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THE RELEASE OF γ-AMINOBUTYRIC ACID DURING INHIBITION IN THE CAT VISUAL CORTEX

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SUMMARY

1. The release of γ-aminobutyric acid (GABA) from the surface of the posterior lateral gyrus of the cerebral cortex was measured by a sensitive enzymic fluorimetric assay procedure. Experiments were performed with anaesthetized cats during resting conditions and during cortical inhibition produced by electrical stimulation of the brain surface or of the lateral geniculate nucleus (l.g.n.).

2. The average resting release of endogenous GABA was 0·20 n-mole/7 min.cm^2 cortex; this was increased during stimulation of both the cortical surface (2·9 times resting release during monopolar stimulation and 7·4 times resting release during bipolar stimulation) and the l.g.n. (5·7 times resting release).

3. Removal of calcium ions from the collection fluid did not affect the resting release of endogenous GABA but prevented the increase in GABA release normally evoked by stimulation of the cortical surface.

4. The stimulus parameters used to increase the release of GABA also inhibited the glutamate-induced firing of single cells in the visual cortex and this inhibition was abolished in the absence of calcium ions.

5. In three experiments the total amino acid content of cortical samples was examined using an amino acid analyser. With the exception of GABA, there were no significant differences between the rates of release of any other detected amino acids during periods with and without electrical stimulation of the cortex.

6. It is suggested that since the release of GABA observed during inhibitory stimulation of the cortex is calcium-dependent and specific, it may originate from inhibitory nerve terminals in the cortex. The

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present findings support the view that GABA is a central inhibitory neurotransmitter.

INTRODUCTION

Interest in the possibility that γ-aminobutyric acid (GABA) might be an inhibitory neurotransmitter was stimulated by the work of Bazemore, Elliott & Florey in 1957 and there is now good evidence that GABA is an inhibitory transmitter at crustacean neuromuscular junctions (Otsuka, Iversen, Hall & Kravitz, 1966; Potter, 1968). Evidence for a similar role for GABA in the mammalian brain, however, has proved difficult to obtain and interpret. Iontophoretically applied GABA is known to produce changes in the ionic conductance of neuronal membranes in the cat cerebral cortex identical with those occurring during post-synaptic inhibition evoked by the natural transmitter (Krnjević & Schwartz, 1967). It is only recently, however, that there has been evidence for an association between the rate of release of GABA from the brain and synaptic inhibition of central neurones (Mitchell & Srinivasan, 1969*a*; Obata & Takeda, 1969; Jasper & Koyama, 1969).

In recent experiments it has been shown that the release of radioactively labelled GABA from the cat cerebral cortex is increased by electrical stimuli which produce synaptic inhibition (Mitchell & Srinivasan, 1969*b*). Since it is not known whether externally applied [^{3}H]GABA is taken up and released by brain tissue in exactly the same way as endogenous GABA, samples collected in a similar way from further experiments have now been analysed for their endogenous GABA content and the properties of the GABA release process have been investigated more fully. The results are consistent with the suggestion that GABA is an inhibitory transmitter at some synapses in the visual cortex of cats.

METHODS

Collection of samples. Adult cats were anaesthetized with Dial compound (allobarbitone + urethane, 0·75 to 0·8 ml./kg) given intraperitoneally and, in most experiments, amino-oxyacetic acid hemihydrochloride (AOAA, 40 mg/kg s.c.) was administered after induction of anaesthesia in order to prevent the break-down of GABA by GABA-glutamate transaminase. The animals were prepared for the collection of samples from the surface of the brain in the way previously described for the collection of other active substances (Mitchell, 1963) and the procedure was completed within an hour. A Perspex cylinder was placed over 1·0 cm^2 of the exposed posterior lateral gyrus, and the cortical surface below the cup was incubated for 60–90 min with a solution of [^{3}H]GABA (40 μc, specific activity 1·65 or 2·00 c/m-mole) in 0·25 ml. of Krebs bicarbonate medium (containing 120 mM-NaCl, 4·75 mM-KCl, 1·2 mM-K_2SO_4, 2·6 mM-$CaCl_2$, 1·2 mM-$MgCl_2$, 25 mM-$NaHCO_3$ and 10 mM glucose, and equilibrated with a 95 % O_2 and 5 % CO_2 mixture). The fluid in the cup was then replaced with 0·7 ml. fresh Krebs medium which was changed every 7 min

for a period of 6–8 hr. The collection procedure was usually repeated on the contralateral cerebral hemisphere of the same animal. The results obtained from the two sides were treated as separate experiments.

The assay of samples. Released radioactivity was measured in 0·2 ml. portions of the fluid from each collection period by liquid scintillation counting, and the remaining 0·5 ml. portions were pooled in groups of from three to five for the assay of endogenous GABA. Pooling of individual samples was necessary, since the assay method was not sufficiently sensitive to yield accurate measurements of the GABA content of individual samples. A portion (0·1 ml.) of each pooled sample was used for the assay of total ninhydrin-positive materials by the method of Moore & Stein (1948) using L-leucine as a standard. The remainder was desalted, and neutral amino acids isolated by ion-exchange chromatography, using a method similar to that described by Otsuka *et al.* (1966). The samples were acidified by the addition of 0·5 ml. 1 M hydrochloric acid and passed through columns (0·6 cm diameter × 2·0 cm) of the strong cation exchange resin, Amberlite CG-120 (200–400 mesh, H^+ form). The columns were washed with 10 ml. deionized water and GABA and other amino acids were eluted with 3·5 ml. 2 M ammonium hydroxide. The eluates were taken to dryness *in vacuo* over phosphorus pentoxide; the residues were dissolved in 1 ml. deionized water before passage through an anion exchange resin (Dowex-1-acetate), and further concentration, as described by Otsuka *et al.* (1966). Portions of the purified samples were used for the assay of GABA by a sensitive enzymic fluorimetric method described by Kravitz & Potter (1965). The recovery of GABA by this isolation procedure was between 70 and 85 %, and the results were corrected for recoveries in this range. The minimum amount of GABA which could be accurately measured (i.e. which yielded a fluorescence reading twice that of the sample blank reading) in a single pooled sample of superfusate was 0·2 n-mole. All the samples assayed in these experiments contained at least 0·4 n-mole GABA. The values for endogenous GABA quoted in this paper were obtained by subtraction of the [^{3}H]-GABA from the total GABA content. The amount of [^{3}H]GABA was calculated from the measured radioactivity and the known specific activity.

In three experiments, the total amino acid spectrum of pooled collection samples was examined before and during bipolar stimulation of the surface of the cortex, as described below. Pooled samples (four to eight individual 7 min collections) were acidified with hydrochloric acid and desalted by adsorption on Amberlite CG-120 and elution with ammonium hydroxide. The samples were dried, dissolved in 0·5 ml. 2 N formic acid and analysed on a Locarte amino acid analyser. Samples (0·5–1·0 ml.) of c.s.f. and plasma were taken from the animals at the end of these experiments and were similarly purified and analysed, after precipitation of proteins with 0·4 M perchloric acid. It was possible to detect 1–2 n-mole of individual amino acids in each pooled sample by this procedure.

Electrical stimulation. The cortical surface enclosed by the Perspex cylinders was stimulated in some experiments with a unipolar silver electrode, insulated except at the tip, the indifferent electrode being a silver plate inserted in the back muscles of the cat. In the majority of experiments, the cortical surface was stimulated with bipolar electrodes (tip separation, 1·0 mm, insulated except at the tips), and in all experiments, the stimulus current and square wave form were monitored on an oscilloscope. In some experiments, in which the cortex was stimulated on two separate occasions using bipolar electrodes, either the first or the second period of stimulation was performed after the cortex had been in contact, for a period of at least one hour, with Krebs medium from which the calcium chloride had been omitted (calcium-free medium).

In experiments in which the dorsal region of the lateral geniculate nucleus was

stimulated, a bipolar electrode (tip separation 1·0 mm, insulated except at the tips) was inserted stereotactically at an angle of 30° to the vertical, to co-ordinates A 6·5, L 10·4, H + 3·4 (atlas of Snider & Niemer, 1961). To check the position of the electrode tips, flashes of light were applied to both eyes and the stimulating electrodes were used for recording local evoked potentials. The position of the tips was adjusted until maximal evoked potentials were obtained.

Electrical recordings. Electrical recordings were obtained from single cells in the visual cortex using twin-barrelled glass micro-electrodes in which the recording barrel contained 2·5 M-NaCl and the second barrel contained 1 M-L-glutamate at pH 7·7. Cortical cell firing was displayed on an oscilloscope and was simultaneously fed to a chart recorder after being converted into firing frequency by a Devices instantaneous rate-meter. The firing of cortical cells was evoked by the iontophoretic application of L-glutamate with an inward current (100 nA) (Krnjević & Phillis, 1963) and was recorded during electrical stimulation of the l.g.n. and the cortex, both in anaesthetized cats and in cats which were anaesthetized, injected with AOAA and had the cortex incubated for 90 min with the same concentration of GABA (0·08 mM) as used in the release experiments. The cortical surface was also incubated with a calcium-free medium for 60 min and recordings were subsequently obtained during bipolar stimulation of the cortex. This was repeated after replacement of the normal medium.

RESULTS

The release of GABA from the visual cortex was studied in twenty-two experiments. The values obtained have been expressed in n-mole/7 min. cm^2 cortex. These represent average values for single 7 min collection periods obtained by dividing the GABA content in each pooled sample by the number of individual samples contained in the pool (3–5). Where the results from several experiments have been compared, the statistical data were obtained using these figures for single collection periods, and n represents the number of pooled samples examined.

The spontaneous release of GABA. The spontaneous release of [^{3}H]GABA was similar to that described previously (Mitchell & Srinivasan, 1969*b*), and showed an exponential fall during the course of an experiment. In contrast, the spontaneous release of endogenous GABA remained steady during the course of each experiment (6–16 hr) and the proportion of [^{3}H]GABA present in the samples therefore progressively decreased. When the slow phase of [^{3}H]GABA efflux had been attained, it accounted for 0·5–15 % of the total GABA recovered. There was considerable variation in the rate of endogenous GABA release from animal to animal. The average spontaneous release of GABA was determined in each experiment from results obtained from two to eight pooled samples; the mean ($\pm$ S.E. of mean) of these average results for sixteen experiments was $0{\cdot}20 \pm 0{\cdot}034$ n-mole/7 min. cm^2 cortex.

In four experiments in which the animals were not treated with AOAA, the rate of efflux of [^{3}H]GABA was initially low and declined to a final level lower than in AOAA-treated animals. The mean spontaneous release

of endogenous GABA in these four experiments was $0{\cdot}13 \pm 0{\cdot}029$ n-mole/7 min.cm^2 cortex. This was not significantly lower than the value for AOAA-treated animals.

The release of GABA during electrical stimulation. Direct stimulation of the cortex (square waves, 1·0 msec duration, 1·5 mA, 200/sec) increased the efflux of endogenous GABA and [^{3}H]GABA (Fig. 1*A* and *B*). The

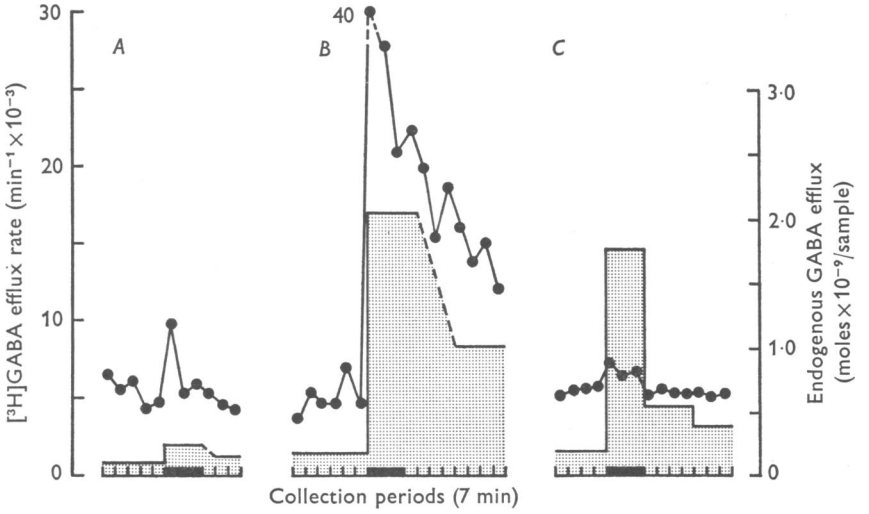

Fig. 1. Individual examples of results obtained for release of endogenous (stippled histogram) and [^{3}H]GABA (filled circles) during electrical stimulation of the cortical surface with monopolar electrodes (*A*), bipolar electrodes (*B*) and stimulation of the l.g.n. (*C*). Horizontal bars indicate periods of stimulation.

The release of [^{3}H]GABA is expressed by efflux rate constants where the rate constant $f = \Delta C/\Delta t \times C_t$, ΔC represents the counts lost in the interval Δt, and C_t is the amount of isotope in the tissue during the interval Δt. C_t is obtained from the difference between C_0 and the total radioactivity lost up to time t. C_0 is determined by integrating the counts released from the tissue during the whole experiment and extrapolating to t_∞.

endogenous GABA contents of samples collected during stimulation periods were compared with those in resting samples taken immediately before the stimulation period, using a paired observations one-tailed t test (Goldstein, 1964), and were found to be significantly higher (Table 1). The mean increase obtained with monopolar stimulation was 2·9 times the resting release. Bipolar stimulation produced a mean increase of 7·4 times the resting release and this ratio was the same in experiments using animals not treated with AOAA (Table 1). Although the mean absolute amounts of GABA in both resting samples and in samples during stimulation were lower in these than in AOAA-treated animals, the differences

between the results from the two groups of animals were not statistically significant (Table 1).

Ipsilateral electrical stimulation of the l.g.n. using square-wave pulses (0·5 msec duration, 2·5 mA) at a frequency of 200/sec during three consecutive 7 min collection periods also increased the output of endogenous GABA from the visual cortex (Fig. 1*C*). The average increase in GABA

TABLE 1. The spontaneous and evoked release of endogenous GABA from the visual cortex of anaesthetized cats

Type of stimulation	*n*	GABA release (n-mole/7 min.cm² cortex) Before stim. (*A*)	During stim. (*B*)	Increase on stim. (*B*/*A*)
Animals treated with AOAA				
Cortex (bipolar)	12	0·30 ± 0·091	2·19 ± 0·611 ($P < 0·01$)	7·4
Cortex (monopolar)	8	0·20 ± 0·043	0·59 ± 0·108 ($P < 0·025$)	2·9
Lateral geniculate nucleus	6	0·22 ± 0·047	1·26 ± 0·240* ($P < 0·01$)	5·7
Animals not treated with AOAA				
Cortex (bipolar)	4	0·15 ± 0·041	1·21 ± 0·249 ($P < 0·025$)	8·1
Cortex (monopolar)	1	0·12	0·64	5·4
Lateral geniculate nucleus	6	0·13 ± 0·033	0·64 ± 0·033* ($P < 0·001$)	4·9

The values for the spontaneous release of GABA (mean ± S.E. of mean) before stimulation (*A*) were obtained from pooled samples of collection fluid from four or five consecutive 7 min collection periods immediately before stimulation. The values for the release during stimulation (mean ± S.E. of mean) (*B*) were from pooled samples of collection fluid from three consecutive 7 min periods of stimulation. The asterisked values (for the release during stimulation of the lateral geniculate nucleus in animals treated or not treated with AOAA) are significantly different ($P < 0·05$).

efflux during stimulation of the l.g.n. was 5·7 times the pre-stimulation resting release and this ratio was the same in animals which were not given AOAA (Table 1). The mean GABA contents of samples collected during stimulation from these animals were significantly lower than those from the AOAA-treated animals (Table 1).

The effect of removal of calcium ions. When the cortex was exposed to calcium-free collection fluid there was no significant change in the spontaneous efflux of GABA. Under such conditions, however, stimulation of the cortex with bipolar electrodes failed to evoke any increase in GABA release (Table 2). This effect was reversible, since in two experiments the cortex was stimulated first in the presence of normal Krebs and then after

treatment with calcium-free medium, and in three experiments this order was reversed; the values for GABA release in the presence and absence of calcium from all five experiments were comparable.

The inhibition of cortical cell firing by electrical stimulation. In the anaesthetized cat few cells in the visual cortex fired spontaneously, but the iontophoretic application of L-glutamate caused a rapid onset of firing in all neurones tested. The glutamate-induced firing of all cells tested (seventy-five cells) was abolished or greatly reduced when the cortical surface or l.g.n. was electrically stimulated using parameters of stimulation

TABLE 2. The effect of the removal of calcium ions on the release of endogenous GABA evoked by bipolar stimulation of the visual cortex

Collection medium	n	GABA release (n-mole/7 min.cm² cortex) Before stimulation (A)	During stimulation (B)	Increase on stimulation (B/A)
$+Ca^{2+}$	5	$0{\cdot}08 \pm 0{\cdot}011$	$0{\cdot}49 \pm 0{\cdot}117$*	6·08
$-Ca^{2+}$	5	$0{\cdot}12 \pm 0{\cdot}024$	$0{\cdot}15 \pm 0{\cdot}040$*	1·27

The release of GABA in samples obtained during stimulation in the presence and absence of calcium (marked with an asterisk) is significantly different ($P < 0{\cdot}05$).

which produced an increase in GABA release (Fig. 2*A* and *B*). This inhibition was of long duration (up to 1 min). Complete recovery of excitability was always obtained within 1·5 min of ending the inhibitory stimulation.

Similar results were obtained when the conditions of the release experiments were reproduced by using cats treated with AOAA in which the cortex was exposed to 0·08 mM-GABA for 60 min (Fig. 2*C* and *D*). When the cortex was exposed to a calcium-free Krebs solution for 60 min the cells remained sensitive to L-glutamate excitation, but direct stimulation of the cortex, which had previously caused a marked inhibition of firing, no longer had this effect and frequently produced an increase in unit activity. Subsequent treatment of the cortex with normal Krebs medium restored the effectiveness of inhibitory stimulation (Fig. 3*A* and *B*).

The release of amino acids and other materials from the cortex. In eight cats the mean resting release of total ninhydrin-positive materials was 0·23 μ-mole/7 min.cm² cortex. The rate of release of such materials was not significantly altered by electrical stimulation (Table 3). Similar values were obtained in animals which were not treated with AOAA and in experiments in which the cortex was exposed to calcium-free Krebs medium (Table 3). Only a small proportion (less than 25%) of the ninhydrin-positive materials could be accounted for as amino acid nitrogen

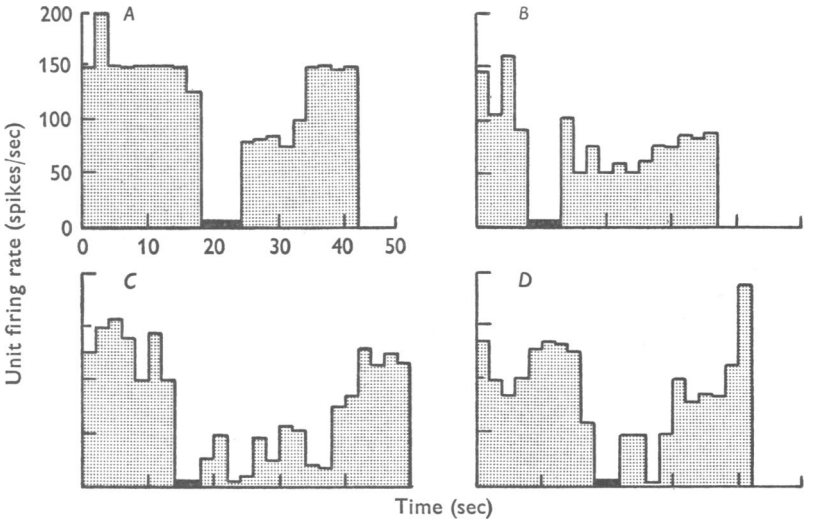

Fig. 2. The rate of firing of cortical cells in the posterior lateral gyrus of an anaesthetized cat. Cell firing was induced by the iontophoretic application of L-glutamate. Upper histograms show inhibition obtained during direct stimulation of the cortex (*A*) and of the l.g.n. (*B*) in a cat which had not had GABA applied to the cortex and which had not been treated with AOAA. Lower histograms show similar inhibition evoked by stimulation of the cortex (*C*) and l.g.n. (*D*) in the same cat after exposure of the cortex to GABA (0·08 mM) for 90 min and injection of AOAA (40 mg/kg s.c.). Horizontal bars indicate periods of electrical stimulation; unit firing was not recorded during these periods.

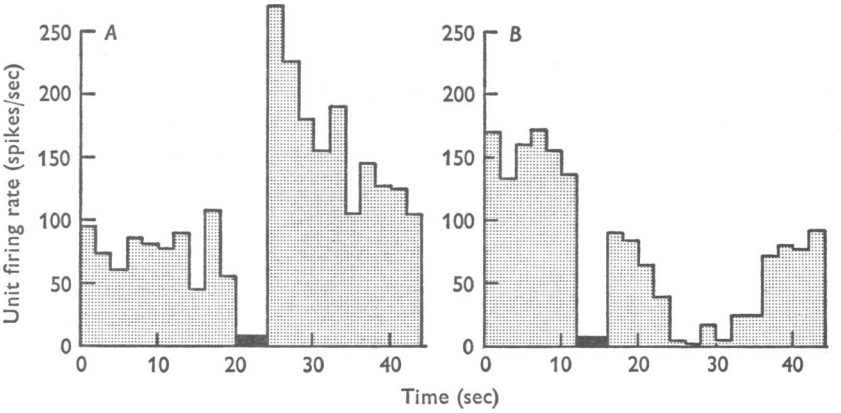

Fig. 3. The rate of firing of cortical cells in the posterior lateral gyrus of an anaesthetized cat treated with AOAA (40 mg/kg s.c.) and after a 90 min exposure of the cortex to GABA (0·08 mM). Cell firing was induced by the iontophoretic application of L-glutamate.

The histograms show the effect of direct stimulation of the cortex after incubation for 1 hr with a calcium-free medium (*A*) and after subsequent incubation with normal medium (*B*). Horizontal bars indicate periods of electrical stimulation; unit firing was not recorded during these periods.

when amino acids were isolated from collection samples by ion-exchange chromatography. The remaining ninhydrin-positive reacting materials were not identified, but urea and ammonium ions are likely to be major components.

The individual amino acids present in pooled resting and stimulated

TABLE 3. The release of total ninhydrin-positive materials from the cortex of control animals (i.e. treated with AOAA), animals not given AOAA, and animals given AOAA in which the cortex was exposed to calcium-free Krebs medium

		Total ninhydrin-positive material (μ-mole/7 min.cm^2 cortex)	
	n	Resting	Stimulated
Control (+AOAA+Ca^{2+})	8	0·23 ± 0·027	0·15 ± 0·027
−AOAA+Ca^{2+}	3	0·15 ± 0·027	0·10 ± 0·020
−Ca^{2+}+AOAA	5	0·33 ± 0·045	0·29 ± 0·060

The mean resting releases of ninhydrin-positive materials were calculated as for endogenous GABA (see Results), and were compared with the mean releases during stimulation (l.g.n. and cortex) calculated in the same way. There are no significant differences between any of the values.

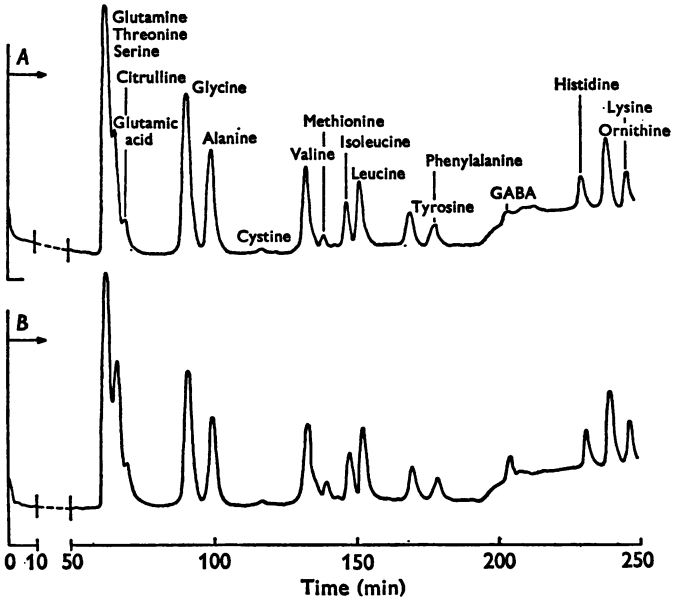

Fig. 4. Amino acid analyses of pooled collection samples from the posterior lateral gyrus of an anaesthetized cat before (*A*) and during (*B*) stimulation of the cortical surface. The lysine peak was followed by a large ammonia peak and subsequently by tryptophan and arginine. The glutamine + serine + threonine peak was preceded by a broad peak (unidentified acids) and aspartate.

samples from one experiment are shown in Fig. 4. The mean results from three similar experiments (Table 4) failed to reveal any consistent changes in the rate of efflux of any of the amino acids in response to cortical stimulation. The amounts of GABA present in these samples were generally too low to be accurately assessed by the amino acid analyser technique,

TABLE 4. Amino acid composition of cortical collection samples

	Resting				Stimulation			
Amino acid	Expt. 1	Expt. 2	Expt. 3	Mean	Expt. 1	Expt. 2	Expt. 3	Mean
Unidentified acids	5·0	7·3	4·1	5·5	3·9	6·6	3·9	4·8
Aspartic	2·0	1·9	1·7	1·8	1·5	2·2	2·2	1·9
Serine, glutamine, threonine	14·8	19·1	13·3	15·8	10·2	17·5	13·9	13·9
Glutamic	4·3	4·5	2·7	3·8	3·6	7·4	2·6	4·5
Citrulline	0·4	1·2	1·2	0·9	0·4	2·0	0·7	1·0
Glycine	5·0	5·5	3·9	4·8	2·9	4·8	3·9	3·8
Alanine	4·7	4·1	3·3	4·0	3·3	3·3	4·3	4·0
Valine	2·1	2·7	2·1	2·3	1·5	3·2	2·4	2·3
Methionine	0·4	0·3	0·2	0·3	0·7	0·5	0·0	0·4
Isoleucine	0·8	0·9	0·9	0·9	0·6	1·1	1·0	0·9
Leucine	1·4	2·2	2·3	2·0	1·3	2·4	1·7	1·8
Tyrosine	0·4	1·3	0·6	0·8	0·1	1·3	0·3	0·7
Phenylalanine	0·7	0·8	0·7	0·7	0·3	0·8	0·4	0·5
GABA*	0·0	0·0	0·1	0·03	0·2	0·4	0·2	0·3
Histidine	1·3	1·3	3·7	2·1	1·3	1·6	2·3	1·8
Ornithine	1·9	2·8	2·4	2·4	2·5	3·1	3·6	3·1
Lysine	0·8	1·0	1·0	1·0	0·4	1·6	1·4	1·1
Tryptophan	3·3	4·9	2·1	3·4	2·9	7·2	1·4	3·8
Arginine	0·8	0·5	0·9	0·7	0·4	0·5	0·8	0·5
Total	56·6	60·8	48·5	55·3	40·8	62·4	51·0	51·4

Values determined from amino acid analysis of pooled samples collected for 30–60 min during bilateral, bipolar stimulation of the cortex, and during a similar period before stimulation (resting values). Values are expressed as n-mole/7 min.cm^2.

* Values for GABA are approximate only, the amino acid levels being at, or below, the threshold sensitivity of the analyser.

although a small peak corresponding to GABA was observed in the stimulated samples of all experiments (Fig. 4). The results of the amino acid analyses of plasma and c.s.f. are given in Table 5. These show that the amino acid composition of the samples collected from the surface of the cortex did not exactly resemble that of either plasma or c.s.f., although it was more similar to the latter than the former.

These results indicate that the release of GABA from the cortex in response to electrical stimulation is a highly specific phenomenon. Stimu-

lation under the conditions used in these experiments failed to evoke any changes in the rate of release of other amino acids or other nitrogenous materials.

TABLE 5. Amino acid composition of cat plasma and cerebrospinal fluid

Amino acid	Plasma	C.s.f.
Unidentified acids	14·9 (5·7–28·4)	33·7 (13·9–53·4)
Aspartic	27·6 (22·5–32·7)	37·3 (20·0–54·7)
Glutamine + serine + threonine	230·2 (185·0–253·0)	325·3 (278·0–372·5)
Glutamic	63·4 (60·0–68·5)	64·3 (23·8–104·8)
Proline	14·0 (5·5–36·5)	36·3 (21·0–51·6)
Glycine	61·3 (54·8–65·7)	50·4 (12·0–88·8)
Alanine	101·6 (94·7–105·5)	45·7 (19·9–71·5)
Valine	95·4 (93·2–97·0)	22·4 (18·4–26·4)
Methionine	9·5 (5·9–15·6)	3·4 (1·7–5·0)
Isoleucine	38·5 (32·6–49·3)	9·8 (6·8–12·8)
Leucine	65·5 (56·7–78·5)	20·5 (20·0–21·0)
Tyrosine	42·3 (32·6–51·0)	16·7 (14·0–19·4)
Phenylalanine	20·6 (17·1–24·3)	7·8 (6·8–8·8)
Histidine	41·8 (35·5–50·8)	15·5 (14·4–16·6)
Ornithine	19·4 (10·6–28·1)	33·7 (13·9–53·4)
Lysine	38·9 (36·6–43·2)	13·0 (12·0–14·0)
Arginine	30·7 (18·9–38·0)	16·4 (12·8–20·0)
Tryptophan	15·3 (14·3–16·2)	22·9 (10·6–35·2)
Other, unidentified	< 10·0	< 5·0

Values are the means for two experiments with c.s.f. and three with plasma, expressed as n-mole/ml. Ranges shown in brackets.

DISCUSSION

At all synapses where the chemical transmitter has been identified a critical part of the evidence has been the demonstration of the release of that transmitter in response to presynaptic stimulation. In the present experiments the effect of stimulation on the release of GABA from the mammalian cerebral cortex has been studied. Early attempts to study GABA release from the brain have shown a spontaneous release but have not provided a correlation with inhibitory synaptic activity (Florey & McLennan, 1955; Jasper, Khan & Elliott, 1965; Crowshaw, Jessup & Ramwell, 1967).

In the present experiments it has been shown that stimuli which produce cortical inhibition and increases in the efflux of [^{3}H]GABA also cause increases in the rate of release of endogenous GABA. The increases in endogenous GABA output which can be evoked by cortical inhibition are larger than those obtained in the same experiment or in previous studies with [^{3}H]GABA. This may indicate that only a small proportion of the

releasable GABA fraction becomes labelled during incubation with [^{3}H]GABA.

In the present experiments on anaesthetized cats the spontaneous release of endogenous GABA from the surface of the posterior lateral gyrus was 0·20 n-mole/7 min.cm^2 cortex. This figure for spontaneous release is similar to those reported for the spontaneous release of GABA under a variety of experimental conditions; from the surface of the brain by Jasper & Koyama (1969) and by Crowshaw *et al.* (1967) and from Purkinje axon terminals in the floor of the IVth ventricle (Obata & Takeda, 1969).

Amino-oxyacetic acid is known to increase brain GABA content by inhibition of GABA-glutamate transaminase (Baxter & Roberts, 1961; Wallach, 1961) and may therefore affect the rate of release. In the present study the amounts of GABA released at rest and during stimulation tended to be higher in animals treated with AOAA. However, the percentage increases in GABA release during stimulation were similar to those observed in animals not treated with AOAA. The increased release of GABA in AOAA-treated animals may be the result of raised tissue GABA levels or of the more ready escape of GABA through pial membranes, which appear to contain transaminase activity (van Gelder, 1968).

The characteristics of GABA release. The increase in GABA release evoked by bipolar surface electrode stimulation was calcium dependent. This is a property shared by all transmitter release mechanisms so far studied (Simpson, 1968). It is interesting that the cortical release of GABA was abolished in the absence of calcium, since application of a calcium-free solution to the surface of the brain is unlikely to remove completely the calcium from the brain tissue itself. In the case of ACh release from the cortex, exposure to a calcium-free medium only reduced release by 40–65% (Hemsworth & Mitchell, 1969). However, electrical recording from cortical units in the present studies showed that exposure of the cortex to a calcium-free solution effectively abolished, and even reversed, the effects of previously inhibitory stimulation. The absence of calcium is known to increase membrane excitability (Curtis, Perrin & Watkins, 1960) but it is unlikely that the reversal of inhibition can be explained solely by a post-synaptic membrane effect since there was no fall in the threshold of cortical units to excitation by L-glutamate.

The electrical stimuli applied to the l.g.n. or to the surface of the cortex were shown to produce inhibition of long duration. The parameters chosen for these stimuli were based on those used by Krnjević, Randić & Straughan (1966) to produce inhibition, but are not necessarily optimal for initiating or maintaining inhibition under the conditions of the present experiments. The stimulation used was unlikely to have produced neuronal damage,

and the observed release of GABA from the cortex could certainly not be a result of local damage during stimulation of the l.g.n.

The electrical stimuli used evoked inhibition but in addition it is possible that this would be accompanied by some cortical excitation. However, Jasper & Koyama (1969) have reported that under conditions which are likely to cause central excitation, there is an increase in the release of glutamic acid from the cortex, and this was not usually observed in the present experiments.

It might be argued that the increases in GABA release observed result from changes in brain metabolism and are unrelated to inhibitory synaptic activity (Vogt, 1969). This seems unlikely since the stimulation used caused no reproducible changes in the release of any amino acids apart from GABA or in the release of other ninhydrin-positive substances. The calcium dependence of the evoked GABA release also makes it unlikely to be due to a metabolic effect. The concentration of GABA in blood and c.s.f. is negligible (Crowshaw *et al.* 1967) and therefore local changes in vascular permeability or blood flow are unlikely to be responsible for the changes in GABA efflux.

Site of GABA release. It is likely that the released GABA originated from within the cortex because the evoked release occurred soon after the beginning of stimulation. Electrophysiological evidence also indicates that intracortical fibres are responsible for inhibition in the posterior lateral gyrus, whether this inhibition is evoked by direct or by remote electrical stimulation (Watanabe, Konishi & Creutzfeldt, 1966; Armstrong, 1968). In addition, it is known that in chronically isolated cortical slabs, in which nerve terminals of extracortical origin would have degenerated, inhibitory post-synaptic potentials can still be produced by surface stimulation and the glutamate-evoked firing of individual cells can still be blocked by GABA (Krnjević, 1969).

The GABA collected in the present experiments is likely to have originated, at least in part, from nerve terminals because previous studies have shown that both [^{3}H]GABA and endogenous GABA are recovered in a synaptosome fraction obtained from cortical homogenates (Neal & Iversen, 1969). It has also been shown that electrical stimulation of isolated synaptosome fractions releases GABA, glutamate and aspartate to a greater extent than other amino acids (Bradford, 1970). The evidence is thus consistent with the view that the GABA collected in the present experiments may have originated from the terminals of inhibitory neurones in the cortex.

The conclusion drawn from the present experiments, that the release of GABA is associated with inhibition of cortical cell firing, is important in assigning a transmitter role to GABA. This is not inconsistent with any metabolic function GABA may have in the brain (Balázs, Machiyama,

Hammond, Julian & Richter, 1970). Other evidence for a transmitter role has accumulated from experiments on the distribution of GABA and the enzymes associated with its metabolism (Roberts & Eidelberg, 1960): the sodium-dependent uptake mechanism for GABA (Elliott & van Gelder, 1960; Iversen & Neal, 1968); the identity of the effect of GABA on post-synaptic membranes with that of the natural transmitter (Krnjević &

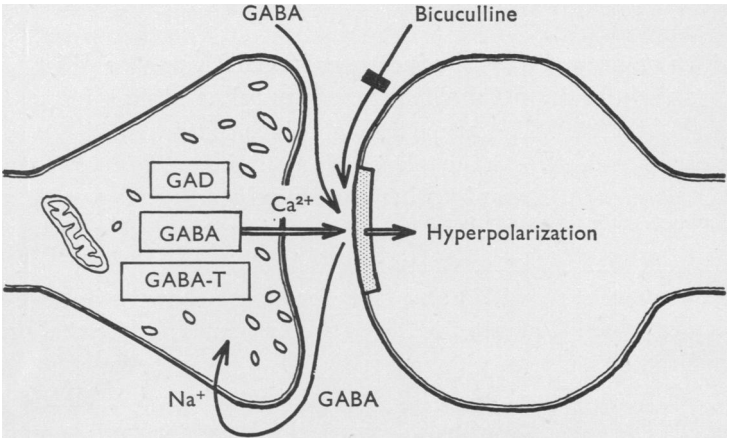

Fig. 5. Diagrammatic summary of the evidence which supports an inhibitory neurotransmitter role for GABA in the mammalian cerebral cortex.

Blocked arrow indicates antagonism by bicuculline. GAD = glutamic acid decarboxylase; GABA-T = GABA-glutamate transaminase. Further explanation in text.

Schwartz, 1967); and the reversible block of this action by the antagonist, bicuculline (Curtis, Duggan, Felix & Johnston, 1970). Taken together, this evidence provides a credible picture of the function of GABA as a transmitter at inhibitory synapses in the mammalian cerebral cortex (Fig. 5).

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