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THE ACTION OF GAMMA AMINOBUTYRIC ACID UPON CORTICAL ELECTRICAL ACTIVITY IN THE CAT

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The presence of γ-aminobutyric acid in brain was discovered in 1950 by Awapara, Landau, Fuerst & Seale (1950), Roberts & Frankel (1950) and Udenfriend (1950). In 1953 Florey (1953, 1954) reported the presence in mammalian brain and spinal cord of a factor, Factor I, which inhibits the generation of impulses by stretch receptor neurons of the crayfish. Preparations from brain containing Factor I have been reported to exert various marked effects on nervous organs in marine organisms (Florey, 1956) and in mammals (Florey & McLennan, 1955, 1956).

An assay procedure for Factor I was developed by Elliott & Florey (1956) who noted that most of the Factor I present in brain is held in an inactive form from which it can be released by mild procedures. Bazemore, Elliott & Florey (1956, 1957) purified Factor I from brain and obtained crystals which showed the highest activity yet obtained and which were identified with γ-aminobutyric (GAB). It was estimated that the amount of GAB found by others to be present in brain could account for the total Factor I activity which is extractable from brain. It is possible, therefore, that this naturally occurring amino acid may be of importance in the regulation of physiological activity in brain tissue.

In the present studies we have observed the effects of GAB upon cortical sensory evoked potentials, recruiting responses, and spontaneous electrical activity of the cerebral cortex in the cat.

METHODS

Experiments were carried out on twenty-four cats. Most of them were immobilized by partial coagulation of the medial portion of the brain stem at the level of the superior colliculus (stereotaxic co-ordinates, frontal 1–2, horizontal –3, lateral 1–3 on both sides of the mid line). Thiopentone (Pentothal, Abbott Laboratories) anaesthesia was employed before brain stem coagulation in

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these animals. Experimentation began at least 2 hr later without further administration of anaesthetic. Pentobarbitone (Nembutal, Abbott Laboratories) anaesthesia was used in a few animals.

The anterior portion of the cortex was exposed on one side. By means of the stereotaxic instrument, the tips of bipolar stimulating electrodes were placed in the somato-sensory nucleus of the thamalus (ventralis posterior) to elicit cortical sensory evoked potentials, and in the intralaminar system of the thalamus for producing cortical recruiting potentials.

A leucite ring with an inside diameter of 4 mm and a depth of 2·5 mm was held with a light pressure upon the cortical surface to be studied, for the purpose of preventing spread of the solutions applied to the surface and to make possible the maintenance of a constant concentration. Evoked potentials were led off by means of a unipolar silver wire electrode placed on the cortical surface inside the ring. Additional electrodes were placed outside the ring for the purpose of control. In some experiments fine electrodes, made of glass pipettes or tungsten wire, were inserted beneath the surface at depths measured with a micromanipulator. The reference electrode was a screw in the skull over the frontal sinus.

For electrical stimulation, square waves of 0·05–0·1 msec were used for sensory evoked potentials and direct cortical stimulation. Durations of 1–2 msec were used for recruiting responses. Shock artifacts were minimized by the use of an isolation transformer. Potentials were recorded by means of condenser-coupled amplifiers (time constant 0·2 sec) led to a Dumont dual beam oscilloscope. Ink-writer tracings were obtained simultaneously by means of an Offner Type T EEG apparatus.

γ-Aminobutyric acid, which will be referred to as GAB, was used in concentrations of 0·02–1 % in physiological saline solution: a 1 % solution was used except when otherwise indicated.

RESULTS

Sensory evoked potentials

The primary evoked potential complex recorded from the surface of the sensory cortex in response to a single brief shock in the sensory nucleus of the thalamus consists of an initial surface positive potential followed immediately by a surface negative wave (see Figs. 1, 2, 9 and 10). One or more brief spikes may be superimposed upon the descending limb of the surface positive deflexion (negative deflexion recorded in an upward direction in all figures). The surface positive phase of the response is considered to represent post-synaptic activity in the depths of the cortex, while the surface negative phase represents conduction of the response toward the cortical surface. The latter wave has been considered to be a dendritic potential by Chang (1953), Bishop (1956), Bishop & Clare (1952), Clare & Bishop (1955) and others. The upward conduction of this wave is believed by Eccles (1951) to take place in a multi-synaptic chain of small interneurones.

Topical application of GAB to the sensory cortex resulted in a rapid decrease in the surface negative phase of the evoked potential with an apparent small increase in amplitude of the surface positive phase, without change in the superimposed spikes. A typical example is shown in Fig. 1. In addition, there was a prolongation of the surface positive wave and the appearance of a slow negativity.

Depression of the surface negative wave occurred within 2 sec after the

application of GAB under most favourable conditions. The prolongation of the surface positive wave, and the appearance of late slow negativity, required a longer time. Washing the substance from the cortex resulted in rapid recovery of the original wave form depending upon how long the substance had been applied. In Fig. 1 the substance, in 1% solution, was applied for 3 min; recovery occurred within 1 min after it was washed off. With prolonged application, up to 90 min, no further change occurred in the primary evoked potential complex. Recovery required as long as 60 min following such a prolonged application.

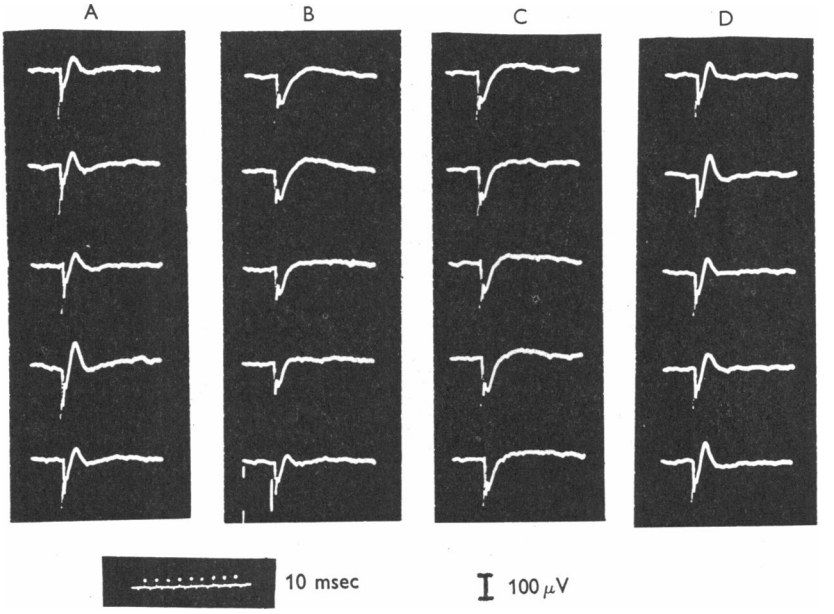

Fig. 1. Primary sensory evoked potential. Each column presents five consecutive sweeps at intervals of 1·75 sec, in order from below upward. A, control records before GAB was applied; B, consecutive records taken immediately after 1% GAB was applied, as indicated by the mark in the left-hand lower corner; negative waves were completely suppressed within 5 sec and positive waves were prolonged and increased slightly in amplitude; C, records at 3 min—no further change in the effect of GAB as compared with B; D, recovery of negative waves after washing off GAB for 2 min.

In order for these changes to occur as described, it was necessary to maintain a good general condition of the animal and of the cortical surface. If the animal was in poor condition, with a low voltage response requiring an increased stimulation voltage, the effects upon the surface negative wave were minimal; the surface positive wave was not increased in amplitude or prolonged in duration.

Following the primary evoked potential complex there occurs, under certain conditions, a repetitive series of waves which has been called the sensory

after-discharge by Adrian (1936) and studied by Bremer & Bonnet (1950), Chang (1950) and Bremer (1952). This after-discharge occurs following a delay of about 100 msec as shown in Fig. 2 C. It is not always present or occurs at a more rapid frequency in the unanaesthetized animal, and is more commonly observed with the animal under barbiturate anaesthesia.

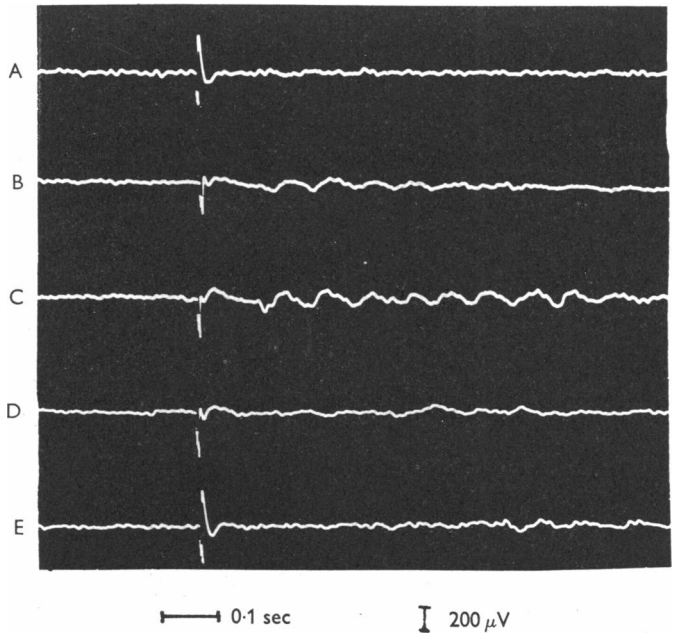

Fig. 2. Enhancement of sensory after-discharge by GAB. A, control record with no after-discharge and prominent negative wave in evoked potential; B, 16 min after the application of 1% GAB after-discharge appeared with small amplitude; C, marked after-discharge with a maximum suppression of the negative wave of the evoked potential at 23 min—GAB was kept in place until 90 min; D, at 30 min after GAB was washed off the after-discharge disappeared while the negative deflexion of primary complex remained still suppressed; E, restoration of the normal form of the primary sensory discharge at 60 min after removal of GAB.

The sensory after-discharge was enhanced after topical application of GAB. This effect appeared later than the depression of the surface negative wave of the primary evoked potential complex (Fig. 2 B, C). The enhancement of the after-discharge disappeared quickly following removal of GAB, before the recovery of the surface negative wave of the primary evoked potential complex (Fig. 2 D). Another example of increased after-discharge is shown in Fig. 4, in relation to increased spontaneous activity in the e.e.g., which will be discussed later.

The recruiting response

The recruiting response of the cortex is produced by repetitive stimulation of the intralaminar system of the thalamus at frequencies of 6–12/sec. It is closely related to rhythmic waves which occur in spindles ('spindle bursts') in the spontaneous activity of the cortex. The recruiting waves are thought to be mediated over separate unspecific projection fibres from the thalamus (Hanbery & Jasper, 1953). They consist of a series of predominantly surface negative waves increasing in amplitude at the onset of repetitive stimulation, then decreasing in a waxing and waning manner with continued stimulation.

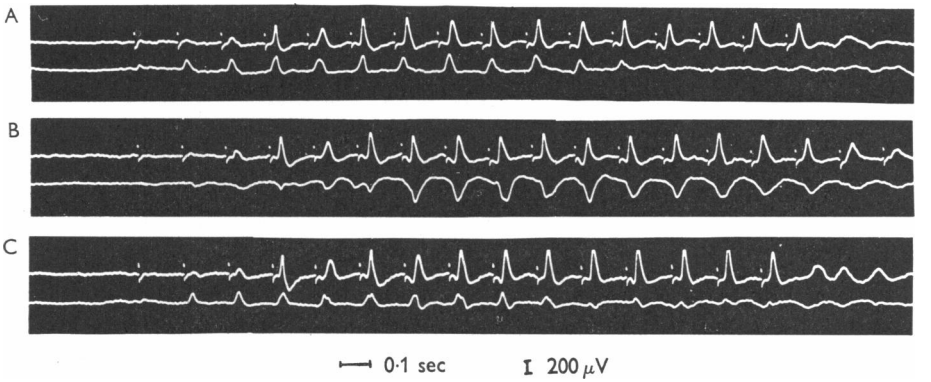

Fig. 3. Effect of GAB on recruiting response. In each record the upper channel was recorded from the suprasylvian gyrus (control point) while the lower channel was from the post-cruciate gyrus (test point). Typical recruiting responses with predominantly negative excursions are seen in both channels in A. The recruiting response from the test area (lower line) was reversed in phase and increased in amplitude at the end of application of 1% GAB for 3 min (B). Recovery 8 min after washing is shown in C.

The surface negative wave of the recruiting response is analogous to, and interacts with the surface negative wave of the specific evoked potential complex, probably utilizing at least some of the same neuronal elements of the cortex. Both have been considered to represent activity in superficial cortical dendrites (Bishop, 1956).

Topical application of GAB reverses the polarity of the recruiting waves, making them surface positive, as is shown in Fig. 3 B. There is also a lengthening in the duration of each wave. The amplitude of the surface positive recruiting waves, under the influence of GAB, is usually greater than the normal surface negative response. These changes, as correlated with alterations in spontaneous activity, are illustrated in Figs. 5 and 6.

Effect of GAB upon spontaneous cortical rhythms

In the cat with a mesencephalic coagulation the spontaneous electrical activity of the cortex is characterized by spindles of rhythmic waves at 6–10/sec, with some rhythmic activity in the background at other frequencies. Following application of GAB 1% solution, in a favourable preparation, there was about a twofold increase in amplitude of all forms of spontaneous activity,

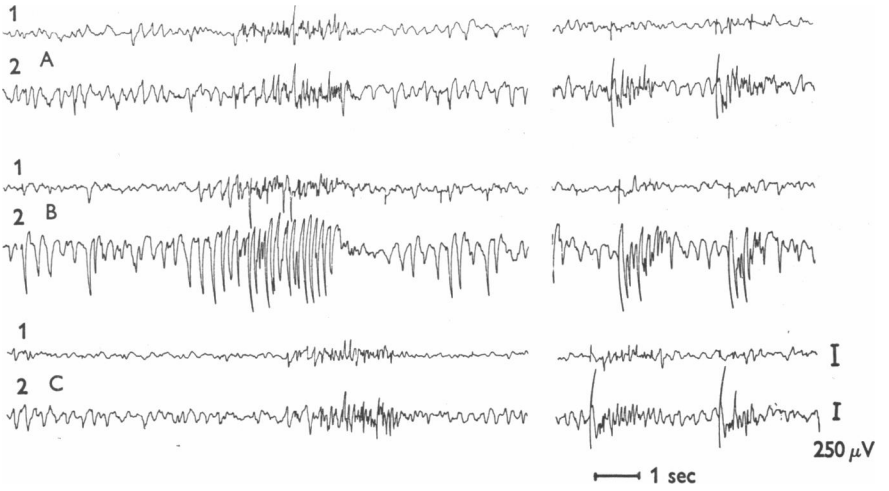

Fig. 4. Augmentation of spontaneous activity and sensory after-discharge due to GAB. Two channels in each record show the activities of two different areas in the somato-sensory cortex. A, control record before the application of 1% GAB; B, 8 min after the application all kinds of activity of the test area showed an augmentation, mainly in the direction of positivity; GAB was left for 30 min on test area (channel 2); C, recovery 30 min after washing.

without a change in frequency (Fig. 4). In some cases large slow waves appeared, similar to those seen in the e.e.g. during sleep (Fig. 5). There was also a reversal in polarity of the spontaneous spindles so that they became mainly surface positive, as shown in Fig. 6B, with no change in other forms of spontaneous activity.

All the above changes in spontaneous activity were completely reversible following removal of GAB and washing the cortex with saline solution. The increase in spontaneous activity was observed to occur in some experiments within 15 sec after application of GAB to the cortical surface.

Coincident with the increase in spontaneous activity there was an increase in voltage of the recruiting response, and an increase in the sensory after-discharge, as shown in Figs. 4–6. In some experiments there was less effect of GAB than shown in these illustrations. In these instances there was also less effect upon the recruiting response. However, the sensory after-discharge

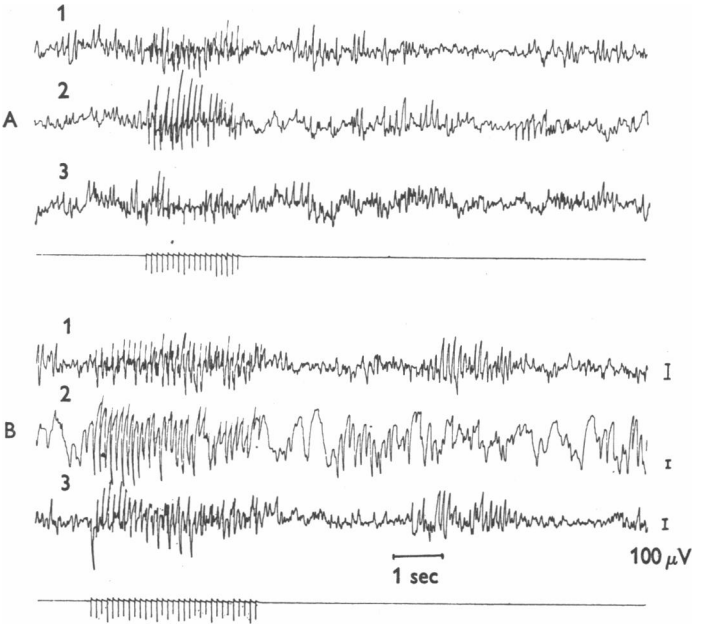

Fig. 5. Activation of slow waves by GAB. Channels 1–3 were derived from the ectosylvian, suprasylvian and post-cruciate gyrus respectively. No marked slow waves can be seen in the control record taken before 1% GAB was applied (A). One minute after the topical application slow waves were recorded in the test area only (channel 2). Note the reversal of phase of recruiting response and of spindle burst.

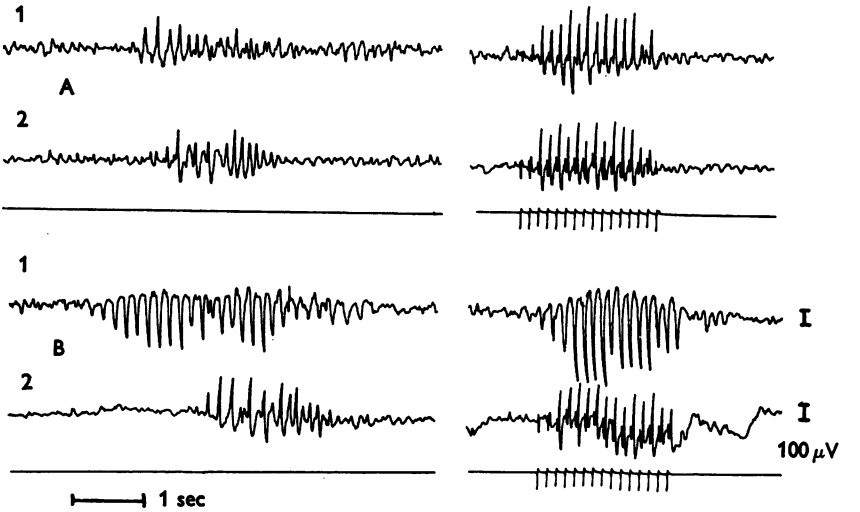

Fig. 6. Spindle burst and recruiting response under the influence of GAB. Channels 1 and 2 were derived from lateral and post-cruciate gyrus respectively. Record A shows the activity before GAB was applied. At 1 min 30 sec after 1% GAB was topically applied, recruiting response and spindle burst were completely reversed in phase and increased in amplitude (B, channel 1), while there was no marked augmentation in the other kinds of spontaneous activity.

was consistently increased in spite of little effect upon spontaneous electrical activity. Effects upon spontaneous activity could be dissociated from the effect upon sensory after-discharge, as was shown in Fig. 2.

Effect upon 'dendritic' response to direct cortical stimulation

The local electrical response of the cortex to direct surface stimulation results in an initial surface negative wave which may be recorded only within 5–10 mm from the site of the stimulating electrodes (Adrian, 1936; Burns, 1950, 1951; Chang, 1951). It is considered to be a response of the apical dendrites of the cortex, either activated directly or via a synapse (Purpura & Grundfest, 1956). With stronger stimulation the surface negative wave is followed by a surface positive wave which is considered to be associated with conduction of activity to deeper layers of the cortex.

In order to determine the effect of GAB upon the dendritic response, a pair of stimulating electrodes, separated by less than 1 mm, were placed on the cortical surface within the enclosure of the ring. A small recording electrode (100 mμ diameter) was placed between them within the ring. Another recording electrode was placed immediately outside the ring to obtain conducted responses in adjacent cortex not directly affected by the application of GAB in the enclosure.

The effect of GAB was to suppress completely the local negative wave leaving only a later surface positive deflexion, as shown in Fig. 7. There was no effect upon the response recorded immediately outside the ring.

The set-up was then reversed, with the stimulating and local recording electrodes outside the ring. The distant recording electrode was then placed within the ring. Placing GAB in the ring then had no effect upon the local response outside, but suppressed completely the distant negative response from within the ring, leaving a small surface positive deflexion, as shown in Fig. 8.

It is apparent that the superficial local dendritic response of the cortex is very sensitive to the effect of GAB, while the surface positive wave, presumably of deep origin, remains present. Furthermore, the effect of GAB remains localized to the site of application. It does not seem to diffuse outside the confines of the ring on the cortical surface, nor does it affect activity in the depths of the cortex.

The distant response obtained outside the ring may have been due to spread of stimulating current under the ring to cortical tissue which was not depressed by GAB, and then conducted to the outside recording electrode about 4 mm distant. The delay of about 3 msec between the inside and outside response in Figs. 7 and 8 would imply a conduction velocity of about 1·3 m/sec, which is of the right order of magnitude for conduction velocity of the 'dendritic response' (Chang, 1952). On the other hand, the conducted wave may be due to

excitation of presynaptic fibres beneath the depressed surface permitting conduction to the surface at a distance where superficial layers were not affected by GAB. This would be in accord with the interpretation of such responses given by Purpura & Grundfest (1956).

Effect upon superficial and deep components of the sensory evoked potential

The form of the primary sensory evoked potential as described above is altered when it is recorded from the depths of the cortex with a fine penetrating electrode. At a depth of 0·7–1·0 mm beneath the cortical surface, the initial surface positive deflexion becomes reversed in polarity to become a larger deep negative wave (Li, Cullen & Jasper, 1956*a*, *b*). There is also a

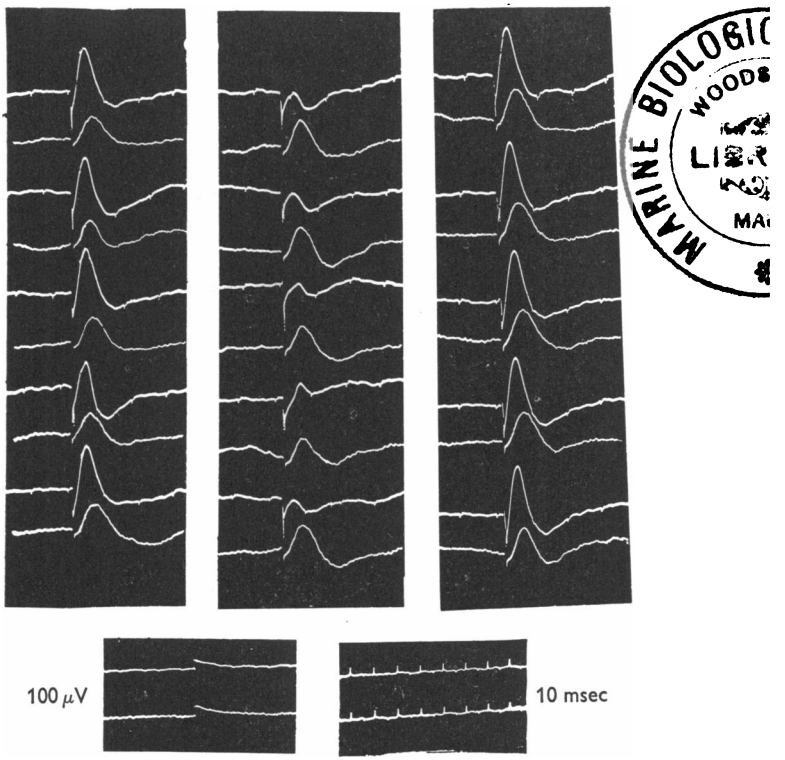

Fig. 7. Dendritic response at the site of stimulation affected by GAB. Each column shows five consecutive sweeps made at an interval of about 0·8 sec (read from below upward). The upper record is from the site of stimulation and the lower record from the point 3 mm distant. The control records are in the left column. 1% GAB was applied at the point of stimulation. At 4 min 20 sec negative waves were completely suppressed, leaving the small positive deflexions at the site of stimulation. The activity at the remote point remained unaffected (middle column). Recovery of normal activity from the suppression at 1 min after the brain was washed (right column).

reversal of the later surface negative wave to become a smaller deep positive deflexion, as illustrated in Figs. 9 and 10, column 1. This deep positive deflexion may be considered to be only a reflexion of the surface negative wave and not a local deep response.

Deep responses were recorded in our experiments by means of a fine, plastic-insulated tungsten wire with an exposed tip of 4–5 μ diameter, mounted in a micromanipulator and connected to a cathode follower input. Surface responses were recorded simultaneously by means of a fine silver

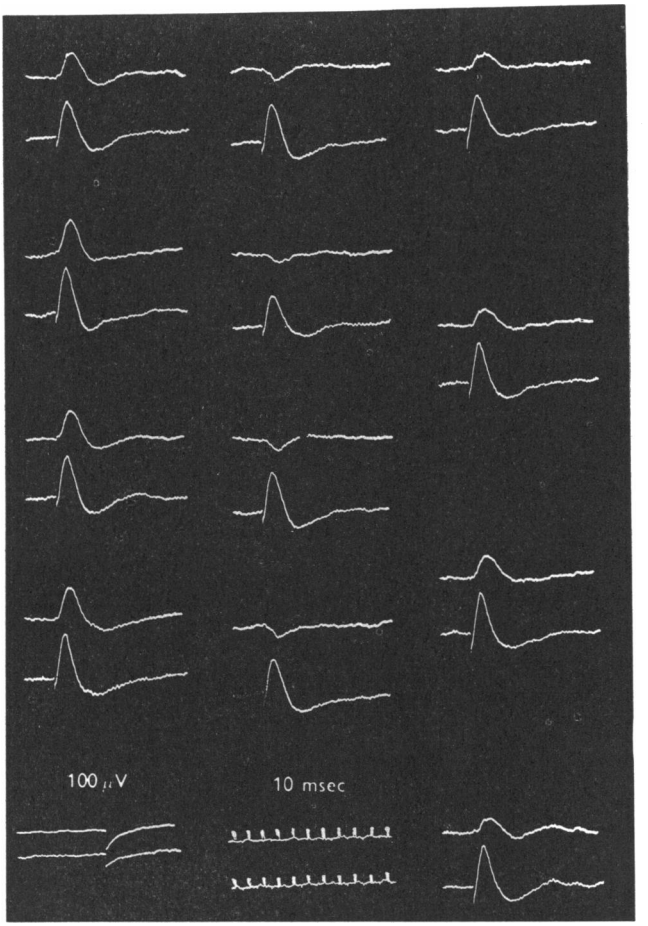

Fig. 8. Effect of GAB on the dendritic potential at 3 mm distant from the stimulating electrodes. Four consecutive pairs of sweeps at intervals of 2·2 sec in the left-hand column are for control: the upper and lower channels show responses from the distant point and the stimulation site respectively. At 1·5 min after 1% GAB was applied to the distant point, the response therefrom was reversed leaving a small positive deflexion (middle column). The right-hand column shows the recovery 11 min after the brain was washed.

electrode placed at the site of insertion of the deep electrode inside the leucite ring. The effect of GAB applied to the surface was then tested at various measured depths in the cortex.

The deep negative evoked response was not depressed in amplitude by surface application of GAB when recording at levels 300 μ or more beneath the surface (Figs. 9, 10). In the example shown in Fig. 9, it will be noted that

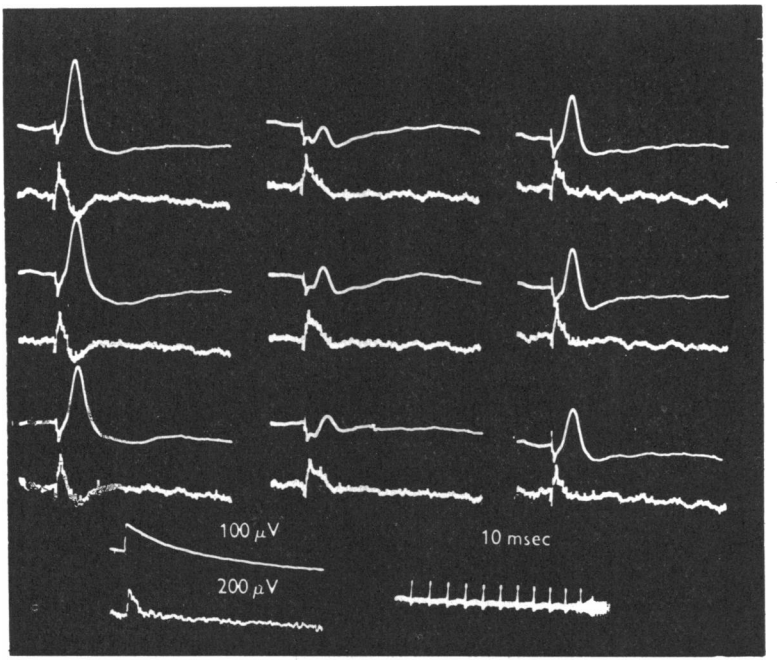

Fig. 9. Surface and deep components of the primary sensory evoked potential under the effect of GAB. The deep recording electrode was at 1·17 mm below the surface shown in lower sweep of each pair. Left-hand column: control before the application of GAB. Middle column: effect of 1% GAB 1 min after application; the surface negative responses were suppressed, while no marked change was seen in the deep responses except for slowing of the descending limbs of the deep negative wave. Right-hand column: recovery after 10 min. Note small spike discharges from the deep point in all the records.

there was a prolongation of the deep negative wave with a disappearance of the later deep positive component. The latter was related to the depression of the surface negative wave. The apparent prolongation of the deep negative wave may have been due only to the depression of the deep positive deflexion, which may be only an inverse reflexion of the surface negative wave generated in surface cortical layers. Consequently, we can assume that there is no essential change in the deep evoked potential subsequent to application of GAB to the cortical surface. This is true even when a 1% solution was allowed

to remain on the cortical surface for as long as 30 min. An example of lack of effect upon a deep response 500 μ beneath the surface is shown in Fig. 10.

In order to test further the action of GAB upon deeper layers of the cortex a glass pipette with an external diameter of 30–40 μ was used as a deep recording electrode. It was connected with a microsyringe which permitted injection of small amounts of solution into the depths of the cortex. A silver wire was placed in the pipette to make possible its use also as a recording electrode.

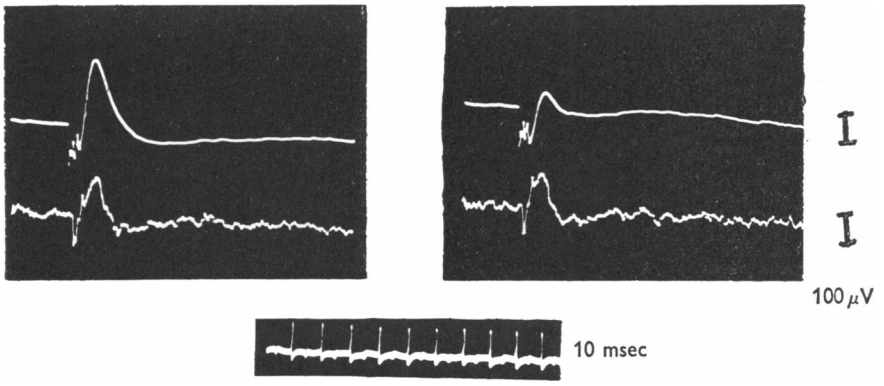

Fig. 10. Simultaneous recording of surface (upper) and deep (lower) component of the primary sensory response. The left-hand record is the control. At 1 min 40 sec after the application of 1% GAB, the right-hand record was obtained; there was suppression of the negative waves on the surface without any marked change in the depth. Recording of deep response was made at 0·5 mm below surface.

In initial control experiments carried out by injection of 0·0035 ml. saline solution at the site of deep recording there was no effect upon deep evoked potentials. The injection of 0·0035 ml. ($3{\cdot}5 \times 10^{-5}$ g) of GAB into the depths of the cortex, while recording deep evoked potentials, produced no effect upon responses obtained from about 1 mm beneath the surface. With a second injection of the same amount or up to 0·01 ml. in the depths, GAB was observed to appear in a small drop at the site of insertion of pipette on the surface. The surface response was then depressed in the manner described above, without evidence of any significant change in the deep response.

From these observations it was apparent that the depressing action of GAB was effective only upon the superficial layers of the cortex, probably chiefly upon the first or molecular layer which is composed principally of the terminations of apical dendrites and small horizontal cells.

DISCUSSION

The sensitivity of the surface negative waves of both the evoked potential and recruiting response of the cortex is well known. A depression of surface negative waves, similar to that with GAB, can be obtained by surface application of procaine or high concentrations of KCl, by anoxia, or by deep barbiturate anaesthesia. Purpura & Grundfest (1956) have recently obtained similar depressing effects by intracarotid injection of tubocurarine.

Although the surface negative wave is the first to be affected by these generally depressing procedures, the surface positive waves with activity in all cortical layers is eventually depressed as well. This is not true of GAB which caused an increase in voltage of certain aspects of cortical response (recruiting waves and spontaneous rhythms) associated with the change in electrical sign from negative to positive. The enhancement of the voltage of spontaneous activity may be considered similar to the effect of light barbiturate anaesthesia or normal sleep, and was also observed by Purpura & Grundfest following small amounts of tubocurarine.

The minimal concentration of GAB which produced appreciable changes in electrical activity when topically applied was between 0·02 and 0·2 mg/ml. Bazemore *et al.* (1956) found the minimal concentration for blocking the crayfish stretch receptor to be 2 μg/ml. The effects upon evoked potentials of neutral saline solutions of alpha-γ-diaminobutyric acid, alpha-aminobutyric acid, L-lysine and glycine in solutions equimolar (0·02 M) with 2 mg/ml. GAB were also tested in preparations giving good responses to 0·2 mg/ml. GAB. No obvious effects were obtained with any of these other substances with the exception of alpha-γ-diaminobutyric acid, which caused similar effects but less marked than those described for GAB.

GAB does not seem to be a general depressant of neuronal function, but seems to depress or block dendritic responses in superficial cortical layers selectively. If this negative wave of the evoked potential complex represents antidromic activation of apical dendrites from the soma initially excited in deeper layers, it may be assumed that GAB acts as a dendritic blocking agent. This would imply that the apical dendrites are susceptible to the action of a substance which does not have a similar action upon their soma or basal dendrites. This may be an unlikely assumption, although it would seem to be in accord with the direct action of GAB upon the crustacean stretch receptor cells and like the effect of an inhibitory transmitter substance (Kuffler & Eyzaguirre, 1955) acting upon a post-synaptic membrane. It may be that some microanatomical structures prevent the access of GAB to deep-lying cell bodies and their dendrites, although this seems unlikely when the substance is injected into the depths of the cortex.

If the surface negative wave or dendritic response is not due to antidromic

conduction from deeper layers, but represents a post-synaptic potential resulting from separate synaptic terminals in the superficial cortical layers of the cortex, GAB may be a specific synaptic blocking agent. This would be the simplest explanation of our results. The present experiments do not permit us to draw any definite conclusions regarding the possibility that GAB may act in the cortex as an inhibitory transmitter substance, though they are not inconsistent with such an hypothesis.

Our results do suggest that GAB itself, or some closely related substance which is normally present in brain tissue, may be of important functional significance in the regulation of cortical function. Furthermore, this substance provides a useful tool for physiological investigation of inhibitory and excitatory processes in the cerebral cortex, since it has a remarkably selective action upon specific structures in the superficial cortical layers.

The depression of surface negative electrical activity coincident with the enhancement of spontaneous rhythms appears to be the opposite of the effects of strychnine which causes a marked increase in surface negative electrical activity, while depressing spontaneous electrical rhythms. The possible antagonism between strychnine and GAB is being studied in greater detail.

SUMMARY

1. The effect of topical application of γ-aminobutyric acid (GAB), in concentrations of 0·02–1·0%, was tested upon various forms of cortical electrical activity in the unanaesthetized cat with partial destruction of the brain stem at the level of the superior colliculus.

2. GAB caused an immediate and reversible depression in the surface negative component of the primary evoked potential in somato-sensory cortex in response to thalamic stimulation. There was a slight increase and prolongation of the initial surface positive wave.

3. There was a marked increase in the repetitive sensory after-discharge which follows a single thalamic volley.

4. The recruiting response was changed from surface negative to surface positive polarity of larger amplitude waves.

5. Spontaneous electrical activity was increased as much as twice in amplitude, and the polarity of 'spindle bursts' was reversed to a predominately surface positive form.

6. Surface negative 'dendritic' responses to local cortical stimulation were abolished at the site of application of GAB without affecting these responses only 3–4 mm distant from the site of application.

7. Sensory evoked potentials recorded 0·5–1·0 mm beneath the surface of the cortex were not affected either by surface application or deep injection of GAB.

8. It is concluded that γ-aminobutyric acid has a selective depressant action upon structures in the most superficial layers of the cortex, perhaps only upon the molecular layer, without affecting deeper structures.

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