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Properties of cholinoceptive neurones in the medial geniculate nucleus

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Summary

1. Acetylcholine (ACh), other cholinomimetics, cholinesterase inhibitors and cholinergic antagonists were administered iontophoretically to medial geniculate (MG) neurones and their effects on chemically or neurally evoked responses recorded extracellularly.

2. Acetylcholine had excitant actions on 45% of the neurones tested. Most of these were of a slow time course. Desensitization to the excitant effects was frequently observed.

3. Acetylcholine excited 91% of neurones activated antidromically by stimulation of the auditory cortex, 71% of neurones activated synaptically from the auditory cortex, 74% of neurones activated from the inferior colliculus and 100% of geniculo-cortical relay neurones.

4. Acetylcholine had depressant effects, which were generally of a rapid time course, on 29% of MG neurones. No desensitization to the depressant effects was observed.

5. On 4% of neurones, ACh had both excitant and depressant effects. Such "dual" effects were manifested either as an initial excitation followed by a depression, or as a depression followed by an excitation.

6. Eserine, neostigmine and edrophonium potentiated both excitant and depressant actions of ACh on many cells. Neostigmine and edrophonium occasionally antagonized the effects of ACh.

7. Atropine, hyoscine, dihydro-β-erythroidine, hexamethonium and (+)-tubocurarine antagonized both excitant and depressant effects of ACh. The muscarinic blocking agents were usually more effective than the nicotinic agents.

8. Carbamylcholine, acetyl-β-methylcholine, nicotine, butyrylcholine, arecoline and pilocarpine had excitant, depressant or no effects on MG neurones. Generally, carbamylcholine was more potent than acetyl-β-methylcholine and ACh, which were more potent than nicotine. Butyrylcholine, arecoline and pilocarpine were even less potent, often having no effect.

9. The cholinomimetics generally had similar effects to those of ACh on the same neurones, but sometimes were quite different. Carbamylcholine, acetyl-β-methylcholine and nicotine antagonized the effects of ACh on some neurones.

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10. The results suggest that cholinoceptive receptors on MG neurones are not homogeneous. Although there are possibly some purely muscarinic and purely nicotinic receptors, the majority appear to be of intermediate muscarinic-nicotinic type. These mediate either excitation or inhibition.

Introduction

Several studies have been made on the types of responses evoked from single medial geniculate (MG) neurones by sound stimulation (Gross & Thurlow, 1951; Galambos, Rose, Bromiley & Hughes, 1952; Nelson & Erulkar, 1963; Adrian, Lifschitz, Tavitas & Galli, 1966; Aitkin, Dunlop & Webster, 1966; Aitkin & Dunlop, 1968). Little is known, however, about the types of responses evoked by stimulation of the auditory cortex and inferior colliculus, because such investigations have only recently been reported (Watanabe, Yanagisawa, Kanzaki & Katsuki, 1966; Aitkin & Dunlop, 1969). Less has been published on the pharmacology of MG neurones (Vernier & Galambos, 1957; Tebēcis, 1967). It was of interest, therefore, to investigate the effects of drugs and stimulation of structures within the brain on neurones of the feline MG nucleus.

Results from biochemical and histochemical studies suggest a possible transmitter function for acetylcholine (ACh) in the mammalian MG nucleus. Acetylcholine is present in the diencephalon of cats (Macintosh, 1941). Its synthesizing enzyme, choline acetyltransferase, is present in the MG nucleus of man, sheep and dog (Hebb & Silver, 1956). Its inactivating enzyme, acetylcholinesterase, has been demonstrated in the MG nucleus of dogs (Burgen & Chipman, 1952) and rats (Koelle, 1954). In particular, Shute & Lewis (1967) have shown (in rats) the presence of fibres containing cholinesterase which originate in the cuneiform nucleus and project directly to the MG nucleus via the dorsal tegmental pathway in the brain stem.

In the investigations described here, information on the possible role of ACh in the MG nucleus was obtained by studying the effects of iontophoretically administered ACh, its antagonists and cholinesterase inhibitors on MG neurones. An attempt was made to characterize the types of cholinoceptive receptors on MG neurones by comparing the effects of several cholinomimetic compounds. Three afferent pathways—from the auditory cortex, inferior colliculus and mesencephalic reticular formation—were examined for the possibility of cholinergic transmission. A brief communication of a part of this work has been presented to the Australian Physiological and Pharmacological Society (Tebēcis, 1968).

Methods

The results presented in this and the following paper (Tebēcis, 1970) were obtained from a total of thirty-five adult cats of both sexes.

Anaesthesia

Anaesthesia was induced in most of the animals by an intravenous dose of thiopentone sodium (30–50 mg/kg; Intraval Sodium, May & Baker). Supplementary doses of this barbiturate were administered when necessary during the dissection,

up to the stage of mounting the cat in the stereotaxic frame, when it was connected to a gas anaesthetic machine (Commonwealth Industrial Gases, Midget Mark I) via the tracheal cannula. Anaesthesia was maintained by a gaseous mixture of oxygen, nitrous oxide and halothane (Fluothane, I.C.I.) or methoxyflurane (Penthrane, Abbott), the levels of which were adjusted to maintain the animal in a lightly anaesthetized state.

Several observations suggested that this combination of anaesthetics did not unduly interfere with the types of pharmacological responses studied. First, although barbiturates often selectively depress the effects of iontophoretically applied ACh and monoamines on thalamic and cerebral cortical neurones, a mixture of nitrous oxide and halothane (or methoxyflurane) apparently has no depressant action on the effects of these drugs (Phillis & Tebēcis, 1967 ; Roberts & Straughan, 1967, 1968). Furthermore, the effects of drugs on neurones of the globus pallidus-putamen complex of gas-anaesthetized and unanaesthetized (*encéphale isolé*) cats appear to be comparable (D. H. York, personal communication).

Ten cats were induced with ethyl chloride followed by ether instead of thiopentone sodium. However, results from cats induced with thiopentone sodium did not differ from those of cats induced with ethyl chloride and ether, indicating that the initial dose of thiopentone had a negligible effect on the responses observed. In any case, usually only a single dose of barbiturate was administered, because the greater part of the dissection was performed when the cat was connected to the gas anaesthetic machine, immediately after tracheotomy.

Despite these precautions, it is possible that the combination of anaesthetics used modified the pharmacology of MG neurones. Mid-collicular sections were not attempted, however, because the use of neuromuscular relaxants (which are essential for obtaining stable long-duration recordings from unanaesthetized animals) may be pharmacologically as hazardous as the use of anaesthetics (Halpern & Black, 1968 ; Galindo, Krnjević & Schwartz, 1968 ; Boyd, Meritt, Aroesty & Celso, 1969).

Dissection, mounting and stimulation

All studies were made on the left MG nucleus. After removal of the appropriate parts of the skull, the overlying cerebral cortex and dorsal hippocampus were sucked away to expose the dorsal lateral geniculate nucleus. The latter was left intact to avoid damaging the vascular supply to the MG nucleus. The animal's head was rigidly fixed in a stereotaxic frame and its abdomen rested on an automatically controlled heating pad maintained at $37° \pm 1°$ C. When the dissection was completed, the exposed surface of the brain (with the exception of the lateral geniculate nucleus) was covered with a 2–4 mm thick layer of 4% agar in physiological saline of the following composition: Na, 152·8 m-equiv./l.; K, 2·65 m-equiv./l.; Ca, 1·05 m-equiv./l.; Cl, 157·6 m-equiv./l.; glucose, 763 mg/l. The lateral geniculate nucleus was continuously perfused by cerebrospinal fluid leaking from the ventricles.

The micropipettes were aligned stereotaxically and driven 3–6 mm through the lateral geniculate nucleus into the MG nucleus between the stereotaxic co-ordinates A3–6 and L8–11, using the atlas of Snider & Niemer (1961). In some experiments three to five concentric bipolar stimulating electrodes were inserted 2–4 mm below the surface of the auditory cortex of the ectosylvian gyrus, in areas AI, AII, Ea and

Ep, as described by Whitfield (1967). The cerebral cortex overlying the hippocampus was sometimes left intact in these cats, although it could usually be removed without damaging the adjacent auditory cortex. MG units were identified by evoking them synaptically, antidromically or by confirming their locations histologically, using " acid lesions " (McCance & Phillis, 1965).

Bipolar stimulating electrodes were also placed in the ipsilateral mesencephalic reticular formation (stereotaxic co-ordinates A3, L3, D–1) and inferior colliculus. The latter was impaled under visual control after removal of the overlying brain tissue and part of the tentorium. When a stereotaxically positioned stimulating electrode was employed, its location was checked histologically after the experiment. Results obtained with incorrectly placed electrodes were discarded. Acoustic stimulation was employed routinely. Clicks were applied simultaneously to both ears by means of two crystal microphones (Zephyr Products, Victoria, of 1–5 MΩ impedance) which were reversely activated by a Grass S4 stimulator.

Recording, micropipettes and drugs

Evoked field and spike potentials were recorded extracellularly through the sodium chloride (2 M) filled central barrel of glass multi- (5, 7 and 9) barrelled micropipettes and the signals displayed on film or by means of a chart recorder. Details of the recording system, the construction and filling of micropipettes and the types of drugs used have been described previously (Phillis, Tebēcis & York, 1967a, b).

One barrel of each micropipette was filled with 0·5–2 M sodium L-glutamic acid (or sometimes 0·2 M DL-homocysteic acid), adjusted to pH 8 with NaOH, for the activation of quiescent neurones. Another barrel was routinely filled with a neutral solution of ACh, usually at a concentration of 1 M. Drugs were generally applied with currents of 20–60 nA and rarely greater than 100 nA. In a series of preliminary experiments it was found that equal currents through barrels containing ACh at concentrations of 10^{-3}, 10^{-1} and 0·5 M, as well as saturated solutions, produced comparable qualitative effects on the same MG neurones. Cationic currents were passed through the sodium L-glutamate-containing barrel to determine whether currents carried by " inert " ions such as Na^+ produced comparable effects to the drugs ejected from the same micropipette. Results were discarded if the drug-induced effects were not obviously different from those produced by equal currents of Na^+. It was often difficult to obtain stable recordings when 7 and 9-barrelled micropipettes containing a series of cholinomimetics were used. This was interpreted as being due to excessive leakage of the cholinomimetics from the barrels, causing either desensitization of ACh-excited, or depression of ACh-depressed, cells (see **Results**). When the " braking " currents were increased (up to 30 nA) to counteract diffusion, there was a tendency for the barrels to become blocked and the latencies of drug-induced effects became more variable.

It is difficult to assess the relative potencies of drugs passed from various barrels of a micropipette because of differences in the physical properties of the separate barrels, the differing transport numbers of the drugs and the different distances of the barrels from the recorded cell (Curtis & Ryall, 1966a). For these reasons, when the potencies of drugs were compared, each drug was tested in at least five different cats, using at least five different micropipettes. In this way the approximate relative potencies of some cholinomimetics were estimated.

Results

Neurally evoked responses

Both antidromically and synaptically evoked responses were recorded in the MG nucleus when the ipsilateral auditory cortex was stimulated. Responses were classified as antidromic if they (*a*) were evoked with an all-or-nothing spike of constant latency which was less than 3·5 ms, (*b*) followed repetitive stimulation at frequencies of 100 Hz or higher, and (*c*) responded to two shocks separated by short intervals (1–2 ms). An example of a response which was evoked antidromically by stimulation of the auditory cortex (area AI) is shown in Fig. 1A. The cell spike was evoked with a constant latency of slightly less than 1 ms when the cortex was stimulated with barely supra-threshold shocks at 1 Hz (A, 1). The spike followed repetitive stimulation at a rate of 200 Hz (A, 2) but began to fail at higher rates.

Most of the antidromic responses recorded in this investigation had latencies ranging from 1 to 2·5 ms, but in a few cases the latencies were as short as 0·5 ms or as long as 3·5 ms. Aitkin & Dunlop (1969) reported that most of the responses of MG units evoked by cortical stimulation had latencies between 1 and 3 ms and that the majority of these were due to antidromic activation. These figures differ from those of Watanabe *et al.* (1966), who recorded latencies no shorter than 3·0 ms, but are similar to those of Andersen, Eccles & Sears (1964), who described latencies of 0·5–3·5 ms for thalamic neuronal responses which were evoked antidromically from the sensori-motor cortex.

Many antidromically evoked spikes were followed by orthodromically evoked responses. An example of this is illustrated in Fig. 1B, which shows two consecutive responses (1 and 2) of a cell to stimulation of the auditory cortex (area AI) at 1 Hz. The first spike was evoked with a constant latency of 2 ms and followed repetitive stimulation at frequencies of up to 250 Hz. The subsequent spikes varied

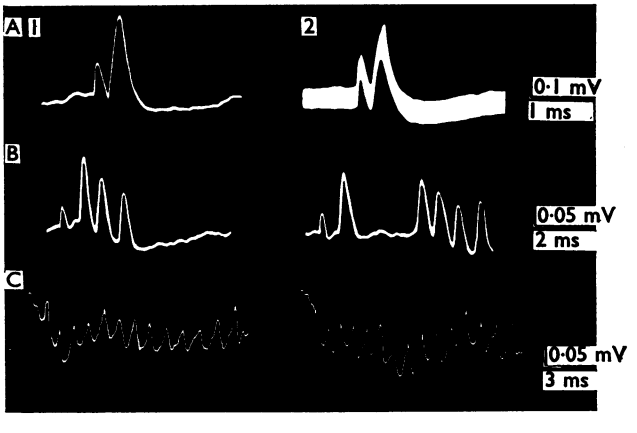

FIG. 1. Examples of responses evoked by stimulation of the auditory cortex. Negativity is upwards in this and subsequent figures of oscilloscope recordings. A: Antidromic spike evoked by stimulation of area AI at 1 Hz (1) and 200 Hz (2). B: Two consecutive responses (1 and 2) of another cell evoked by stimulation of area AI at 1 Hz. The first spike was evoked antidromically; the subsequent spikes, synaptically. C: Two consecutive responses (1 and 2) evoked from a third cell by stimulation of area AII at 1 Hz.

in latency and number and did not follow repetitive stimulation at frequencies greater than 8 Hz.

Responses which were evoked only synaptically by stimulation of the auditory cortex varied considerably in latency, duration and pattern of firing. The most frequently observed synaptic responses had latencies of 3–5 ms, although responses with latencies of 20 msec or more were also recorded. The latter were invariably repetitive discharges.

Fig. 1C (1 and 2) shows two consecutive repetitive responses evoked in the MG nucleus when the auditory cortex was stimulated at a rate of 1 Hz. These responses had a latency of 3 ms and a duration of up to 30 ms. It was not determined whether the first spike in the responses was evoked antidromically or synaptically. In either case the latency was short enough to indicate a direct pathway from the auditory cortex to the MG nucleus.

The responses of MG neurones evoked by stimulation of the ipsilateral inferior colliculus usually consisted of one or two spikes of 1·5–3·0 ms latency. This is similar to the observations of Aitkin & Dunlop (1969). However, several other types of responses were recorded. One response (of 4 ms latency) had a total duration of 45 ms and consisted of six to nine spikes, when the inferior colliculus was stimulated at 1 Hz. Some repetitive discharges had latencies of 50–100 ms. Repetitive stimulation (2–20 Hz) either facilitated or depressed spontaneous or L-glutamate-induced firing of several neurones. Three units fired in bursts when the colliculus was stimulated at 0·2 Hz, but were completely inhibited with higher frequencies of stimulation. Collicular stimulation (1 Hz) inhibited four spontaneously firing neurones for periods of 150–300 ms.

Effects of acetylcholine

Acetylcholine was administered iontophoretically to more than six hundred MG neurones. The effects observed are summarized in Table 1. (Results which were ambiguous or variable were not analysed.) These effects were classified according to whether ACh affected neuronal firing when applied with currents of up to 60 nA. The figures in the table are, at best, only an approximate estimate of the proportions of ACh-induced effects on MG neurones because of the variability between cats, electrodes and areas of recording. Attempts were therefore made to assess the actions of ACh on physiologically identified neurones. Electrode positions were chosen to penetrate all areas of the parvo- and magno-cellular nuclei, but the more central regions of the MG nucleus were sampled more frequently.

Excitant effects of acetylcholine

Although ACh excited a relatively large proportion of cells (45%) its effects on most of these were weak and slow in onset and decline. On some cells, however, it was extremely potent with a time course comparable with that of L-glutamate, as has

TABLE 1. *Summary of the effects of ACh applied to 586 neurones of the MG nucleus*

	Total	Excitation	Depression	Dual	Nil
Number	586	265	169	24	128
%	100	45	29	4	22

Figures refer to the number (top row) and percentage (bottom row) of neurones. The effects are described in the text.

been observed for some cholinoceptive neurones in the lateral geniculate nucleus (Phillis *et al.*, 1967b).

An example of the facilitatory effect of ACh on glutamate-induced firing is illustrated in Fig. 2A. This unit responded to bilateral click stimulation (1 Hz) with a spike of approximately 6 ms latency superimposed on a field potential (A, 1). The increased firing frequency produced by glutamate (60 nA) was tripled during two successive applications of ACh (60 nA), but this increased excitability did not persist when the application of ACh was terminated (A, 2). ACh alone did not appear to increase the firing rate of this neurone, as registered with the pen-recorder. However, neurones of this type were sometimes observed to fire at a low frequency when viewed on the cathode ray oscilloscope. Because it was difficult to assess the effects of other drugs on low-frequency discharges produced by ACh, the method of recording glutamate-induced firing on the chart recorder was usually preferred.

Figure 2B is an example of a neurone which was fired directly by ACh. Stimulation of the auditory cortex (1 Hz) evoked a repetitive response consisting of three to five spikes (B, 1). Two applications of glutamate (60 nA) caused the cell to fire at a maximum spike frequency of 100 Hz. ACh (40 nA) caused a comparable increase in firing rate after it had been applied for 35 s.

Cells often became desensitized to the excitatory effects of ACh. Thus, although the first application of ACh to such cells caused a significant increase in firing rate, successive applications a short while afterwards had little or no effect. After a 5–10 min rest, an application of ACh again produced a significant effect. Similarly, pro-

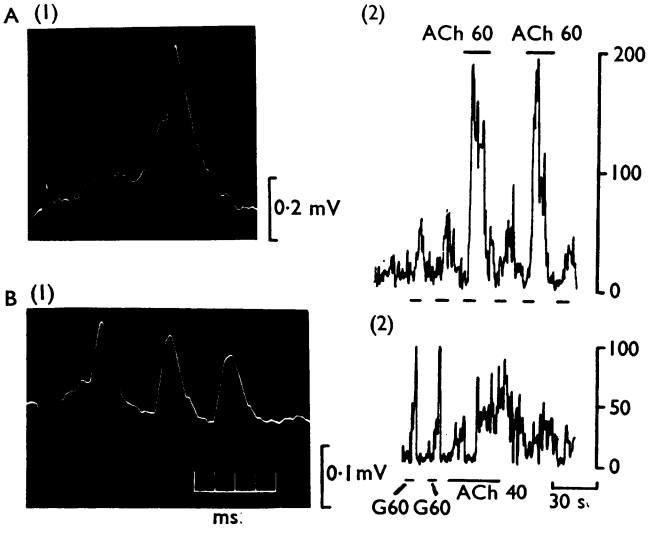

FIG. 2. Excitatory effects of ACh on two different cells (A and B). A(1), response of the cell to click stimulation (1 Hz). A(2), chart recording showing the action of ACh on this cell. In this and subsequent figures of chart recordings periods of drug application are represented by horizontal bars above or below the tracings. In A(2) L-glutamate was applied with a current of 60 nA at periodic intervals. The vertical scale on the left or right of chart recordings in this and subsequent figures refers to the firing frequency of the neurone, in spikes/s. B(1), repetitive response of another neurone evoked by cortical stimulation at 1 Hz. B(2), chart recording showing the effects of L-glutamate (G 60 nA) and ACh (40 nA) on the firing frequency of this cell. Time calibration for A(1) and B(1) is in ms; for A(2) and B(2), 30 s. In subsequent figures of chart recordings the time calibration scale on a particular figure applies to all pen tracings on the figure, unless indicated otherwise.

longed applications using high currents (80–100 nA) of ACh sometimes caused a progressive decrease in the maximum firing frequency attained. Many ACh-excited cells did not become desensitized, even on repeated applications of ACh with high currents.

Depressant effects of acetylcholine

ACh depressed 29% of the synaptically or chemically evoked responses of MG neurones tested. Typically, ACh-induced depressions were rapid in onset and decline, although some cells remained depressed for 1–2 min after an application of ACh. Figure 3 illustrates three methods used for testing the depressant effects of ACh.

Stimulation of the brachium of the inferior colliculus (1 Hz) evoked a short-latency spike in a neurone in the ipsilateral MG nucleus (Fig. 3A, 1). ACh (80 nA) blocked the spike 10 s after the beginning of its application (A, 2) and recovery was complete 15 s after the application was terminated (A, 3). A cationic current of Na^+ (80 nA) from the glutamate-containing barrel did not affect the spike.

Figure 3B is a chart-recorder tracing of the same cell as in A, showing the depressant effect of ACh on glutamate-firing. ACh (20 and 40 nA) reduced glutamate-firing, but with these currents did not block the synaptically evoked response shown in A. In fact, it was often observed that drugs depressed chemically evoked responses more readily than neurally evoked responses. This might be explained if it is assumed that a response evoked synaptically by presynaptic endings which terminate over a large area of the soma membrane has a higher safety factor than one

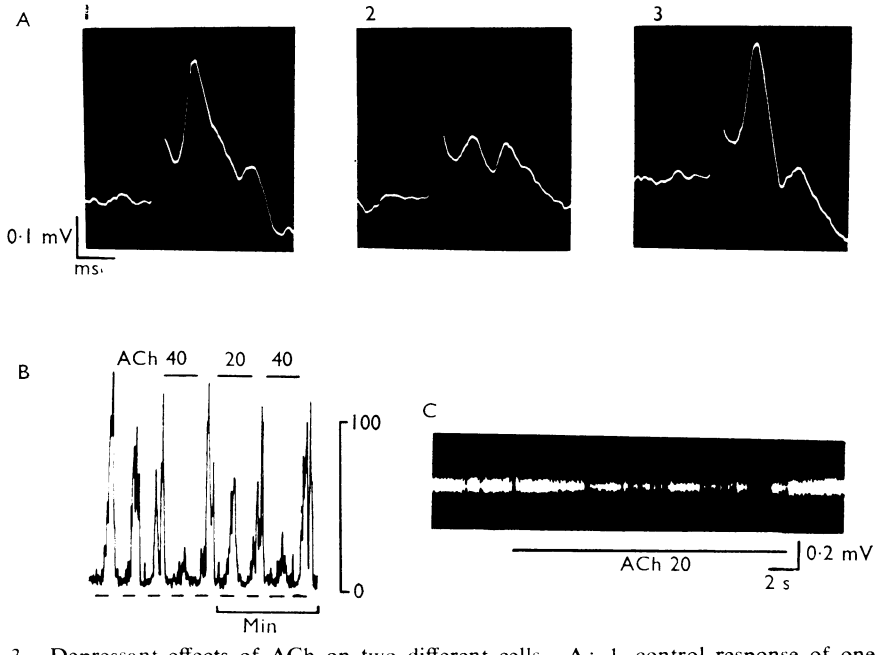

FIG. 3. Depressant effects of ACh on two different cells. A: 1, control response of one cell evoked by stimulation of the brachium of the inferior colliculus (1 Hz). 2, 10s after the beginning of a 20 s application of ACh (80 nA). 3, recovery 15 s after the current ejecting ACh had been terminated. B: Depressant effect of ACh on glutamate (50 nA)-firing of the same cell as in A. C: Effect of a 14 s application of ACh (20 nA) on the spontaneous firing of another cell, recorded on moving film.

evoked with an excitant amino-acid acting on a localized area of the soma membrane. Moreover, drugs ejected from different barrels of the same micropipette probably act on approximately the same area of membrane, thus facilitating their interaction.

Some spontaneously firing neurones were studied using moving-film records of the cathode ray oscilloscope (Fig. 3C). ACh (20 nA) began to reduce the firing rate of the neurone after 2 s and almost completely abolished it after approximately 10 s. When the application was terminated the cell recommenced to fire at its maximum frequency within 1 s. A current of Na^+ (20 nA) had no effect on the cell's firing rate.

ACh-induced depressions were generally pronounced. The diffusional efflux of ACh which resulted from a cessation of the " braking " current was sufficient to cause depression of some neurones. Desensitization to the depressant effects of ACh was not observed.

" Dual " excitant-depressant effects of acetylcholine

ACh had both excitant and depressant effects on a small proportion of cells. Two examples are illustrated in Fig. 4.

L-Glutamate (60 nA) was applied at regular intervals to the cell in Fig. 4A. ACh (20 nA for 15 s) reduced glutamate-firing during its application. When the application was terminated the firing frequency of the cell increased above control level and recovered 1·5 min after ACh was turned off. Hyoscine was ejected from another

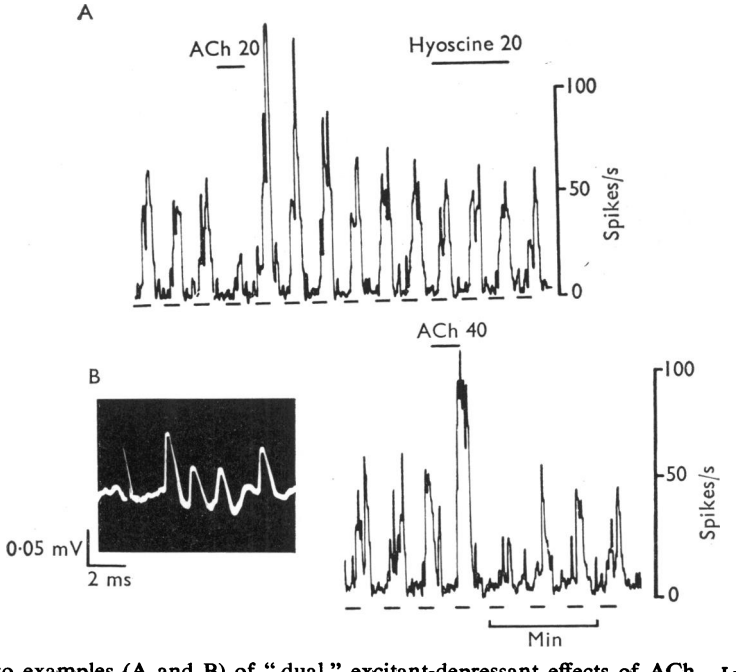

FIG. 4. Two examples (A and B) of " dual " excitant-depressant effects of ACh. L-Glutamate was applied with currents of 60 nA to both cells. The oscilloscope record shows a repetitive response of the cell in B to stimulation of the auditory cortex (area AII) at 1 Hz. 4 s trains of repetitive stimuli at 100 Hz inhibited all but the initial spike for 2 min. The first spike followed repetitive stimulation at frequencies above 100 Hz.

barrel of the micropipette in order to determine whether a cationic current of 20 nA could depress this cell. As the figure indicates, hyoscine did not alter glutamate-firing. In some " dual " cells of this type the excitant phase following the application of ACh lasted for up to 3 min.

The other type of " dual " effect observed was an excitation during the application of ACh, followed by a depression after it (Fig. 4B). Stimulation of area AII of the auditory cortex (1 Hz) evoked an antidromic spike, followed by three synaptic spikes. ACh (40 nA) facilitated glutamate-firing during its application. When the application of ACh was terminated glutamate-firing was depressed for approximately 30 s. The post-excitant depressant phase lasted for up to 1 min in some cells of this type.

Continuous monitoring of the cell's firing frequency on the cathode ray oscilloscope revealed that " dual " responses to ACh occurred even though the amplitude of the spikes remained constant. This suggests that ACh was not having opposing actions on two adjacent cells near the tip of the micropipette, although it does not exclude the possibility that ACh had opposite effects of differing time course on two cells which fired with spikes of equal amplitude.

Effects of acetylcholine on neurally identified cells

The effects of ACh on MG responses evoked by stimulating the auditory cortex and inferior colliculus are summarized in Table 2. Responses which were evoked both antidromically from the auditory cortex and synaptically from the inferior colliculus were defined as geniculo-cortical relay responses (GCR). This is in accordance with the definition for thalamo-cortical relay cells employed by Andersen *et al.* (1964).

ACh had excitant actions (which were generally direct) on a large proportion of cells which responded to neural stimulation. ACh excited 91% of the cells antidromically activated from the auditory cortex. It excited 71% and 74% of the cells synaptically activated from the cortex and inferior colliculus, respectively, and also had depressant actions on a small proportion of them. In particular, ACh excited all thirty-three GCR cells recorded.

The typical effects of ACh on a GCR neurone are illustrated in Fig. 5. The spike followed repetitive stimulation of the cortex (150 Hz) with a constant latency. Stimulation of the inferior colliculus evoked a similar spike which followed repetitive stimulation at 8 Hz with a variable latency. With higher rates of stimulation the synaptic spike failed. ACh (50 nA) caused the cell to fire after a latency of approximately 20 s. The neurone continued to fire for 12 s after the application was terminated. A second application of ACh had a comparable effect, although the

TABLE 2

	Total	Excitation	Depression	Dual	Nil
Cortex (A/D)	74	64	0	3	7
Cortex (syn)	35	24	3	1	7
Colliculus (syn)	53	39	10	0	4
GCR	33	31	0	2	0

Figures refer to number of neurones. The types of evoked responses are indicated at the beginning of each row. Abbreviations are: A/D (antidromic), syn (synaptic). Units whose responses were evoked both antidromically and synaptically by stimulation of the auditory cortex are represented twice, once in row 1 and again in row 2. The figures do not distinguish between the different types of synaptically evoked responses.

latency was reduced. Dihydro-β-erythroidine (100 nA for 2 min) had a slight excitatory effect on the cell but did not reduce the excitant action of ACh (not shown on figure). Atropine was not present in the micropipette used to record this cell but was found to be an effective antagonist of ACh on other GCR neurones. On two GCR neurones studied by pulses of glutamate it was observed that ACh caused an initial depression before excitation.

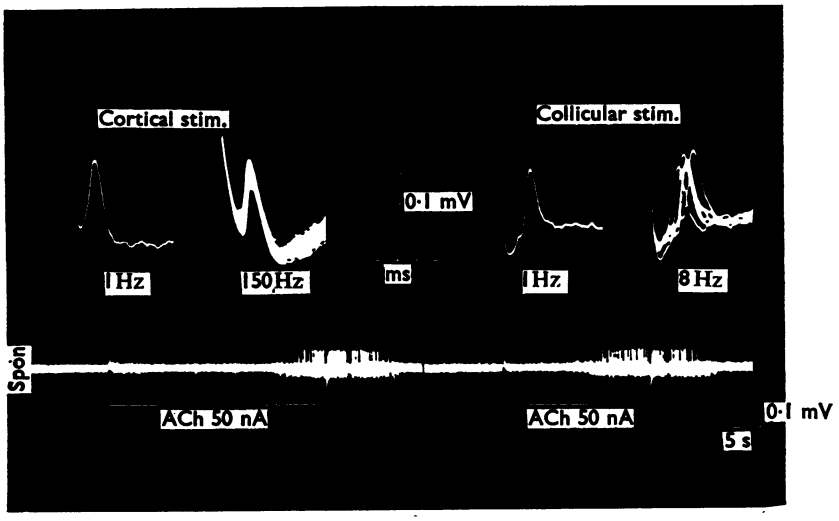

FIG. 5. Excitatory effect of ACh on a cell which was identified as a GCR neurone. The records on the top left show that the cell followed repetitive stimulation of the auditory cortex (150 Hz) wth a constant, short latency. Those on the right show that a comparable spike was evoked by stimulation of the inferior colliculus. The spike failed to follow collicular stimulation at frequencies higher than 10 Hz. The moving film record shows the action of ACh (50 nA) on the background activity of the neurone.

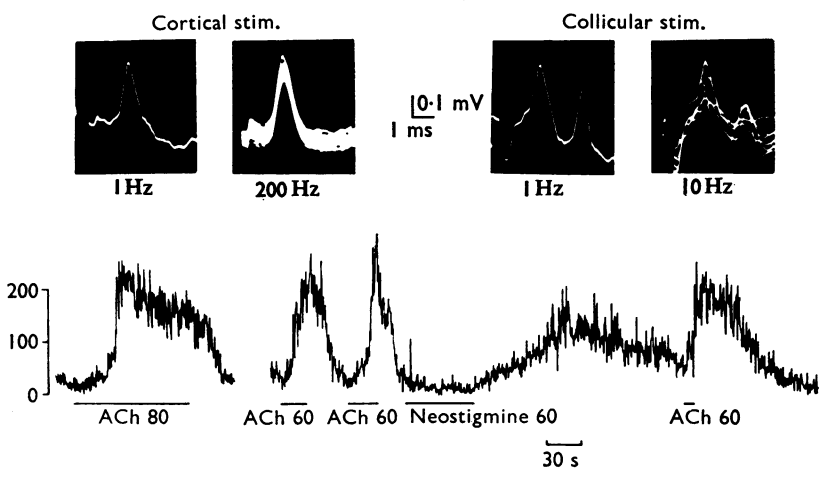

FIG. 6. Potentiating effect of neostigmine on the action of ACh on a GCR neurone. Stimulation of the auditory cortex (1 Hz) evoked a spike of constant short latency, which followed repetitive stimulation up to a frequency of 200 Hz. Stimulation of the inferior colliculus (1 Hz) evoked two consecutive spikes which began to fail when the colliculus was stimulated at a frequency of 10 Hz. The chart tracings below the oscilloscope records show, firstly, a progressive decrease in firing frequency of the GCR neurone to a prolonged application of ACh (80 nA), and, secondly, the potentiated excitant effect of ACh (60 nA) after neostigmine (60 nA) had been applied.

Effects of cholinesterase inhibitors

The three cholinesterase inhibitors, eserine, neostigmine and edrophonium (Tensilon), potentiated both the excitant and depressant effects of ACh on many neurones.

Figure 6 shows the potentiating effect of neostigmine on the excitant action of ACh applied to a GCR neurone. ACh (80 nA) fired the cell at a maximum frequency of 250 Hz after approximately 40 s, but this frequency declined when the application was continued (possibly due to desensitization). Applications of ACh with a current of 60 nA caused no desentitization (not shown on figure). Neostigmine (60 nA) also excited the cell, and when this excitation had nearly subsided, a briefer application of ACh (60 nA) caused a more rapid onset of, and longer lasting, excitation.

The neurone illustrated in Fig. 7A fired chiefly in bursts to applications of glutamate. ACh (40 nA for 30 s) reduced glutamate-firing. Neostigmine (50 nA) had little effect on the cell's firing rate, but after the application was terminated ACh had a more pronounced and longer lasting depressant effect. A third application of ACh, approximately 2 min after neostigmine had been applied, was equipotent to the first application, suggesting a rapid recovery of cholinesterase activity in the vicinity of the cell. The potentiating effects of the cholinesterase inhibitors on ACh-induced depressions of other cells usually lasted longer.

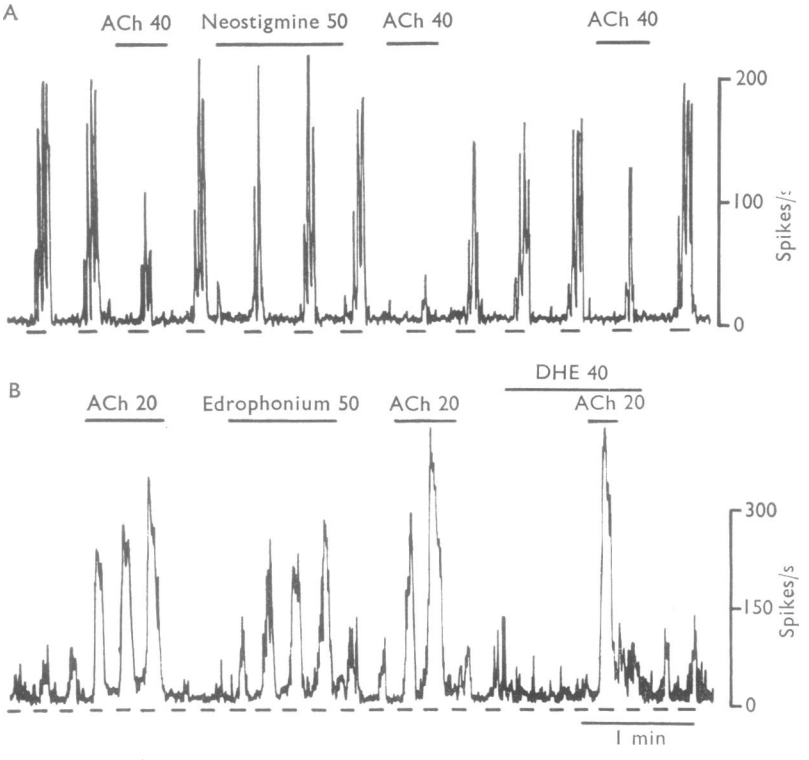

FIG. 7. Chart recordings showing the potentiating action of neostigmine (50 nA) on the depressant effect of ACh (40 nA) on one cell (A), and that of edrophonium (Tensilon) 50 nA on the excitant effect of ACh (20 nA) on another (B). L-Glutamate was applied with currents of 60 nA (A) and 50 nA (B). Dihydro-β-erythroidine (DHE, 40 nA) did not reduce the excitant effect of ACh in B.

On the cell represented by Fig. 7B ACh (20 nA) facilitated glutamate-firing during its application and depressed it after the application (see Fig. 4B). Edrophonium (50 nA) had only a facilitating effect on glutamate-firing. After edrophonium had been applied ACh had a more pronounced excitant action but no depressant action was apparent. Dihydro-β-erythroidine (40 nA for 70 s) did not antagonize the excitant action of ACh on this cell.

On some cells the cholinesterase inhibitors did not increase the maximum firing frequency evoked by ACh but clearly prolonged its excitant action. It was not possible to demonstrate a potentiation of the effects of ACh on all cells to which the cholinesterase inhibitors had been applied. Generally, cells which were excited or depressed by ACh were also excited or depressed, respectively, by the cholinesterase inhibitors. On some cells, however, the cholinesterase inhibitors had either no effect on the cell's firing frequency or had effects which were opposite to those of ACh. For example, on five cells edrophonium blocked the depressant effects of ACh and on two cells neostigmine reduced the excitant effects of ACh.

Effects of cholinergic antagonists

The muscarinic compounds atropine and hyoscine were effective antagonists of both excitant and depressant effects of ACh on many neurones. Iontophoretically administered atropine and hyoscine usually caused depression during their applications, although they had no effect on some cells and, more rarely, atropine facilitated glutamate-firing of a few cells which were also excited by ACh. Typically, the blocking action of atropine was long-lasting (up to half an hour or more) and hence recovery was difficult to demonstrate. It was possible to conclude, however, that atropine had blocked ACh when the effect of the latter remained depressed even though cell excitability had returned to control level. The antagonistic effects of atropine were considered specific for cholinomimetics because atropine did not antagonize the effects of iontophoretically applied monoamines and amino-acids. Examples of the blocking actions of the muscarinic compounds are given in the following paper (Tebēcis, 1970).

The nicotinic compounds, dihydro-β-erythroidine, hexamethonium and (+)-tubocurarine, were usually less effective than the muscarinic compounds as antagonists of ACh, although they clearly reduced or abolished excitant and depressant effects of ACh on some cells. The blocking actions of the nicotinic antagonists usually lasted for only a few min. Hexamethonium was generally more effective than dihydro-β-erythroidine, which was more effective than (+)-tubocurarine. Each compound also exhibited agonist (excitant and depressant) actions on neurones.

Two examples of the blocking actions of the nicotinic antagonists are illustrated in Fig. 8. On one cell (A) dihydro-β-erythroidine (100 nA for 3 min) blocked the facilitatory action of ACh (40 nA) on glutamate-firing. On another cell (B) hexamethonium (80 nA) reduced the depressant action of ACh (20 nA). Both cells recovered within 10 min after the currents ejecting the nicotinic antagonists had been terminated.

Effects of other cholinomimetics

The cholinomimetics, carbamylcholine, acetyl-β-methylcholine, nicotine, butyrylcholine, arecoline and pilocarpine, had excitant, depressant or no effects on MG

neurones. Carbamylcholine, acetyl-β-methylcholine and nicotine also had " dual " excitant-depressant effects on a small proportion of neurones, as has been observed for ACh (Fig. 4). Although the cholinomimetics usually mimicked the effects of ACh on the same neurones, there was sometimes no correlation between their actions.

Carbamylcholine was more potent than ACh (either as an excitant or depressant) on most of the neurones on which their effects were compared. On some neurones, however, it was clearly less potent or had no effect at all. On three cells carbamylcholine caused depression, whereas ACh caused excitation, and on five cells carbamylcholine blocked the effects of ACh. The blocking action of carbamylcholine on the excitant effects of ACh on two different cells is shown in Fig. 9.

Two applications of ACh (60 nA) to the cell illustrated in Fig. 9A caused comparable effects, indicating that the cell did not become desensitized to the substance. After an application of carbamylcholine (60 nA) the effect of ACh was blocked, but began to recover approximately 5 min later. Recovery was still incomplete 15 min after the end of the carbamylcholine application. A spike could be evoked antidromically before and after the application of carbamylcholine, indicating that the latter had not unduly depressed cell excitability. In Fig. 9B (recorded from another cell) are shown a similar series of events.

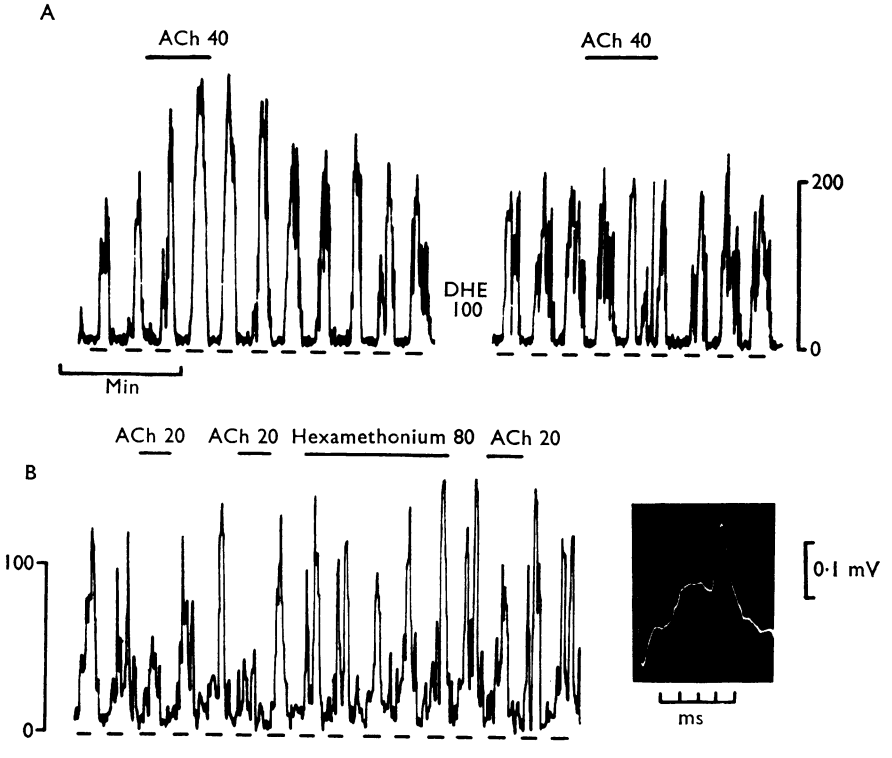

FIG. 8. Chart recordings showing the antagonistic action of dihydro-β-erythroidine (DHE, 100 nA for 3 min) on the facilitatory effect of ACh (40 nA) on glutamate (80 nA)-firing of one cell (A), and that of hexamethonium (80 nA) on the depressant effect of ACh (20 nA) on glutamate (70 nA)-firing of another (B). The latter could be activated by stimulating the inferior colliculus (oscilloscope record on right).

As it is difficult to block an antidromic spike by a depressant compound, the possibility remains that carbamylcholine " blocked " the action of ACh by depressing cell excitability even though it did not affect the antidromic spike. This explanation is unlikely, however, because of the long duration of the depression. Typical depressions by carbamylcholine never lasted longer than 3 min. The most plausible explanation is that carbamylcholine competitively inhibited ACh.

Acetyl-β-methylcholine also antagonized ACh on some cells. It had effects comparable with or different from those of ACh on various neurones. When their effects were comparable, acetyl-β-methylcholine was usually less potent than ACh, but often their potencies were similar.

Nicotine excited nine, depressed seventeen, had " dual " actions on three, and was without effect on eight of thirty-seven cells tested. Typically, its excitant effects were long-lasting and its depressant effects brief. When both ACh and nicotine caused excitation, the latter never increased the firing frequency as high as did ACh, but usually caused a longer lasting excitation. When their effects differed, nicotine generally caused depression, whereas ACh caused excitation.

Butyrylcholine always produced similar, though less marked, effects than those of ACh. Arecoline and pilocarpine usually had no effect, but sometimes caused a slight excitation or depression.

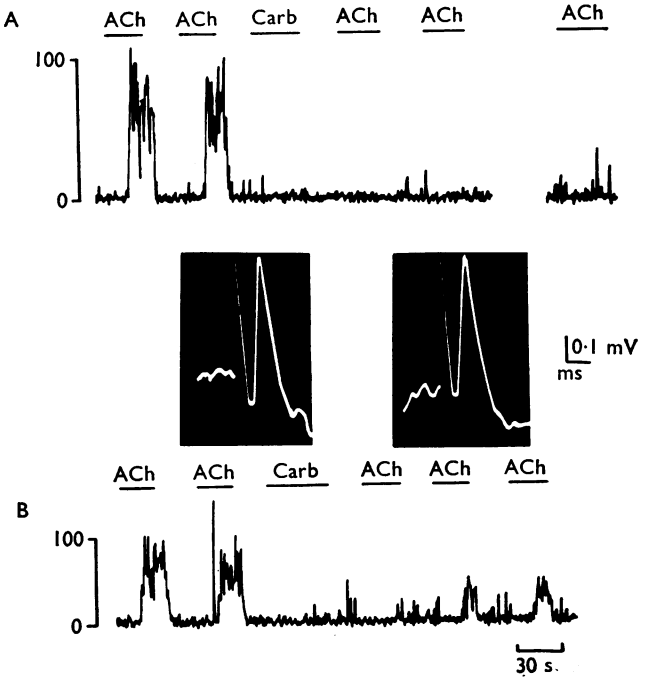

FIG. 9. Examples of the antagonistic action of carbamylcholine (Carb) on the excitatory effects of ACh on two different cells (A and B). Antidromic responses could be evoked from both neurones by cortical stimulation. A: Each application of ACh and carbamylcholine was with a current of 60 nA. The gap in the tracing represents a period of 4 min. Below are two records of the antidromic spike, recorded 1 min before (on left) and 2 min after (on right) the application of carbamylcholine. B: Each drug application was with a current of 80 nA. The antidromically evoked spikes for this cell are not shown.

Comparisons of the effects of the cholinomimetics were difficult to interpret because of the phenomenon of desensitization and the antagonistic properties of these substances. It was therefore necessary to apply them in random order on many neurones before generalizations could be made. When the various cholinomimetics each produced either excitation or depression, the relative order of potency (in descending order) generally was carbamylcholine, ACh, acetyl-β-methylcholine, nicotine and arecoline or pilocarpine. The difference in potency between ACh and

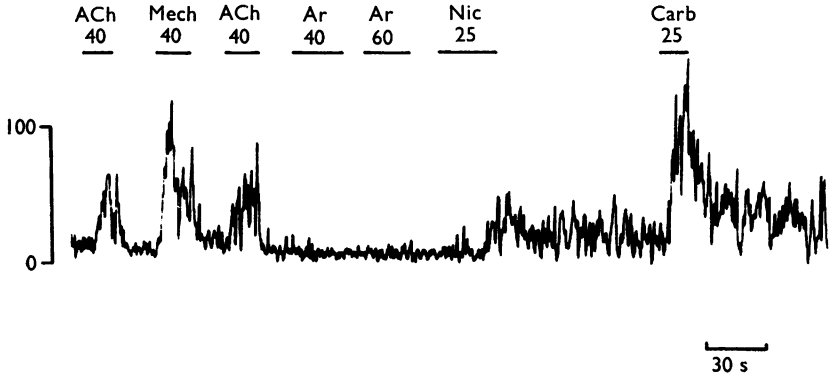

FIG. 10. Effects of ACh, acetyl-β-methylcholine (Mech), arecoline (Ar), nicotine (Nic) and carbamylcholine (Carb) on a GCR neurone. Higher currents than 25 nA could not be passed through the nicotine and carbamylcholine-containing barrels.

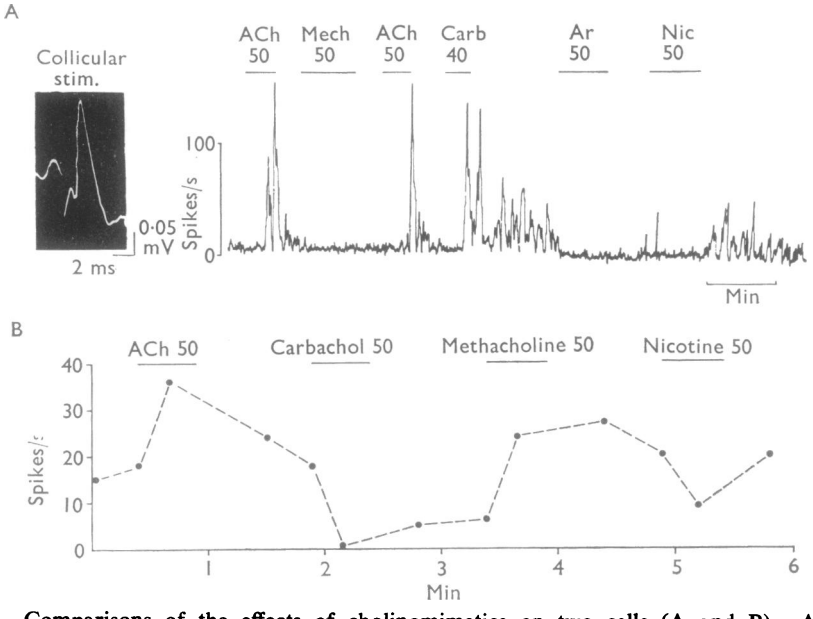

FIG. 11. Comparisons of the effects of cholinomimetics on two cells (A and B). A: The oscilloscope record shows the response of one cell to stimulation of the inferior colliculus (1 Hz). The chart record shows the effects of ACh, acetyl-β-methylcholine (Mech), carbamylcholine (Carb), arecoline (Ar) and nicotine (Nic), each administered with a current of 50 nA to this cell. B: Graph showing the effects of four cholinomimetics on another (spontaneously firing) cell. Each compound was applied with a current of 50 nA for 30 sec at intervals of 1 min. The firing frequency of the cell at the beginning, during and between the applications was plotted as shown.

acetyl-β-methylcholine was usually small, the latter often being more effective. Such an example is illustrated in Fig. 10, which shows a recording from a GCR neurone. The pooled results suggested that cholinoceptive receptors on most MG neurones are intermediate (muscarinic-nicotinic) in type.

Occasionally, however, distinct differences between the effects of the muscarinic and nicotinic compounds were observed. For example, the cell illustrated in Fig. 11A was excited by ACh, carbamylcholine and nicotine, but not by acetyl-β-methylcholine or arecoline, suggesting that the receptors for ACh excitation were purely nicotinic. Carbamylcholine and nicotine had longer duration effects than ACh, probably because neither compound is affected by cholinesterase. Acetyl-β-methylcholine and arecoline may have had depressant effects on this cell (which would suggest the presence of muscarinic inhibitory receptors), but this possibility was not investigated. On another MG neurone (Fig. 11B) ACh and acetyl-β-methylcholine increased the cell's firing frequency, but carbamylcholine and nicotine decreased it. This suggests that the receptors for ACh excitation were purely muscarinic and those for depression, purely nicotinic. Alternatively, it is possible that carbamylcholine and nicotine competitively blocked ACh receptors on this cell. Thus, if the cell's background firing was caused by ACh and acetyl-β-methylcholine diffusing from their barrels, blocking actions by carbamylcholine and nicotine would account for such a depression. However, the short durations of the depressant effects make this possibility unlikely.

Discussion

Effects of acetylcholine

The results of this investigation indicate that iontophoretically administered ACh has excitant or depressant effects on a large proportion of neurones in the feline MG nucleus. Characteristically, the excitant effects are weak and slow in onset and decline, although on some cells it is a powerful excitant with a rapid time course of action. The depressant effects of ACh are generally strong and rapid in onset and decline.

Of particular interest in this study are the large proportions of cells which are excited by both ACh and neural stimulation, for the finding that a compound excites neurones of a particular functional type raises the possibility that the compound is a transmitter acting on such neurones. More specifically, ACh excites most MG neurones which project to the auditory cortex. Similar results were obtained by Satinsky (1967) and Phillis *et al.* (1967b), who found that lateral geniculate neurones which could be activated antidromically by stimulating the visual cortex were also excited by ACh. Other examples of neurally or anatomically identified cells which are excited by ACh are Renshaw cells (Curtis & Eccles, 1958a, b), Betz cells (Krnjević & Phillis, 1963a, b ; Crawford & Curtis, 1966), hippocampal pyramidal cells (Salmoiraghi & Stefanis, 1965 ; Biscoe & Straughan, 1966), Purkinje cells (Crawford, Curtis, Voorhoeve & Wilson, 1966), cells of the paramedian reticular nucleus (Avanzino, Bradley & Wolstencroft, 1966), Deiter's neurones (Salmoiraghi & Stefanis, 1965 ; Yamamoto, 1967), thalamo-cortical relay cells (McCance, Phillis & Westerman, 1968) and ventral cochlear neurones (Comis & Whitfield, 1968). These examples support the contention that cells of a particular physiological type are pharmacologically homogeneous.

The " dual " excitant-depressant effects of ACh may be explained in more than one way. One possibility is that the effect (excitation or depression) observed during drug application was due to the direct action of ACh on the neurone being recorded, and the effect following the application was due to ACh-induced bombardment from neighbouring neurones which were too far from the electrode tip to be recorded satisfactorily. For example, ACh may have excited the neurone being recorded and then diffused away and excited other neurones which inhibited the recorded neurone synaptically (either directly or via a system of interneurones). Another possibility is that some MG neurones have both excitatory and inhibitory receptors for ACh. For example, Fig. 11 suggests that some neurones may have nicotinic and muscarinic receptors which mediate opposite effects of ACh. Furthermore, there is evidence for more than one type of receptor for ACh on central neurones of vertebrates (Curtis & Ryall, 1966a, b, c) and invertebrates (Wachtel & Kandel, 1967 ; Kehoe, 1969).

Effects of cholinesterase inhibitors and cholinergic antagonists

A potentiation of the effects of ACh after applications of cholinesterase inhibitors must be interpreted with caution, because these substances often have agonist actions. However, they probably increase the effectiveness of ACh, at least in part, by inactivating acetylcholinesterase in the vicinity of the recorded cell, for the excitant effects of ACh are facilitated to a greater extent than those of glutamate, and the depressant effects of ACh are potentiated when glutamate firing is unaffected. Moreover, on some neurones the cholinesterase inhibitors potentiate the effects of ACh even though they have slight opposite effects on glutamate firing. The antagonistic effects of edrophonium and neostigmine (both quaternary ammonium compounds) on the effects of ACh may be explained in terms of competitive inhibition for the cholinoceptive receptor sites. Curtis & Ryall (1966b) sometimes observed that edrophonium depresses the sensitivity of Renshaw cells to ACh after an initial slight excitant action.

The cholinergic antagonists reduce or block both excitant and depressant effects of ACh on many MG neurones. Recovery from the blocking action of atropine is often not seen and so it is difficult to estimate the proportion of cells on which the alkaloid specifically blocked ACh. The actions of cholinergic antagonists on synaptic responses are described in the following paper (Tebēcis, 1970). Agonist actions of the nicotinic blocking agents have been reported by other investigators and were also observed in the present study. In addition, it was found that iontophoretically applied atropine sometimes excites neurones which can be excited by ACh.

Possible types of cholinoceptive receptors

The diverse types of effects of the cholinomimetics and cholinergic antagonists indicate that cholinoceptive receptors on MG neurones are heterogeneous. Although some evidence was obtained for the presence of purely nicotinic or muscarinic receptors (Fig. 11) most of the observations suggest the presence of receptors intermediate between the two types. The latter varied in the degree of nicotinic or muscarinic properties. For example, atropine and hyoscine block the effects of ACh more readily and more often than do dihydro-β-erythroidine, hexamethonium and (+)-tubocurarine, suggesting that the receptors on MG neurones are pre-

dominantly muscarinic. However, carbamylcholine is more potent than acetyl-β-methylcholine on most cells, suggesting that nicotinic receptors predominate. The two observations taken together suggest that the receptors are intermediate between the two types, as have been described for brain stem (Salmoiraghi & Steiner, 1963; Bradley & Wolstencroft, 1965), thalamic (Andersen & Curtis, 1964a, b; McCance, Phillis, Tebēcis & Westerman, 1968) and lateral geniculate (Phillis *et al.*, 1967b) neurones.

GCR cells resemble Betz cells in that the excitant action of ACh has a relatively slow time course (Krnjević & Phillis, 1963a, b). ACh sometimes causes an initial depression before exciting GCR cells, as occurs with Betz cells (Crawford & Curtis, 1966). The effects of ACh on both types of cells are readily blocked by atropine but not by dihydro-β-erythroidine (Krnjević & Phillis, 1963a, b; Crawford & Curtis, 1966). These properties are characteristic of muscarinic synapses. However, GCR neurones are excited by carbamylcholine and nicotine, two nicotinic compounds. Moreover, the time course of action of ACh on GCR neurones is briefer than that on Betz cells, and dihydro-β-erythroidine has a slight excitant action on GCR neurones. All these observations suggest that the cholinoceptive receptors on GCR cells are of an intermediate muscarinic-nicotinic type.

It is possible that the "intermediate" type effects observed may have been due to the presence of both muscarinic and nicotinic receptors, both of which mediate excitation (or inhibition) to the same neurone. For example, Renshaw cells have nicotinic and muscarinic receptors, both of which mediate excitation (Curtis & Ryall, 1966a, b, c). Probably the most satisfactory way of elucidating the types of cholinoceptive receptors on neurones is to determine the effects of a series of cholinomimetics on physiologically identified neurones, before and after applications of muscarinic and nicotinic antagonists. Such attempts on MG neurones were abandoned because of the difficulty in obtaining stable recordings when 7 or 9-barrelled micropipettes were filled solely with cholinomimetics.

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