine had an action upon cortical neurones similar to that already reported for neurones in the spinal cord (Curtis *et al.*, 1959; Curtis & Watkins, 1960a, b). The action of taurine persisted for 10 to 15 sec after the termination of the ejection, a duration which was intermediate between those of GABA and 3-amino-1-propanesulphonic acid.

Guanidino-acetic acid (glycocyamine) and β-guanidino-propionic acid. These compounds were not tested as depressants of the synaptic responses of cortical neurones, but guanidino-propionic acid was slightly more active than glcocyamine as a depressant of the firing of these cells induced by DL-homocysteic acid.

It might be expected that, because of the efficacy of synaptic excitation, the post-synaptic action of a weak depressant amino acid would not be sufficient to affect such responses, although this substance might interfere with the access of an excitant amino acid to membrane receptors (see Curtis & Watkins, 1960b) and so reduce its effectiveness. A re-examination of the action of guanidino-acetic acid upon spinal interneurones has confirmed the lack of action of this compound upon the synaptic responses of these cells, but has demonstrated a reduction in the frequency of firing produced by concurrent administration of an excitant amino acid. On the other hand, β-guanidino-propionic acid was found to be a more potent depressant since it diminished both the synaptic and amino acid evoked firing of spinal interneurones.

Other compounds related to these amino acids

ε-Aminocaproic acid and ω-amino-caprylic acid. These were found, in agreement with Krnjević & Phillis (1963a), to have "somewhat ambiguous" actions when ejected electrophoretically. Solutions of *p*H 3 were necessary to increase the degree of ionization of these compounds and so permit sufficiently large ejecting currents to be passed. Under these conditions ε-aminocaproic acid had a slight depressant action on cortical neurones but, after a latency of 15 to 90 sec, paroxysmal high frequency firing was produced by both compounds and continued for up to 15 sec after termination of the current flow. This excitatory phenomenon could not be reproduced by ejecting neutral solutions (*p*H 6.7 to 7.7) of either of these compounds by means of pressure (up to 300 mm Hg for 3 min), although in most experiments when ε-aminocaproic acid was so administered a depression of the firing of cortical neurones induced by DL-homocysteic acid was observed.

Thus the delayed excitation appears to be related to the effects of local *p*H changes produced when the cationic form of the ejected amino acid releases its extra proton on reaching the medium around the micropipette tip (see Curtis & Watkins, 1960b, p. 119). GABA and closely related short chain ω-amino acids are presumably sufficiently potent depressants to mask this delayed effect.

D- *and* L-*Asparagine.* These were inactive when administered electrophoretically (currents of 30 nA) or by pressure, unless the *p*H of the solutions was below 4. In such experiments a delayed excitation was observed with subsequent depression of the sensitivity of the cell to DL-homocysteic acid which lasted for 3 to 4 min. A similar delayed firing was observed with spinal interneurones (Curtis & Watkins, 1960b) but, as with the longer chain ω-amino acids, was considered to result from local *p*H changes.

Antidromic invasion of Betz cells

If the mode of action of GABA, and related depressants, upon cortical neurones is the same as that which has been found for spinal motoneurones, namely an increase in membrane conductance which reduces the effectiveness of depolarizing currents (Curtis *et al.*, 1959), it would be expected that the antidromic invasion of Betz cells would be depressed by these substances.

Even in the absence of spontaneous firing the safety factor for the antidromic invasion of the body and dendrites of Betz cells is extremely high (see Phillips, 1959) and it was anticipated that some difficulty would occur when attempting to test the effect of GABA upon this form of firing. This proved to be the case, and only in rare instances, using currents of the order of 100 to 200 nA, could sufficient GABA be ejected electrophoretically to block antidromic firing. In several instances GABA converted a soma-dendritic (A or SD) spike to a smaller initial segment (B) spike, but more often there was complete suppression of the soma-dendritic spike to leave a small axonal or M spike. The depression of these antidromic responses was readily measured by the "firing index," which expressed the number of soma-dendritic spikes as a percentage of ten supramaximal axonal stimuli.

3-Amino-1-propanesulphonic acid was more effective than GABA as a depressant of cortical neurones, and the action of this amino acid upon the antidromic spikes of one cell are illustrated in Fig. 3. Throughout the series the stimulus applied

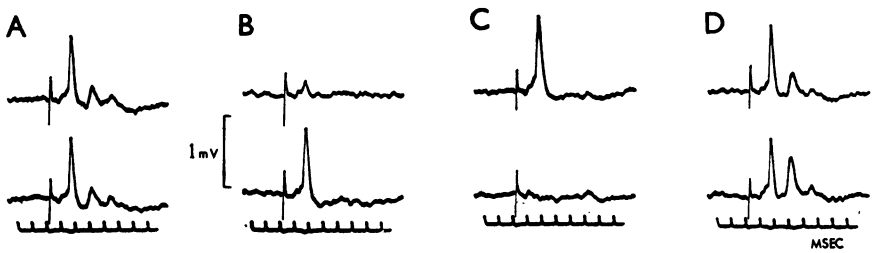

Fig. 3. The effect of 3-amino-1-propanesulphonic acid (3APS) upon the antidromic invasion of a Betz cell in the postcruciate area. Each column shows two successive responses to supramaximal pyramidal stimuli delivered at a rate of 1 shock/sec. Negativity is recorded upwards. A, control ; firing index 100%. B, approximately 10 sec after a current of 80nA began to eject 3APS ; firing 80%. C, 10 sec after this current was increased to 150nA ; firing index 50%. D, 10 sec after the electrophoretic ejection of 3APS was terminated : firing index 100%. Voltage scale, 1 mV, time scale in msec.

to the pyramids was supramaximal for the axon of this Betz cell, and the antidromic spike potential (latency 1.27 msec) was followed by a complex field potential produced by the antidromic and synaptic excitation of other nearby neurones. The firing index of this cell was initially 100% (A), but 10 sec after a cationic current of 80 nA began to eject 3-amino-1-propanesulphonic acid the index was 80% (B). When the electrophoretic current was increased to 150 nA the firing index fell to 50% (C), and full recovery to the original value was observed after the termination of the current (D). The depression, and recovery, of the field potentials generated by other nearby neurones is also apparent in this series.

DISCUSSION

The importance of the present results, and those of Krnjević & Phillis (1963a), lies in the evidence which has been obtained regarding the sensitivity of unidentified pericruciate neurones and Betz cells towards electrophoretically administered amino acids. Although this method of drug administration has some disadvantages (see

Curtis, 1964), particularly in respect to the concentrations which are attained, it is clear that when amino acids are tested by ejection from multibarrel micropipettes there are no essential differences between the amino acid sensitivity of cortical neurones and that of spinal interneurones (Curtis *et al.*, 1959, 1960; Curtis & Watkins, 1960a, b, 1961, 1963). A limited study of spinal Renshaw cells, lateral geniculate neurones (Curtis & Watkins, 1963) and neurones of the ventrobasal thalamus (Andersen & Curtis, 1964a) has also failed to reveal serious discrepancies in this pattern. This type of amino acid sensitivity is in marked contrast to the behaviour of neurones in the various regions towards acetylcholine, cholinomimetics and cholinergic blocking agents (Curtis, Phillis & Watkins, 1961; Curtis & Koizumi, 1961; Krnjević & Phillis, 1963b; Curtis, Ryall & Watkins, 1964; Crawford *et al.*, 1963) or towards indole and phenylethylamine derivatives (Curtis *et al.*, 1961; Curtis & Davis, 1962; Krnjević & Phillis, 1963c; Andersen & Curtis, 1964b).

There are, however, differences between the results reported here, and those of Krnjević & Phillis (1963a). Some of these discrepancies are no doubt due to differences in technique, and it is thus relevant that the method used to assess amino acid actions and potencies in the present investigation were identical with those used in recent studies in this laboratory upon spinal and thalamic neurones (see particularly Curtis & Watkins, 1963). In particular, the use of a rate meter and paper recorder permitted a close analysis to be made of firing frequency and a ready assessment of the maximum action for a particular current used to eject an amino acid. These refinements in technique led to a recent revision (Curtis & Watkins, 1963) of an earlier study of the action of amino acids upon spinal interneurones (Curtis & Watkins, 1960b).

The present findings on the relative potencies of the D- and L-isomers of aspartic and glutamic acid are similar to those of Krnjević & Phillis (1961, 1963a), but, although high accuracy cannot be claimed for such estimations, we do not agree with these investigators that D-glutamic acid was always "much less effective" than L-glutamic acid since the potency difference is of the order of only 0.5 to 0.8. The difference in the evaluation of these two amino acids has presumably arisen from the different methods used to assess excitant amino acid potency, as outlined in Results. The report that *N*-methyl-D-aspartic acid desensitized cortical neurones to subsequent doses of L-glutamic acid (Krnjević & Phillis, 1963a) has not been substantiated, since any such interaction between different amino acids upon cortical and spinal neurones appears to be associated with the presence of a "depolarization block" due to excessive concentrations of a powerful depolarizing agent, rather than to a specific interference of access to receptor sites. However, the slow recovery of amino acid sensitivity after the production of such a "block" (see Fig. 1) warrants additional investigation.

The failure of Krnjević & Phillis (1961, 1963a) to demonstrate a depressant action of electrophoretically administered taurine and of 3-amino-1-propanesulphonic acid is rather surprising since, like GABA, each of these sulphonic amino acids readily depresses cortical responses when applied topically in solution to the exposed cortical surface (Curtis & Watkins, 1961; but see also Purpura, Girado, Smith, Callan & Grunfest, 1959). Furthermore, when injected intraventricularly

into mice, all three amino acids result in a diminution of spontaneous activity, a loss of righting reflexes and eventually a state resembling sleep (Crawford, 1963). Again the relative potencies of the amino acids were similar to those observed in electrophoretic studies upon cortical neurones and spinal interneurones.

In view of the inability to reproduce the delayed excitation of ω-aminocaprylic acid and asparagine by pressure ejection from neutral solutions, it cannot be stated with any degree of certainty that these amino acids excite neurones, or that the results obtained electrophoretically agree with those of Hyashi (1956), Purpura *et al.* (1959) and van Harreveld (1959) who administered amino acid solutions topically to the exposed cortex. On the other hand, if indeed these amino acids do excite central neurones, then the effects upon cortical and spinal neurones are practically identical, since the report by Curtis & Watkins (1960b) that ω-aminocaprylic acid and L-asparagine were inactive upon spinal interneurones was made on the basis that the delayed excitation was not directly associated with these amino acid molecules.

The finding that γ-amino-*n*-butyric acid, and particularly 3-amino-1-propane-sulphonic acid, block the antidromic invasion of Betz cells does not lend support to the postulate of Purpura, Girado & Grundfest (1957) and Purpura *et al.* (1959) (see also Grunfest 1958, 1960; Purpura, 1959, 1960) that GABA, and presumably also closely related amino acids, specifically block superficial excitatory axodendritic synapses. It is clear that, as with spinal motoneurones (Curtis *et al.*, 1959), GABA produces an alteration in the membrane conductance of cortical neurones (see also Bindman, Lippold & Redfearn, 1962). The difficulty which was experienced in blocking the antidromic invasion of Betz cells by pyramidal volleys was presumably associated with the high safety factor for this type of excitation, and with the limited proportion of the membrane of any one cell which was affected by the locally administered amino acid.

The failure of γ-hydroxybutyric acid to affect central neurones, both cortical and spinal, suggests that the alterations in behaviour, in the electroencephalogram and in spinal reflexes which are produced by this compound are unlikely to be due to a direct action upon central cells (Laborit *et al.*, 1960, 1961; Drakontides *et al.*, 1962; Basil *et al.*, 1964). Indeed the latent period between intravenous administration and the depression produced by γ-hydroxybutyrate (Drakontides *et al.*, 1962; Basil *et al.*, 1964) suggests that it has an indirect action, possibly as a result of interfering with the metabolism of GABA. It is pertinent to point out that a related substance, γ-butyrolactone, which also has central depressant effects (Giarman & Schmidt, 1963) does not increase the brain level of GABA. However total brain levels of GABA may not necessarily be related to the state of excitability of central neurones (see also Kamrin & Kamrin, 1961; Medina, 1963) and it may be more relevant to these studies to ascertain differences in the distribution of amino acids between intra- and extra-cellular compartments (see Curtis & Watkins, 1960a).

In conclusion, it may be stated that the actions of individual amino acids upon neurones within the spinal cord and cerebral cortex (and thalamus—Andersen & Curtis, 1964a) are very similar in both potencies and time courses. This similarity suggests either that some of these substance (or structurally related compounds)

are synaptic transmitters common to synapses upon all of these various types of neurone, or that amino acid receptors are components of the membrane of all neurones but are not directly associated with transmitter receptors, in which case the amino acid action could be considered to be nonspecific. Despite the impressive and striking action of amino acids such as GABA and L-glutamic acid, arguments against a role of either of these compounds as synaptic transmitters have already been presented (Curtis *et al.*, 1959, 1960 ; Curtis & Watkins, 1960a, 1963), and the recent findings of Ryall (1962) and of Weinstein, Roberts & Kakefuda (1963) are relevant in that the the subcellular distribution of GABA and L-glutamic acid in mammalian brain does not indicate any special aggregation of these amino acids in synaptic endings, as is found for acetylcholine (see Ryall, 1964). It is thus very probable that the action of these excitant and depressant amino acids is unrelated to the presence of specific transmitter receptor sites.

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