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Studies on cholinergic transmission in the medial geniculate nucleus

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Summary

1. Studies were made on the effects of iontophoretically and intravenously administered cholinergic antagonists on the synaptic responses of medial geniculate (MG) neurones evoked by stimulation of the auditory cortex, inferior colliculus and mesencephalic reticular formation.

2. Atropine specifically blocked a proportion of the excitatory responses evoked by stimulating the auditory cortex, inferior colliculus and reticular formation, although it was without effect on some of them.

3. Neostigmine and eserine facilitated some excitatory synaptic responses evoked by inferior collicular stimulation.

4. It is suggested that the feline MG nucleus receives excitatory cholinergic, as well as non-cholinergic, pathways from the auditory cortex, inferior colliculus and lower brain stem. The cholinergic pathways from the auditory cortex may be either corticofugal fibres or recurrent axon collaterals of afferent projections from the MG nucleus to the cortex. Those from the lower brain stem are possibly the cholinesterase-containing fibres described by Shute & Lewis (1967).

Introduction

In the previous paper (Tebēcis, 1970) it was shown that acetylcholine (ACh) can have excitant or depressant effects on medial geniculate (MG) neurones and that these are often potentiated by cholinesterase inhibitors and antagonized by cholinergic blocking agents. Moreover, ACh has excitant actions on most of the neurones which can be activated by stimulation of the auditory cortex and/or inferior colliculus. The presence of cholinoceptive neurones in the MG nucleus suggested that cholinergic fibres may terminate in this structure.

In this investigation, the possible presence of cholinergic pathways from the auditory cortex and inferior colliculus to the MG nucleus was studied by determining the effects of cholinergic antagonists on synaptic responses evoked by cortical and collicular stimulation. Similar studies were made on the responses evoked by stimulating the mesencephalic reticular formation, as cholinesterase-containing pathways from the lower brain stem to the MG nucleus have been described (Shute & Lewis, 1967). Some evidence for cholinergic pathways from the brain stem to the lateral geniculate nucleus (Phillis, Tebēcis & York, 1967 ; Deffenu, Bertaccini &

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Pepeu, 1967) and thalamus (McCance, Phillis & Westerman, 1968 ; Phillis, Tebēcis & York, 1968) increases the likelihood of cholinergic transmission in the MG nucleus, for all three structures are phylogenetically related and receive cholinesterase-containing fibres from a common source in the brain stem (Shute & Lewis, 1967).

Methods

The methods are as described in the preceding paper.

Results

Stimulation of the auditory cortex

Figure 1 shows the effects of intravenously administered atropine on the repetitive responses evoked from an MG neurone when the auditory cortex (area AII) and inferior colliculus were stimulated at a rate of 1 Hz. Cortical stimulation evoked a synaptic response consisting of four to eight spikes which fired at 470 Hz. Collicular stimulation evoked a similar response, although the latency was longer and more variable. Atropine sulphate (2 mg/kg) was then injected intravenously. After 19 s the cortically evoked response had decreased to four spikes, after 50 s it consisted of only the first spike, and after 1 min the response was completely blocked, as shown in Fig. 1. The response evoked by collicular stimulation, however, was unaltered. These responses were observed for a further 35 min, during which time the cortically evoked response remained blocked and the collicularly evoked response remained unaltered. The stability of the collicularly evoked response indicates that atropine did not abolish the cortically evoked response non-specifically. These results suggest the presence of a cholinergic pathway between the auditory cortex and the MG nucleus.

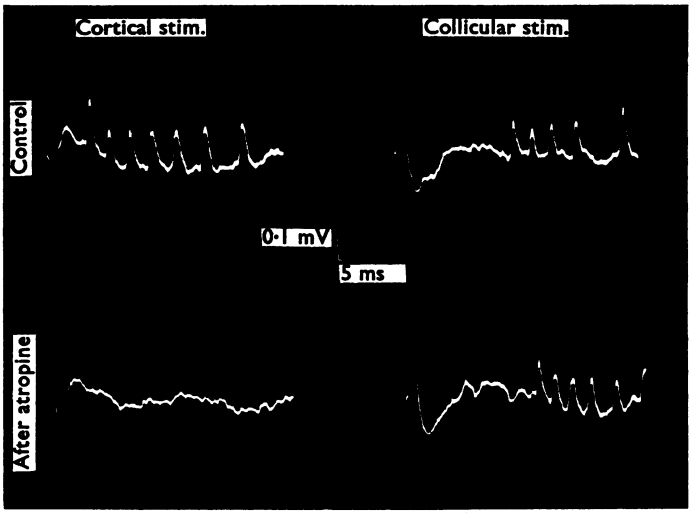

FIG. 1. Effects of intravenously administered atropine (2 mg/kg) on the repetitive responses evoked by stimulation of the auditory cortex and inferior colliculus (1 Hz). The top records are of control responses. The bottom left-hand record was obtained 1 min after atropine had been injected. The bottom right-hand record was obtained 75 s after atropine. Atropine abolished the effects of cortical, but not of collicular, stimulation.

Evidence for such a pathway was obtained from several other neurones. Fig. 2A shows three consecutive synaptically evoked responses of a cell during stimulation of the auditory cortex (area Ep) once every 2 s. One minute after an intravenous dose of atropine (0·6 mg/kg) the response was completely blocked and remained blocked for as long as it was observed (25 min). In another cell (Fig. 2B) cortical stimulation at 1 Hz evoked a single spike superimposed on a field potential. This spike was blocked by an iontophoretic application of atropine (60 nA for 2 min) and remained blocked for 15 min after the current ejecting atropine had been terminated. Although recovery from the effects of atropine could not be demonstrated, it was thought that the alkaloid did not depress the cells (A and B) non-specifically because both could be excited by L-glutamate after atropine had been applied.

Extensive testing revealed that not all cortically evoked synaptic responses could be blocked by iontophoretically applied atropine, even when the latter was ejected with currents of up to 200 nA for several minutes. Most of these responses were depressed during such applications, but usually recovered within 3 min of the termination of the applications, suggesting that depression had been due to a reduction in cell excitability. The lack of a blocking action of synaptic responses by iontophoretically administered atropine, however, need not necessarily indicate the presence of non-cholinergic synapses, as the drug may not reach a sufficient number of synapses on the cell soma and dendrites when applied by this method. Studies involving intravenous injections, however, confirmed the finding that some cortically evoked synaptic responses are resistant to atropine.

The first response illustrated in Fig. 3A was evoked by stimulation of the auditory cortex and consisted of an antidromic, followed by an orthodromic, spike. Two to three synaptic spikes were evoked when the inferior colliculus was stimulated (Fig. 3A, second record). Neither response was altered after intravenous injections of

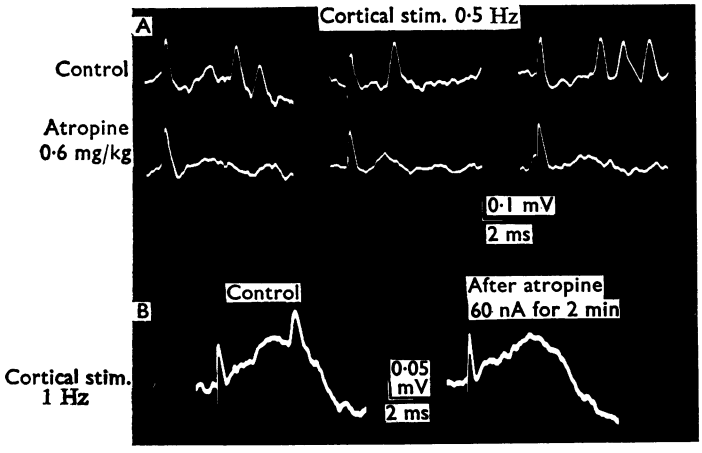

FIG. 2. Effects of atropine on the cortically evoked responses of two different cells (A and B). A: The top row shows three consecutive control responses evoked by 0·5 Hz cortical stimulation. The bottom row shows three consecutive responses recorded 1 min after atropine (0·6 mg/kg) had been injected intravenously. B: The first record is a control response of another cell (of another cat) evoked by 1 Hz cortical stimulation. The second record was obtained 3 min after an iontophoretic application of atropine (60 nA for 2 min). Atropine abolished the cell spike but not the field.

atropine (0·5 mg/kg (B) or 2 mg/kg (C)), indicating that they were mediated non-cholinergically. These results also indicate that not all fibres from the auditory cortex or inferior colliculus to geniculo-cortical relay (GCR) neurones are cholinergic, even though all such neurones appear to be excited by ACh (Tebēcis, 1970).

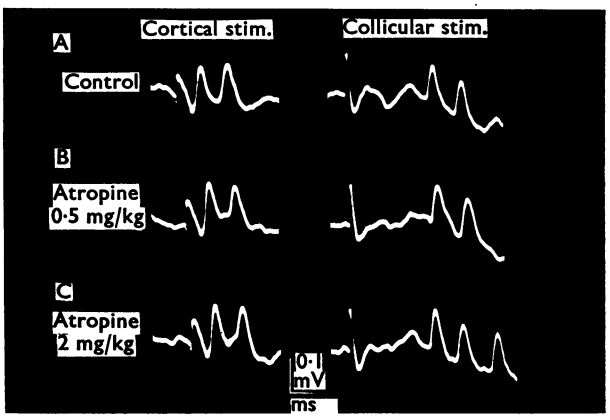

FIG. 3. The resistance of responses of a GCR neurone to atropine. A: Control responses of the neurone to stimulation (0·5 Hz) of the auditory cortex (first record) and inferior colliculus (second record). The cortically evoked response consisted of an antidromic, followed by a single synaptic, spike. The collicularly evoked response consisted of 2–3 synaptic spikes of variable latency. B: Responses 2 min after atropine (0·5 mg/kg) had been administered intravenously. C: Responses 3 min after an additional dose of atropine (1·5 mg/kg) had been injected.

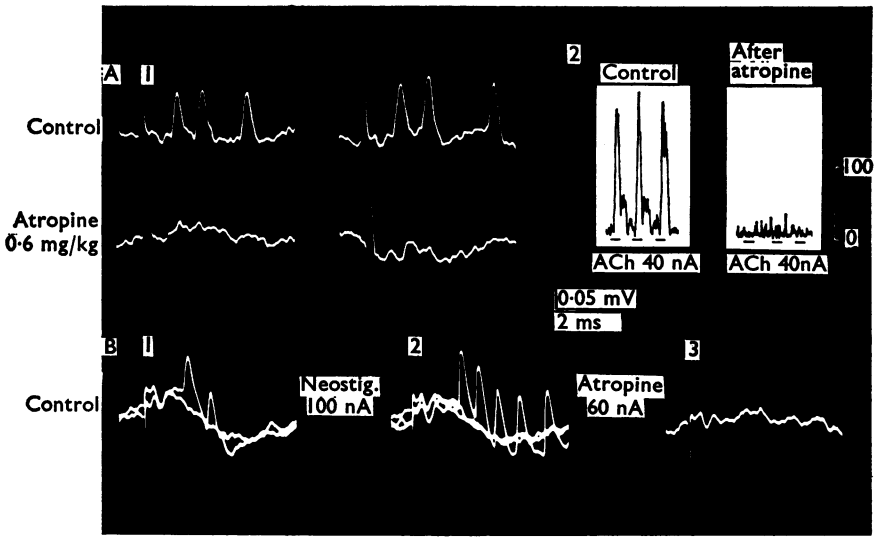

FIG. 4. Evidence for cholinergic pathways from the inferior colliculus to the MG nucleus. A: 1 (top records), two consecutive control responses of a cell evoked by 1 Hz collicular stimulation. 2 (first record), chart tracing showing the rapid time course of the excitatory effects of three 6 s applications of ACh (40 nA) to the cell. The bottom records in 1 and the second record in 2 were obtainable 60 and 100 s, respectively, after an intravenous dose of atropine (0·6 mg/kg). B: 1, control response of another neurone evoked by 1 Hz collicular stimulation. The stimulus strength was on threshold. Two superimposed sweeps are shown. 2, three superimposed sweeps of the response recorded 15 s after an application of neostigmine (100 nA for 60 s). 3, 3 min after an application of atropine (60 nA for 2 min). The response could not be evoked by increasing the stimulus strength.

Stimulation of the inferior colliculus

Although Figs. 1 and 3 indicate that not all pathways from the inferior colliculus to the MG nucleus are cholinergic, some evidence for such pathways was obtained in several instances (Figs. 4–7). It is noteworthy, also, that the brachium of the inferior colliculus stains more densely for acetylcholinesterase than the adjacent areas of the MG nucleus (unpublished observations).

Figure 4A shows neuronal responses which were evoked synaptically with two to three spikes by inferior collicular stimulation (A, 1). Chart recordings showed that ACh (40 nA) had potent excitatory effects with a time course comparable with that of glutamate (A, 2). One minute after atropine (0·6 mg/kg) had been administered, the synaptic response and the excitant effect of ACh were blocked. No recovery was apparent during the next 15 min, even though the cell was firing spontaneously (A, 2).

The response of the cell illustrated in Fig. 4B was evoked with one to three spikes when the inferior colliculus was stimulated (B, 1). After an application of neostigmine (100 nA for 1 min) the number of spikes per response increased (B, 2) and after atropine (60 nA for 2 min) the response was blocked (B, 3). It was not possible to determine whether atropine had a specific blocking action on the synaptic response because no glutamate was available for testing cell excitability and recovery was not seen. The response remained blocked for at least 15 min after atropine had been applied.

Figure 5 is of a cell for which it was possible to determine whether iontophoretically administered atropine had a specific blocking action on the synaptic response. The response of this GCR unit was evoked with an antidromic spike from the cortex, and repetitively from the inferior colliculus. After atropine (130 nA) had been

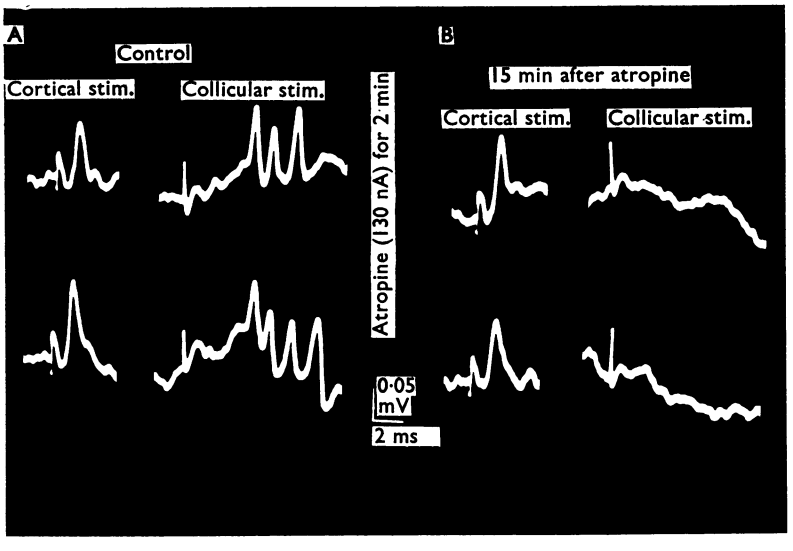

FIG. 5. Evidence for cholinergic fibres from the inferior colliculus to a GCR neurone. The top and bottom records in A are two consecutive control responses of the neurone to cortical (left) and collicular (right) stimulation at 0·5 Hz. The cortically evoked response was identified as an antidromic spike. Collicular stimulation evoked a repetitive response consisting of two to four spikes. The records in B were obtained 15 min after an application of atropine (130 nA for 2 min). Atropine abolished only the collicularly evoked response.

applied for 2 min, the collicular response was blocked but the antidromic spike remained unaltered, indicating that the cell had not been inactivated during the application of atropine.

Figure 6 shows the facilitatory effects of eserine on a response evoked by stimulating the inferior colliculus. Collicular stimulation (1 Hz) evoked a field potential on which a cell spike occasionally appeared (A). After an intravenous injection of eserine sulphate (1 mg/kg) the field potential increased in amplitude (B). The cell

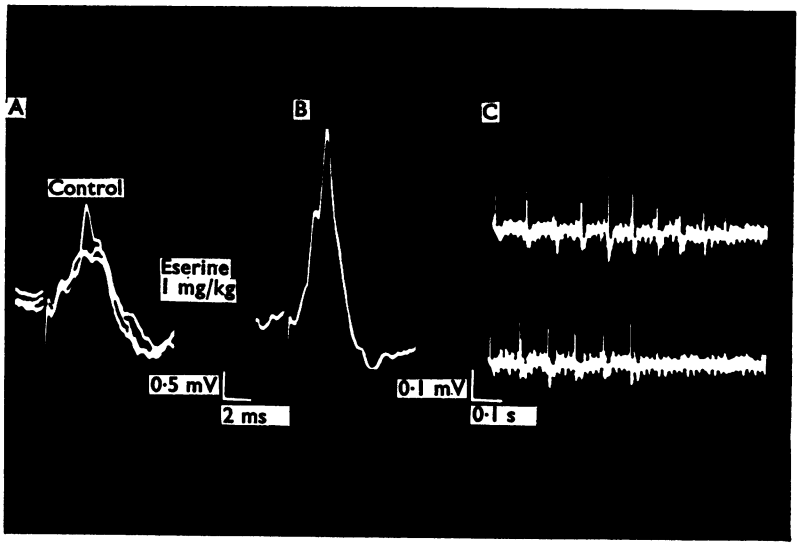

FIG. 6. Effects of intravenous eserine on a collicularly evoked response. A: Three superimposed control responses of the field potential evoked by 1 Hz stimulation of the inferior colliculus. A cell spike was evoked on one of these. B: Typical response recorded 5 min after eserine (1 mg/kg) had been administered. C: Two consecutive responses evoked by stimulation identical to that in A and B. These were recorded 7 min after eserine had been applied, using a much slower sweep speed.

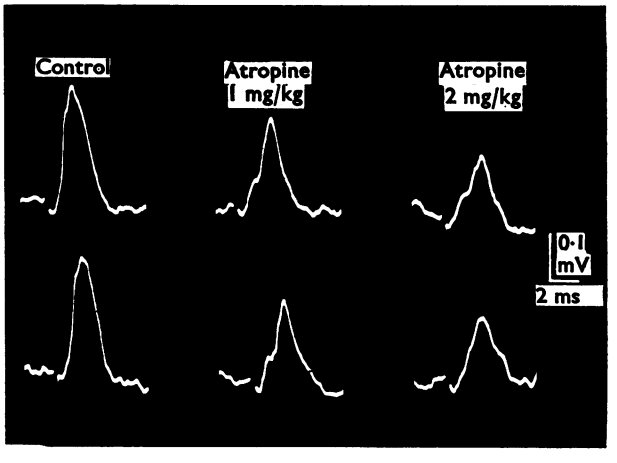

FIG. 7. Effects of atropine on a field potential evoked by stimulation of the inferior colliculus at 1 Hz. The top and bottom records are of consecutive responses. The first records are control responses. The second records were obtained 2 min after an injection of atropine (1 mg/kg). The third records were obtained 2 min after an additional dose of atropine (1 mg/kg) had been administered.

spike, which had also increased in amplitude, was now evoked by every shock. As well as the short-latency response (B), a long-latency (up to 100 ms), long-duration (500–700 ms) response was apparent after eserine had been administered (C). The latter response was noticed only because it could be heard clearly on the audio amplifier. The two consecutive sweeps in C (1 Hz) reveal that the neurone discharged in bursts of one to three spikes separated by intervals of approximately 90 ms. Eserine often has excitant effects on MG neurones (Tebēcis, 1970), so it was not possible to conclude whether the effects illustrated in Fig. 6 were due to direct excitant effects of eserine or to its cholinesterase-inhibiting action.

The results illustrated in Fig. 7, which shows the effect of atropine on a field potential evoked by stimulating the inferior colliculus, confirmed the observation that only a proportion of fibres from the colliculus to the MG nucleus terminate at cholinergic synapses. Atropine (1 mg/kg) reduced the amplitude of the field potential. At a dose of 2 mg/kg it reduced the amplitude even more, but had no further effect when the concentration was increased to 5 mg/kg.

Stimulation of the reticular formation

Repetitive stimulation (10–30/s) of the mesencephalic reticular formation had excitant, depressant or no effects on spontaneous or glutamate-induced firing of MG neurones. Three cells which responded with one to three spikes of 5–16 ms

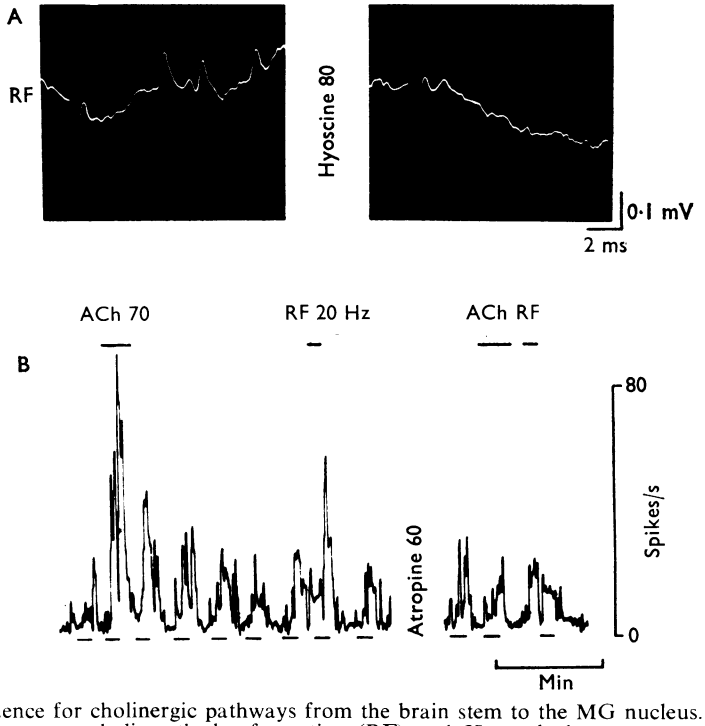

FIG. 8. Evidence for cholinergic pathways from the brain stem to the MG nucleus. A: Stimulation of the mesencephalic reticular formation (RF) at 1 Hz evoked a response consisting of two to three spikes (first record). The record on the right was obtained 5 min after hyoscine (80 nA) had been applied for 2 min. "A.C. hum" is superimposed on these records. B: Chart recording of another cell, showing the effects of ACh (70 nA) and stimulation of the reticular formation (20 Hz) before and 4 min after an application of atropine (60 nA for 70 s). L-Glutamate was applied with a current of 50 nA.

latency to 1 Hz stimulation of the reticular formation were also recorded. Some evidence was obtained for the presence of excitatory cholinergic pathways from the brain stem to the MG nucleus.

Figure 8A shows a short-latency repetitive response evoked by stimulation of the mesencephalic reticular formation (1 Hz). The record on the right was made 5 min after an application of hyoscine (80 nA for 2 min), showing a block of the synaptic response. On another cell (B) the excitant effects of ACh (70 nA) and repetitive stimulation of the reticular formation (20 Hz) on glutamate (50 nA)-firing were blocked by atropine (60 nA for 70 s).

Several cells on which the effects of ACh (excitant or depressant) were opposite to those of reticular formation stimulation were recorded, indicating that excitatory and inhibitory non-cholinergic fibres also project from the brain stem to the MG nucleus. Some of these may be monoaminergic pathways (Tebēcis, 1967).

Dihydro-β-erythroidine, hexamethonium and (+)-tubocurarine did not block the synaptic responses evoked by stimulation of the auditory cortex, inferior colliculus and reticular formation. The nicotinic antagonists were not tested on synaptic responses as extensively as was atropine, however, because they are generally less effective in blocking the effects of ACh (Tebēcis, 1970).

Discussion

The hypothesis that ACh is a transmitter in the mammalian MG nucleus was suggested by the findings that the synthesizing and inactivating enzymes of ACh are present in the MG nucleus of various mammals (Burgen & Chipman, 1952; Koelle, 1954; Hebb & Silver, 1956) and that cholinesterase-containing fibres project from the cuneiform nucleus to the MG nucleus (Shute & Lewis, 1967). This hypothesis was supported by evidence for the presence of cholinoceptive neurones in the MG nucleus (Tebēcis, 1970). In particular, ACh excites most MG neurones, the responses of which can be evoked by stimulating the auditory cortex and inferior colliculus (Tebēcis, 1970).

In the present investigation it was observed that atropine blocked a proportion of the synaptic responses evoked by stimulation of the auditory cortex, inferior colliculus and reticular formation. These results must be interpreted with some caution, for it was rarely possible to demonstrate recovery from the blocking actions of atropine. Block by atropine was considered specific for cholinergic synapses when it could be shown that the compound depressed synaptic responses without unduly altering the sensitivity of the neurone to glutamate or other types of neural stimulation. If this assumption is correct, these results, as well as the observations that neostigmine and eserine sometimes increase the amplitude and duration of responses evoked by collicular stimulation, suggest that the feline MG nucleus receives cholinergic fibres from the auditory cortex, inferior colliculus and reticular formation. Because atropine blocks only a proportion of the synaptic responses, the remainder must be mediated by either non-cholinergic or nicotinic (cholinergic) synapses. The latter possibility was not investigated thoroughly.

The cholinergic pathways from the auditory cortex may be either corticofugal fibres, which have been described by Otani & Hiura (1962) and Watanabe, Yanagisawa, Kanzaki & Katsuki (1966), or recurrent axon collaterals of afferent projections from the MG nucleus to the cortex, as described by Mettler (1932) and

Papez (1936). Aitkin & Dunlop (1969) have recently presented evidence for recurrent inhibition in the MG nucleus. If recurrent axon collaterals of afferent fibres to the auditory cortex release ACh synaptically on to inhibitory interneurones, the situation is analogous to the spinal cord, in which motor axon collaterals to Renshaw cells are cholinergic (Eccles, Fatt & Koketsu, 1954 ; Eccles, Eccles & Fatt, 1956 ; Curtis & Eccles, 1958a, b). However, some of the cortically evoked responses which were blocked by atropine did not resemble the typical repetitive discharges of inhibitory interneurones, raising the possibility that it is the corticofugal fibres which are cholinergic. The present evidence does not distinguish between these two possibilities.

Figure 1 is an example of cholinergic and non-cholinergic excitation impinging on the same neurone, as has been described for the spinal cord and thalamus. Although Renshaw cells are excited by synaptically released ACh from motor axon collaterals, their activation by dorsal root volleys does not involve cholinergic mechanisms (Curtis, Phillis & Watkins, 1961 ; Curtis & Ryall, 1966). In the thalamus, synaptic responses may be evoked by both limb nerve and reticular formation stimulation, only the latter type being blocked by cholinergic antagonists (McCance *et al.*, 1968).

The cholinergic pathways from the brain stem to the MG nucleus may be the cholinesterase-containing pathways described by Shute & Lewis (1967), as the stimulating electrode used to evoke the atropine-sensitive responses was positioned to interrupt the dorsal tegmental pathway. This is comparable with the evidence obtained for cholinergic pathways from the brain stem to the lateral geniculate nucleus (Phillis *et al.*, 1967) and thalamus (McCance *et al.*, 1968).

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