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Acetylcholine inhibition in the intact and chronically isolated cerebral cortex

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Summary

1. Some spontaneously firing cells in the cerebral cortex of cats can be depressed by iontophoretically applied acetylcholine or acetyl-β-methylcholine, and this depression is antagonized by atropine. Thirteen per cent of 101 spontaneously active neurones tested were depressed by cholinergic agents and 64% were excited.

2. Single stimuli applied to the adjacent cortical surface excited 132 neurones orthodromically. Acetylcholine or acetyl-β-methylcholine depressed this synaptic firing in 18% of the cells. The depression was blocked by atropine.

3. The population of neurones in which cholinergic agents depressed spontaneous or synaptic firing was located within the superficial half of the cortex.

4. Glutamate-induced firing was depressed by cholinergic agents in 41% of 211 cells tested; atropine and strychnine strongly antagonized this depressant action, while dihydro-β-erythroidine was a weaker antagonist.

5. Long duration inhibition of glutamate-induced firing evoked by repetitive stimulation of the cortical surface could be blocked by atropine or strychnine in both the intact and chronically isolated cortex. This provides strong evidence for a system of intracortical cholinergic neurones which make direct inhibitory contacts with neurones in the superficial layers of the cortex.

Introduction

The existence of an inhibitory cholinergic system within the cerebral cortex has been suggested by various investigators. The concept was initiated by Chatfield and his colleagues to account for the augmentation of primary cortical responses by topically applied atropine (Chatfield & Purpura, 1954; Chatfield & Lord, 1955). More recent observations on the actions of acetylcholine (ACh) antagonists on cortical excitability have been interpreted as effects on transmission at inhibitory synapses in the cortex (Bhargava & Meldrum, 1969; Bannerjee, Feldberg & Georgiev, 1970).

Iontophoretic studies have demonstrated that ACh can depress the firing of single neurones in the cat cerebral cortex (Randić, Siminoff & Straughan, 1964; Phillis & York, 1967a, 1968a, b; McLennan, 1970; Crawford, 1970b; Falchetto, Kato & Provini, 1971). Phillis & York proposed that ACh is an inhibitory transmitter in the cerebral cortex and presented evidence to show that the inhibition of cortical neurones evoked by repetitive stimulation of various regions of the brain, including the adjacent cortical surface, was mediated by ACh. This inhibition, which frequently had a duration of over one minute, was blocked by atropine

and hyoscine. Strychnine, which blocks the depressant action of ACh on cortical neurones, also abolished the inhibition evoked by repetitive stimulation of the adjacent cortical surface (Phillis & York, 1967b). The fact that long duration inhibition could be evoked by stimulation of a variety of brain structures suggested that cholinergic cortical interneurones were involved, and this interpretation was supported by the finding that similar long duration inhibition could be evoked in acutely isolated cortical slabs (Phillis & York, 1968a).

Ilyutchenok & Gilinsky (1969) have since confirmed the existence of an inhibitory cholinergic synapse in the cerebral cortex by demonstrating that topical application of the ACh-antagonists benactyzine and atropine abolishes the inhibition of spontaneously active cortical neurones induced by stimulation of the reticular formation. The electroencephalographic arousal reaction produced by stimulation of the reticular formation was also abolished. An intracortical inhibitory cholinergic interneurone has also been postulated by Vazquez and his colleagues (Vazquez, Krip & Pinsky, 1969 ; Krip & Vazquez, 1971) to account for the effects of cholinergic drugs on the duration of the epileptiform afterdischarge evoked by surface stimulation of chronically isolated cortical slabs.

Some doubt has been cast by McLennan (1970) on the evidence used to support the assertion that ACh is an inhibitory transmitter in the cerebral cortex. McLennan was able to find only a few ACh-depressed neurones in the cerebral cortex and was unable to demonstrate blockade by atropine or strychnine of inhibition evoked by surface stimulation.

The aim of the present study was to investigate further the phenomenon of cholinergic inhibition in the cerebral cortex. Much of the evidence favouring inhibition of cortical neurones by ACh has been obtained with cells which were excited by application of glutamate. Since objections to the use of glutamate as a test of cell excitability have been raised (cf., Johnson, Roberts, Sobieszek & Straughan, 1969), the first objective of this study was to test the action of ACh on cells firing in response to synaptic activation and on cells firing spontaneously. Secondly, in order to investigate the intracortical nature of atropine-sensitive long duration inhibition, experiments were performed to determine whether this type of inhibition persists in chronically isolated cortical slabs.

Methods

These experiments were performed on 23 adult cats. Anaesthesia was initiated with sodium thiopental (Pentothal, Abbott), a tracheotomy was performed and a tracheal cannula inserted. Anaesthesia was maintained during the remainder of the experiment with a mixture of nitrous oxide, oxygen and methoxyflurane (Penthrane, Abbot). Methoxyflurane was chosen because it does not appreciably alter chemical sensitivity of cortical neurones (Crawford, 1970a). The animal was placed in a stereotaxic frame, and body temperature was maintained at 37°–38° C with an electric heating pad controlled by a feed-back circuit using a rectal probe. The skull was opened, the dura reflected, and a perspex pressor foot positioned on the surface of the brain to reduce pulsations. The exposed muscle and skin and the remaining surface of the brain were covered with warm 4% agar in Ringer solution to prevent drying.

Five-barrel microelectrodes with overall tip diameters of 6–12 microns were inserted into the pericruciate or suprasylvian cortex through a small hole in the

pressor foot. The central recording barrel was filled with 2 M NaCl, and 1 M solutions of ACh, acetyl-β-methylcholine (AβM), atropine and L-glutamate normally occupied the outer barrels. Strychnine (10 mM in 200 mM NaCl), gamma-aminobutyric acid (GABA), eserine or dihydro-β-erythroidine were occasionally substituted for atropine. Drugs were injected using a constant-current polarizer, and retaining currents of 5–10 nA were maintained when drugs were not being ejected.

Extracellular action potentials recorded through the central recording barrel were amplified and displayed on an oscilloscope for photography. Alternatively, the spikes were distinguished from background noise through the use of a window discriminator consisting of a variable threshold Schmidt trigger and a pulse-shaping monostable multivibrator. The rate of firing was then determined by a Hewlett-Packard 5214L electronic counter, converted to analogue form, and displayed on a pen recorder.

Cells firing spontaneously, in response to brief pulses (5–10 s) of glutamate, or following synaptic activation were studied in these experiments. Neurones were fired synaptically by stimulating the cortical surface with either of two bipolar electrodes (enamel coated silver wire, 200 μm diameter) protruding from the pressor foot on either side of the recording electrode, or by stimulation of a coaxial electrode (overall diameter=0·5 mm) positioned just beneath the cortical surface and within 3 mm of the recording electrode. The stimuli were 0·1 ms in duration and ranged from 5 to 15 V in magnitude. The N-wave direct cortical response (DCR) was recorded as a monitor of the efficacy of stimulation (Phillis & Ochs, 1971). The coaxial electrode was always used when attempting to induce long duration inhibition.

A portion of the left suprasylvian gyrus was surgically isolated in 5 cats, by means of the technique described by Vazquez *et al.* (1969). The surgery was performed under sterile conditions. Animals were allowed to recover for 1 week to 4 months; then the cortex was re-exposed and recordings were made in the usual manner. At the end of each experiment the operated hemisphere was removed and fixed in 10% formol-saline for subsequent histological examination.

Results

Spontaneously firing cells

Spontaneously firing cells were located in all layers of the pericruciate cortex between 300 and 2,000 microns from the surface. A proportion of these neurones were depressed by ACh; the firing rate was reduced following iontophoretic application of ACh and recovered fully after the ACh current was terminated. Records from two such cells are shown in Fig. 1. The depressant action was often slow in onset and persisted after the passage of current ceased, as shown in Fig. 1A. The dose-dependent nature of the depressant action is illustrated in Fig. 1B. The depressant action of ACh was not accompanied by reduced spike heights, and therefore could not be due to depolarization block. In confirmation of earlier reports in which glutamate-fired cells were studied (Randić *et al.*, 1964; Phillis & York, 1967a, 1968a), the depressant action of ACh was readily antagonized by atropine. This action of atropine is apparent for both cells in Fig. 1. The fact that the ACh inhibition can be antagonized by a blocking agent eliminates current effects as a possible cause of the reduction in firing.

A total of 101 spontaneously firing cells were studied in 13 of the cats. Thirteen (13%) of these cells were depressed by ACh, and sixty-five (64%) were excited; the remainder were unaffected by ACh. Clearly, the majority of the spontaneously firing cells were excited by ACh, in agreement with the results of Krnjević & Phillis (1963a, b). Those cells depressed by ACh tended to be located in the more superficial layers of the cortex, whereas those excited by ACh were usually

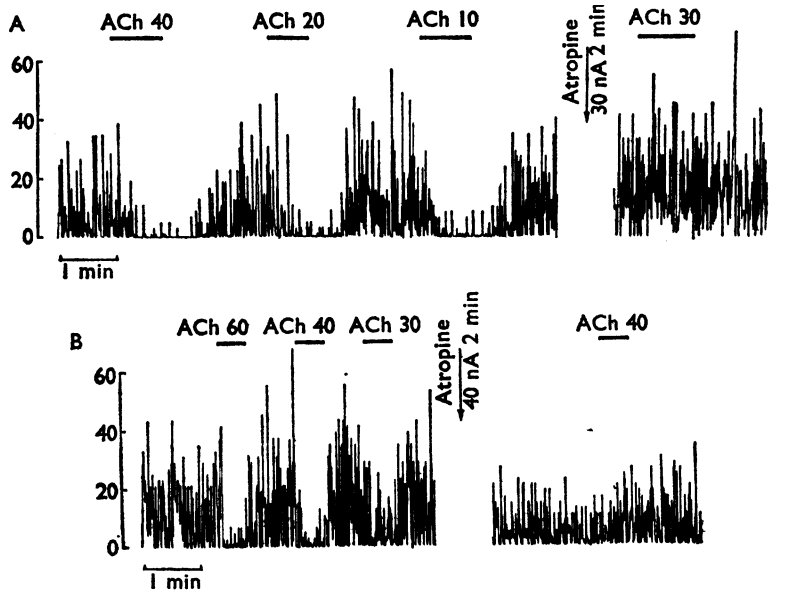

FIG. 1. ACh inhibition of two spontaneously firing cortical cells (A and B). The ordinate shows the firing rate in spikes per second. Horizontal bars above the trace represent the periods during which drugs were injected; the current in nanoamperes used for drug injection is shown above the horizontal bar in each case. The period during which atropine was applied has been deleted from the record.

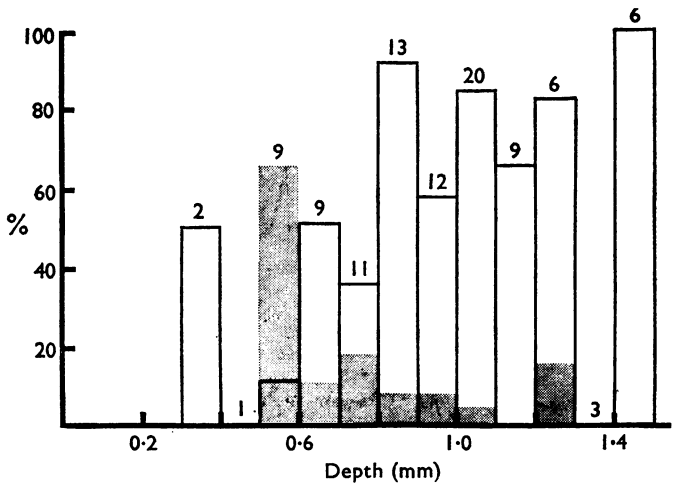

FIG. 2. Summary of the actions of ACh and AβM on spontaneously firing cells. The percentage of cells inhibited by ACh or AβM at each depth within the cortex is shown by the stippled areas. The vertical bars enclosed by heavy black lines show the percentage of cells at each depth which were excited by the drugs. The last vertical bar represents the cells found below 1·4 mm. The number of spontaneously firing cells found at each depth is indicated above each vertical bar.

found in the deeper layers. These features of ACh action on spontaneously firing cells are summarized in Fig. 2. The depth distribution of cells depressed by ACh is similar to that described by Phillis & York (1967a), who found them most frequently in layers II, III and IV.

Synaptically excited cells

Neurones at all depths in the cerebral cortex can be excited orthodromically by stimulation of the adjacent cortical surface (Phillis & Ochs, 1971). A total of 132 cells at depths ranging from 200 to 1,000 microns beneath the cortical surface were fired by surface stimulation, and the action of ACh or acetyl-β-methylcholine (AβM) was tested on this synaptic firing. The orthodromic nature of these responses was established by the tendency of the spike latency to vary between consecutive stimuli (cf. Phillis & Ochs, 1971). The synaptic firing of 24 cells (18%) was depressed by ACh or AβM. Depression was manifested as disappearance of the spike or a marked increase in spike latency. Only those cells showing complete recovery from drug action were considered to be depressed. Examples of cells in which ACh or AβM blocked synaptically-evoked firing are shown in Fig. 3. In each instance, a response to every stimulus applied to the cortical surface did not reappear until several seconds after current injection was terminated, indicating that the blockage of the spikes was due to drug action rather than to current effects. Recovery usually occurred within 1–5 minutes. The depression of the synaptically-evoked firing by ACh was blocked by atropine.

Although cells at all depths in the cortex were found which could be fired synaptically by stimulating the cortical surface, only two of those depressed by ACh or AβM were found at depths greater than 700 microns. This finding is in agreement with the superficial distribution of ACh-depressed cells found for

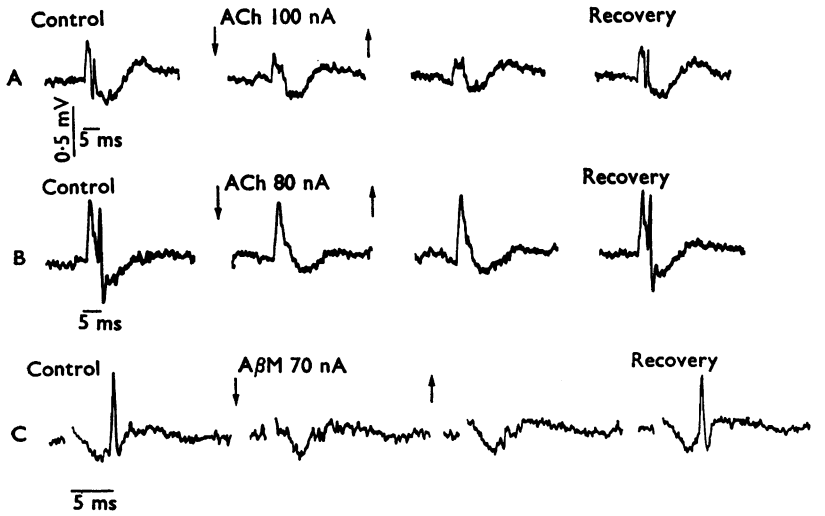

FIG. 3. Inhibition of synaptically-evoked firing by cholinergic agents. Action potentials produced in three cells following suprathreshold surface stimulation are shown in the control and recovery oscilloscope traces. Block of the action potential during drug administration (following the downward-pointing arrow) and after termination of the injecting current (following the upward-pointing arrow) occurred in each cell. Cells A, B and C were found 718, 631, and 1,000 microns respectively, below the surface of the cortex.

spontaneously firing cells in this study and also for cells firing in response to the application of glutamate, described in earlier studies (Randić, *et al.*, 1964; Phillis & York, 1967a).

During the course of recording from a few cells it was possible to compare the action of ACh on spontaneous, glutamate-induced and synaptic firing of a single neurone. Results from such a cell are shown in Fig. 4. The depression of synaptic firing by ACh in two trials is shown in Fig. 4A. During the control period the cell was firing consistently in response to surface stimulation; occasionally there was a second spike of longer latency. Acetylcholine blocked firing completely and after the injecting current ceased the cell fired with an increased latency. Recovery was complete within a few minutes. Spontaneous firing (Fig. 4B) and glutamate firing (Fig. 4C) were also depressed by ACh. These results illustrate that small amounts of ACh can inhibit synaptic firing. In most cases, however, synaptic firing was more resistant to depression by ACh, while glutamate excitation was the most susceptible. Depression of synaptic firing by ACh or AβM usually required larger ejecting currents than did depression of glutamate-induced or spontaneous firing with these agents. Tests with gamma-aminobutyric acid (GABA) revealed that much higher currents were also required to cause depression of synaptic firing with this substance than were required to depress spontaneous or glutamate-induced firing. Often 80 nA or more of GABA current was required to block synaptically-evoked firing of the cell, whereas 20–50 nA was generally effective in abolishing the firing of cortical neurones in response to L-glutamate. It is there-

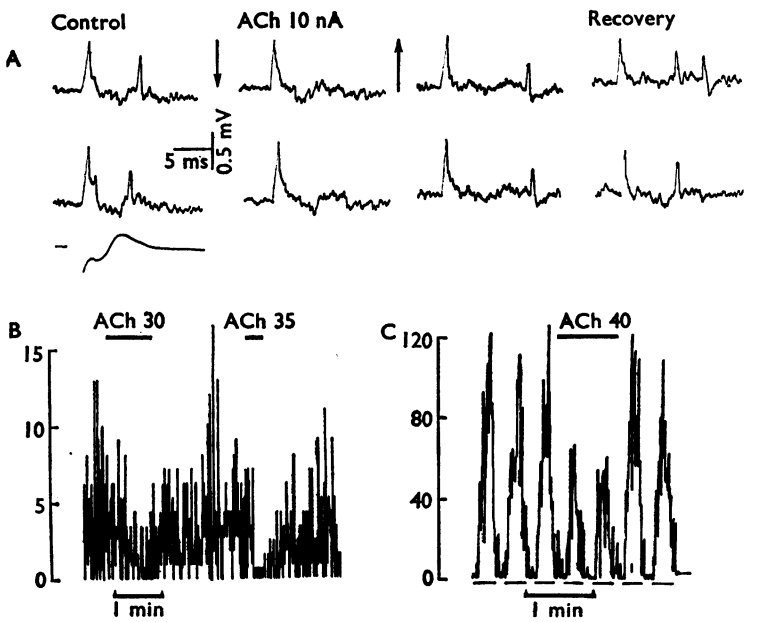

FIG. 4. ACh inhibition of synaptically-evoked, spontaneous and glutamate-induced firing in a single cell. Blockage of synaptic firing during two separate applications of ACh (10 nA) is shown in A. The third trace in the control column is the N-wave direct cortical response recorded from the surface of the cortex. B and C show ACh inhibition of spontaneous and glutamate-induced firing, respectively. The ordinates represent firing rate in spikes per second, and the periods during which drugs were applied are indicated above the traces. Glutamate pulses (40 nA) were applied during the periods indicated by horizontal bars below the trace in C.

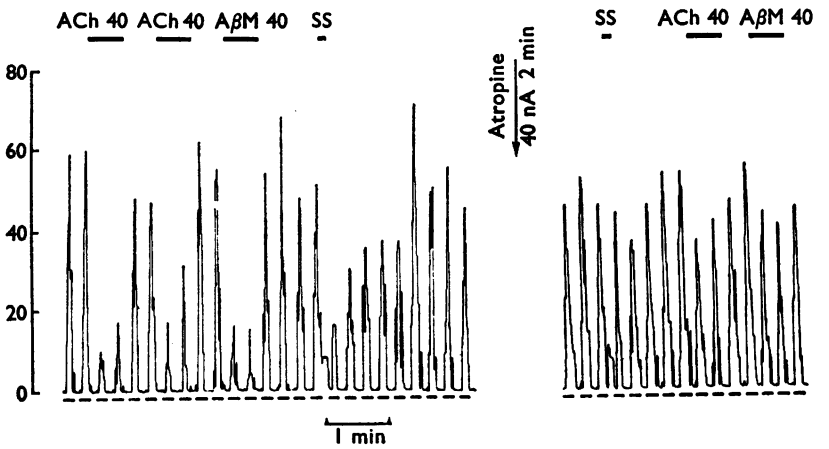

FIG. 5. Antagonism by atropine of ACh and AβM inhibition and of long duration inhibition produced by surface stimulation (SS). The surface of the cortex was stimulated at a rate of 15/s for 5 s during the period indicated. The vertical scale shows spikes per second, and the applications of glutamate pulses (40 nA) are shown by horizontal bars beneath the trace.

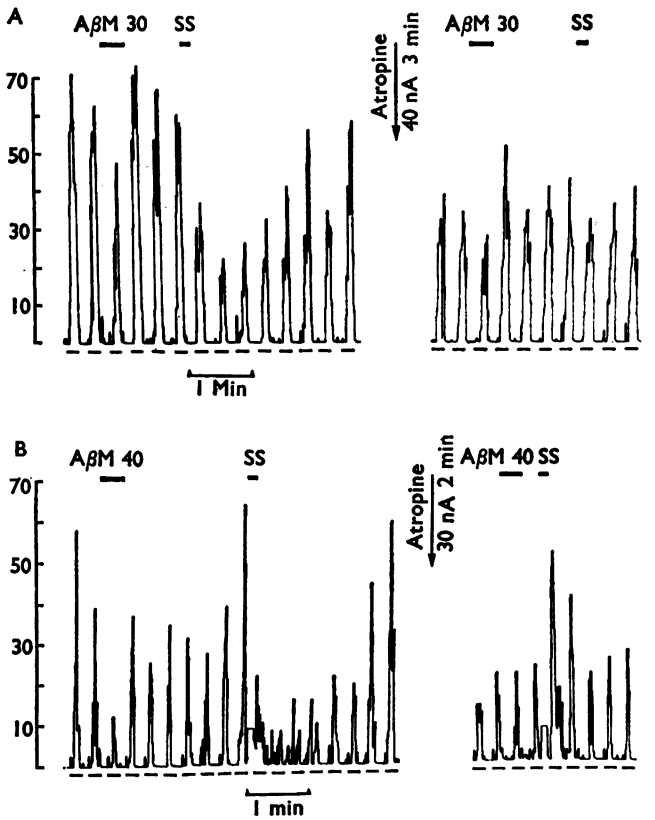

FIG. 6. Cholinergic and long duration inhibition in chronically isolated cortical slabs in the suprasylvian gyrus. Glutamate firing of a cell (A) 570 microns below the surface of a slab isolated for 14 days was inhibited both by AβM (30 nA) and by surface stimulation. A cell (B) located 777 microns below the surface of a slab isolated for 4 months was also inhibited by AβM (40 nA) and by surface stimulation. The glutamate current was 40 nA in both cases. The inhibition induced by drug application and the long duration inhibition produced by surface stimulation (SS) were both antagonized by atropine in these cells.

fore not surprising that ACh depression of synaptic firing often required larger currents than was necessary to block other types of firing.

Glutamate excited cells

The action of ACh or AβM was tested on 211 cells at depths ranging from 100–1,800 microns which were firing in response to brief pulses of glutamate. Forty-one per cent of these cells were depressed by one of the drugs. This is in good agreement with the previous observation (Phillis & York, 1968b) that 46% of cortical cells firing spontaneously and in response to glutamate were depressed by ACh. Figures 5 and 6 show examples of the depression by ACh of glutamate firing. The depressant action was antagonized by atropine as shown in Fig. 5. Strychnine (10 to 40 nA) has a similar antagonistic action which has been illustrated elsewhere (Phillis & York, 1967b). Dihydro-β-erythroidine was found to be considerably less effective as an antagonist of ACh than were atropine or strychnine. Eserine often mimicked the action of ACh on the ACh-depressed cells, but its effects were usually weaker. It potentiated the action of ACh on ACh-excited cells and had no effect on ACh-insensitive cells.

Long duration inhibition in intact cortex

Repetitive stimulation of the surface of the cortex at a rate of 15 per second for 5 s frequently caused prolonged inhibition of firing of cells in the pericruciate cortex. It was possible to block this type of inhibition with atropine (Fig. 5) and with strychnine, in agreement with the findings of Phillis & York (1967a, 1968a, b). Of 36 cells depressed by ACh, 23 (64%) were inhibited for a long period by cortical surface stimulation. In several instances it was also possible to block synaptically-evoked firing by repetitive stimulation of the cortical surface. This type of inhibition resembled long duration inhibition of glutamate firing; it lasted several seconds and could be antagonized by atropine.

Experiments on isolated cortical slabs

Five experiments were done on cats with a portion of the left suprasylvian gyrus chronically isolated from the rest of the brain. Sections through the isolated slabs subsequent to the experiment revealed that isolation was complete. The pial circulation was always left intact, and many viable cells were found in each slab, indicating that tissue preservation had been satisfactory. Long periods of isolation tended to increase the chance of producing paroxysmal activity following surface stimulation (cf., Krnjević, Reiffenstein & Silver, 1970b), and such activity obscured the appearance of long duration inhibition. Often the paroxysmal activity was followed by depression of glutamate firing, but this depression was insensitive to atropine and may have been due to post-excitation depression. For these reasons it was difficult to study long duration inhibition in cortical slabs which had been isolated for more than 2 weeks.

Nevertheless, repetitive stimulation of the surface of the isolated cortex depressed glutamate firing in 31 of 44 cells (70%). Figure 6A shows recordings from such a cell, located within a slab that had been isolated for 14 days. Glutamate firing of this cell was clearly depressed by AβM, and surface stimulation produced inhibition that recovered slowly and lasted for well over a minute. This response to

surface stimulation is identical to long duration inhibition occurring in the intact cortex. Figure 6B shows records from a cell within a slab which had been isolated for 4 months. The firing of this cell by glutamate was depressed by AβM, and surface stimulation evoked a pronounced long duration inhibition. In both cells of Fig. 6, the depression by AβM, as well as the long duration inhibition, was antagonized by atropine. Atropine blocked the long duration inhibition of 75% (6 out of 8) of those cells in which the duration of recording was sufficient to allow a test of its action. Clearly, atropine-sensitive long duration inhibition persists in chronically isolated cortical slabs.

The cells in isolated slabs which exhibited long duration inhibition that was atropine-sensitive were all depressed by ACh. Eserine also depressed these cells, although less effectively than did ACh, and it potentiated the action of ACh.

Discussion

Evidence in favour of the existence of cholinergic interneurones in the cerebral cortex has been forthcoming from experiments on the cholinergic systems in intact and isolated cerebral cortex. After isolation, the choline acetyltransferase and acetylcholinesterase contents of cat cortical slabs fall rapidly for three days and then reach fairly stable levels (choline acetyltransferase at 35% of control value; cholinesterase at 43% of control) which are sustained for at least a further two weeks, the duration of isolation in these particular experiments (Green, Halpern & Van Niel, 1970).

Acetylcholinesterase-staining fibres are found ramifying throughout the various layers of the intact cortex although the majority occur deeply in relation to the pyramidal cells of layer V and the polymorph cells of layer VI. These branches originate from a system of fibres which form a predominantly tangential system running beneath the cortex (Krnjević & Silver, 1965). Although no cerebral cortical neurones in the cat show intense staining, some of the spindle or polymorph cells in the deepest layer of the cortex often have relatively large amounts of intracellular enzyme and may therefore give rise to some of the fibres described above. Other fibres reach the cortex from the corpus striatum and septal area (Krnjević & Silver, 1965); a system which may be comparable with that described by Shute & Lewis (1967). Histochemical observation of large isolated cortical slabs has revealed that there is substantial preservation of the normal staining pattern with a clearly visible system of fibres running beneath and invading the cortex. Small slabs, with damage to the deeper layers of the cortex, were almost devoid of histochemically demonstrable acetylcholinesterase activity (Krnjević, Reiffenstein & Silver, 1970a). These observations on isolated slabs all strongly suggest that there are both intrinsic and extrinsic components of the cortical cholinergic system: an intracortical system, which may originate in the polymorph cells of layer VI, and an extracortical system from the corpus striatum and septal area.

The results of the present study lend further support to the assertion that cholinergic inhibitory synapses are present in the more superficial layers (II, III and IV) of the cerebral cortex. Such a distribution of cholinergic inhibitory terminals would be consistent with the finding (Sastry, 1956) that the highest concentrations of ACh in the feline cerebral cortex are found in layers II, III and IV.

Thirteen per cent of the spontaneously firing cells and 18% of the cells firing in response to synaptic activation were depressed by ACh or AβM. It is therefore apparent that cholinergic inhibition in the cortex is not dependent upon the use of glutamate to evoke neuronal firing. Indeed, it is evident that glutamate-induced firing, at least with regard to ACh inhibition in the cortex, is a valid test of cell excitability. This conclusion appears to justify the use of glutamate-induced firing in order to demonstrate inhibitory or excitatory synaptic responses in the central nervous system.

Although a higher percentage of cells firing in response to glutamate was depressed by ACh or AβM than spontaneously firing cells, the lower percentage in the latter case is undoubtedly due to the fact that many spontaneously firing cells are excited by ACh. Those cells which were excited by ACh were excluded from the population studies on glutamate-excited cells and this would account for the higher percentage of ACh-depressed cells observed.

In agreement with Phillis & York (1967a, b, 1968a, b), these studies revealed that the depressant effects of ACh can be antagonized by muscarinic and nicotinic blocking agents and by strychnine. The antagonist action of dihydro-β-erythroidine, however, was not as powerful as that of atropine, indicating that the receptors are basically muscarinic in nature. Even so, the pharmacological properties of the inhibitory receptor are clearly distinct from those of the muscarinic excitatory receptor on cortical neurones (Krnjević & Phillis, 1963c; Crawford & Curtis, 1966). This distinction argues against the proposition that the depressant effects of ACh are in fact secondary to an excitant action on adjacent neurones which in turn inhibit the cell under observation.

The depressant action of eserine in these experiments was often less powerful than that of ACh, whether the test was conducted on an intact or a chronically isolated cortex and, moreover, eserine only affected neurones which were depressed by ACh. This is in contrast to the report by Krnjević *et al.* (1970a) in which eserine is described as a more powerful depressant of neuronal firing than ACh in isolated cortical slabs. Krnjević *et al.* (1970a) cited the absence of histochemically detectable cholinesterase as evidence in support of their assumption that eserine had a direct depressant action on neuronal excitability. As stated above, Green *et al.* (1970) find 45% of the original cholinesterase content in isolated cortical slabs and the possibility that histochemical procedures may be unreliable as an indication of the presence or absence of cholinesterase must be considered. Phillis (1968) has described the failure of some histochemical techniques to detect cholinesterase in the cerebellar cortex.

Phillis & York (1968a) have previously reported that long duration inhibition can be evoked by direct stimulation of acutely isolated cortical slabs. Although demonstrating that other areas of the brain were not involved in a feedback loop to produce long duration inhibition, this procedure did not eliminate the possibility of direct activation of the terminal portions of incoming axons. Chronic isolation, on the other hand, should cause degeneration of all incoming cholinergic fibres, so that stimulation of the slab can induce activity only in intracortical neurones. This conclusion is justified by the rapid decrease to a stable plateau in the choline acetyltransferase and cholinesterase contents of cat cortical slabs which occur after isolation (Green *et al.*, 1970). Hence, if long duration inhibition were due to activation of fibres arising in other areas of the brain, it should have been abolished

in the chronically isolated slabs. The fact that inhibition comparable to that found in the intact cortex was preserved in the chronically isolated slab strongly supports the hypothesis that the inhibition studied is mediated by intracortical cholinergic interneurones.

Studies on ACh release from the cerebral cortex also support this conclusion, since Collier & Mitchell (1967) have shown that ACh release from chronically undercut cortical slabs is reduced to one-fifth that observed in the intact cortex. Approximately 20% of the ACh release must, therefore, originate from local intracortical neurones.

The long duration inhibition of glutamate-induced firing observed in this study was identical in time course and drug susceptibility to that described by Phillis & York (1967a, 1968a, b). The failure of McLennan (1970) to observe atropine or strychnine antagonism of long duration inhibition is possibly attributable to his selection of large cells in the deeper layers of the cortex which were unaffected or excited, rather than depressed, by ACh. This problem of electrode sampling bias has been discussed by Towe & Harding (1970), who pointed out that extracellular microelectrode sampling in the cerebral cortex is largely a function of neurone size. Conclusions on the presence or absence of cholinergic inhibitory synapses based on studies of neurones which are unaffected by ACh may be misleading. The results reported in the present and earlier papers have been attained by studying neurones which were depressed by ACh. It is clear that the surface evoked inhibition described by Krnjević, Randić & Straughan (1966a, b, c), amongst others, is not cholinergically mediated. The long duration cholinergic inhibition is a supplementary phenomenon which affects only a limited number of the neurones in the cerebral cortex, and especially those in the upper half of the cortex.

Neuropharmacological evidence in support of the concept of an intracortical cholinergic inhibitory synapse has been forthcoming from the recent work of other investigators. Ilyutchenok & Gilinsky (1969) have shown that the firing of some spontaneously active cortical neurones can be facilitated by anticholinergic agents and that the inhibition of such neurones produced by repetitive stimulation of the reticular formation can be abolished by intravenously administered or topically applied benactyzine, benzacine or atropine. This phenomenon is quite analogous to atropine's antagonism of long duration inhibition described in this paper. Vazquez and his associates (Vazquez *et al.*, 1969; Krip & Vazquez, 1971) have confirmed the involvement of cholinergic interneurones in cortical inhibition by demonstrating that cholinergic agents shorten, and anticholinergic muscarinic agents lengthen, the duration of the epileptiform afterdischarge in chronically isolated cortical slabs.

In association with our own results, these findings demonstrate the existence of cholinergic inhibition in the cerebral cortex.

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