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THE PHARMACOLOGY OF ACETYLCHOLINE-EXCITATION OF THALAMIC NEURONES

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An excitant action of acetylcholine on thalamic neurones and its relationship to synaptic transmission was described in the preceding paper (McCance, Phillis & Westerman, 1968). Evidence in support of the suggestion that acetylcholine has a transmitter function was obtained by comparing the pharmacology of synaptic excitation with that of iontophoretically applied acetylcholine. The results of a simultaneously conducted investigation into the actions of various cholinomimetic agents, and potentiators and antagonists of acetylcholine on thalamic neurones are presented in this report.

METHODS

Most of the methods used have been described in detail in the preceding paper (McCance *et al.*, 1968).

Diffusion of excessive amounts of cholinomimetic agents from the nine-barrelled micropipettes used in this investigation was controlled by using quarter-saturated aqueous solutions and "braking" currents of 5–10 nA. The latency before an excitant action of the various cholinomimetic compounds became apparent was frequently increased after the uninterrupted passage of a "braking" current for several minutes. Provision was made for this effect, which was attributed to removal of drug from the immediate vicinity of the electrode tip, when relative potencies were to be compared. Before testing different drugs in an electrode on a neurone, brief pulses of drug-releasing current were passed through each barrel; drugs were usually applied at least twice on to each neurone. Because of the possible variations in the properties of different micropipettes (Krnjević, Mitchell & Szerb, 1963), all substances were tested by release from at least two pipettes and observations were made on neurones in several animals.

RESULTS

Excitation by cholinomimetics

The distribution and identification of cholinoceptive neurones in the thalamus has been described in the preceding paper (McCance *et al.*, 1968). These were most frequently encountered in the ventro-basal thalamic complex (VBC), and so most of the pharmacological investigations were made in this area. The protracted period during which stable recordings could be obtained from large thalamocortical relay neurones also facilitated comparisons of the relative potencies of several cholinomimetic agents on the same neurone.

An example of a thalamocortical neurone that was extremely sensitive to acetylcholine is presented in Fig. 1. The unit responded antidromically with a short latency spike (0.8 msec) to stimulation of the precruciate cortex and would follow cortical stimulation at 200/sec, thus satisfying the criteria discussed in the preceding paper (McCance *et al.*, 1968). Acetylcholine had a powerful excitatory action on this neurone. After termination of the "braking" current through the barrel containing acetylcholine, diffusion of acetylcholine was sufficient to fire the neurone at frequencies in excess of 200/sec. When several cholinomimetics were present in an electrode, diffusion of above-threshold amounts of excitant was difficult to control and led to the adoption of the precautions mentioned.

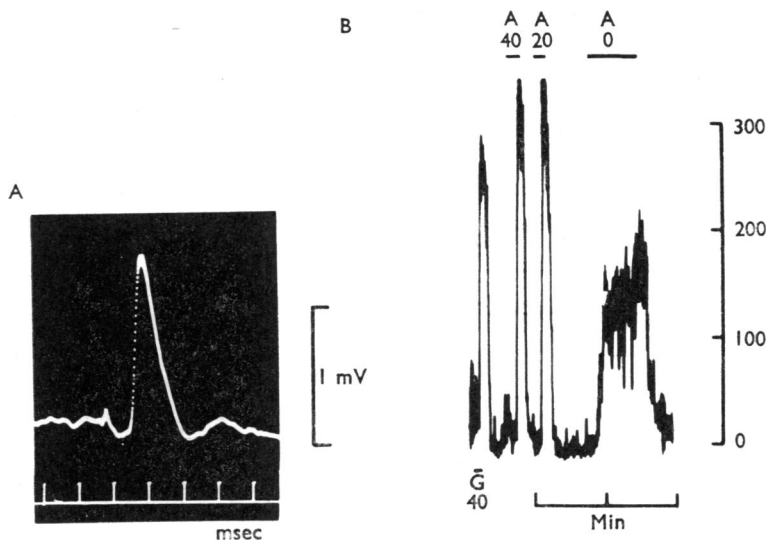

Fig. 1. A thalamocortical neurone extremely sensitive to acetylcholine. A: Antidromically evoked response to stimulation of the precruciate cortex. B: Horizontal bars above and below trace in this and subsequent figures represent duration of drug applications. Ordinates represent firing frequencies of the cells in spikes/sec. L-glutamate (G, 40 nA) did not fire the cell as intensely as acetylcholine (A, 40 and 20 nA). (Maximum recordable frequency was 340 spikes/sec.) Diffusion of acetylcholine when the "braking" current was switched off (A, on A) was sufficient to fire the cell.

The actions of acetylcholine and six other cholinomimetic agents on a VBC neurone are illustrated in Fig. 2. All compounds were applied by a current of the same magnitude (60 nA) and it is evident that acetylcholine, acetyl-β-methylcholine and carbachol were considerably more effective than the other compounds tested.

A comparison of the activities of the choline esters and other cholinomimetics tested is presented in Table 1. The activities are expressed relative to that of acetylcholine, which was arbitrarily assessed as having an activity of $^{+++}$. Activity was assessed by comparing the magnitudes of currents of uniform duration that had to be passed to induce a similar frequency of firing. Substances such as arecoline and nicotine had a longer duration of action than acetylcholine and the onset of excitation was frequently slower.

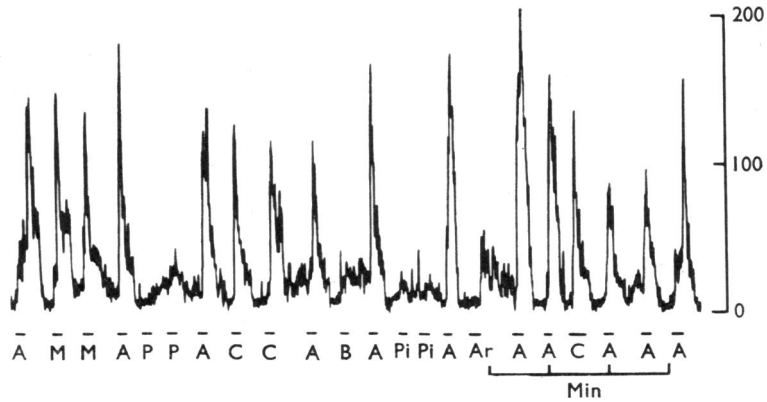

Fig. 2. Excitant effects of a number of cholinomimetics applied to a thalamic neurone recorded at a depth of 4,810 μ below the dorsal surface of thalamus. All applications are at currents of 60 nA. Acetylcholine (A), acetyl-β-methyl choline (M) and carbachol (C) were approximately equieffective and were much more active than propionylcholine (P), butrylcholine (B), pilocarpine (Pi) and arecoline (Ar) on this unit. After the second, longer, application of carbachol, excitation induced by acetylcholine was significantly reduced.

After an application of carbachol, the subsequent excitant actions of both carbachol itself and other cholinomimetics were frequently depressed for periods of up to 2 min. An example of this effect is evident in Fig. 2. After carbachol had been applied, the excitant effect of the subsequent two applications of acetylcholine was reduced. Recovery of the acetylcholine response to control magnitude had occurred 90 sec after the application of carbachol. A comparable reduction in excitability was also observed to follow the application of muscarone (the quaternary methyl ammonium salt of 2-methyl-3-keto-5-(aminomethyl)tetrahydrofuran).

Investigations are continuing into this action of carbachol, which may be related to the initial depressant action of acetylcholine on some thalamic neurones that are ultimately excited by this compound. The phenomenon may also provide an explanation for the observation that acetylcholine had, on some neurones, an initial powerful excitant action that was not observed during subsequent applications (McCance *et al.*, 1968).

TABLE 1

RELATIVE ACTIVITIES OF CHOLINOMIMETIC SUBSTANCES

Potencies expressed relative to that of acetylcholine (+++).

Carbachol (carbamyl–choline chloride)	++++
Acetylcholine chloride	+++
Acetyl-β-methylcholine chloride	+++
Arecoline hydrobromide	++
Nicotine hydrogen tartrate	++
d,l-Muscarine iodide	++
Succinylcholine chloride	++
Propionylcholine chloride	+
Butyrylcholine iodide	+
Pilocarpine hydrochloride	+
d,l-Muscarone iodide	0

Cholinesterase inhibitors

During the initial experiments on the sensitivity of thalamic neurones to acetylcholine, cholinesterase inhibitors were routinely carried in the exploratory micropipettes to permit a readier identification of potentially responsive cells. Both potentiation of acetylcholine-excitation and direct excitant actions were observed when these compounds were tested on neurones in the VBC.

The tertiary compound, physostigmine (eserine) was used as a cholinesterase inhibitor in most experiments because it has less effect on acetylcholine-receptors than the quaternary inhibitors such as neostigmine and edrophonium (Riker & Wescoe, 1946; Werner & Kuperman, 1963).

An example of the potentiating action of eserine on acetylcholine excitation is shown in Fig. 3A. This VBC cell was excited by acetylcholine (60 nA) but failed to respond to acetylcholine (30 nA). After application of eserine (40 nA) for 40 sec, acetylcholine (30 nA) had quite a marked effect, although the response to L-glutamate was unaltered. During the succeeding 3 min, acetylcholine was tested twice and had a progressively smaller effect.

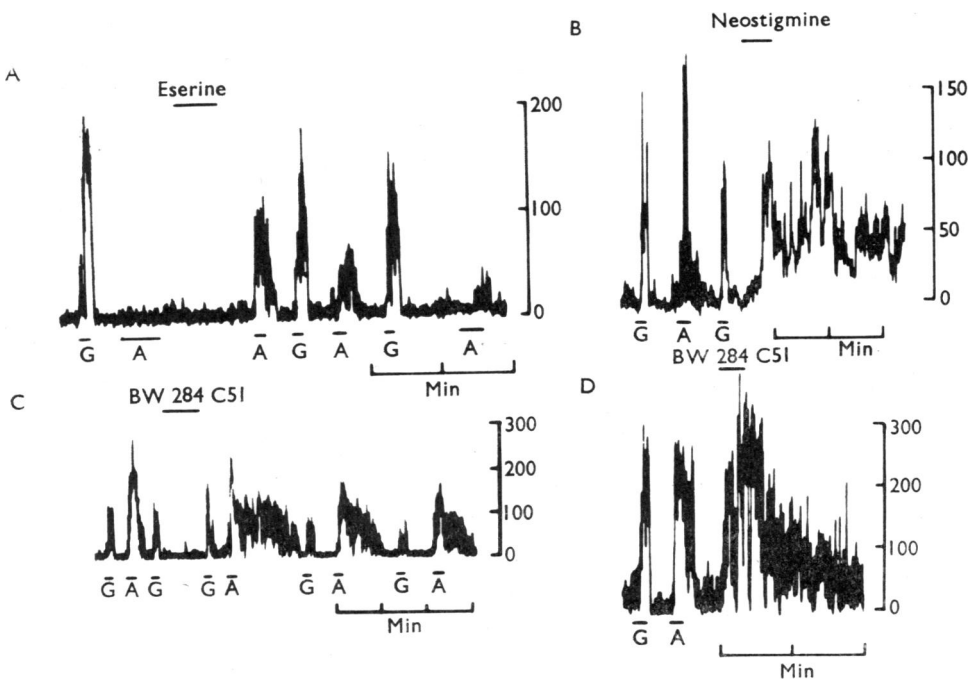

Fig. 3. Effects of acetylcholine potentiating compounds. A, B, C, D represent neurones recorded at depths of 5,740, 6,980, 1,760 and 3,660 μ respectively. A: This neurone was excited by L-glutamate (G, 40 nA) but not by acetylcholine (A, 30 nA). After eserine (40 nA), acetylcholine (30 nA) had a marked excitant effect that declined during 3–5 min after the application of eserine. B: Compared with neostigmine (80 nA) both L-glutamate (80 nA) and acetylcholine (80 nA) had excitant actions of short latency and rapid decline. C: BW 284 C51 (40 nA) slightly reduced the excitant effect of L-glutamate (40 nA) but prolonged the excitant effect of acetylcholine (30 nA). D: On this cell, L-glutamate (40 nA), acetylcholine (40 nA) and BW 284 C51 (40 nA), all had excitant effects, those of the BW 284 C51 being the most prolonged.

Eserine frequently induced firing in the absence of an application of acetylcholine. Characteristically, these effects developed slowly, taking about 35 sec to commence and reached a maximum about 60 sec after the commencement of application. The effect then declined slowly over a period of several minutes. This type of response was observed when eserine was applied with currents of 20–30 nA, and is therefore unlikely to have been the result of a direct action of the compound on acetylcholine receptors on the cell. Inactivation of the cholinesterase in the vicinity of the electrode tip is likely to have allowed locally released acetylcholine to accumulate to suprathreshold concentrations.

The quaternary cholinesterase inhibitors, neostigmine, edrophonium and BW 284 C51 (1.5-*bis*-(4-allyldimethyl ammonium-phenyl) pentan-3-one dibromide) often had direct excitant actions on thalamic neurones as well as potentiating the effects of acetylcholine. Examples of this are presented in Fig. 3B and D. Both cells were excited by acetylcholine and the application of neostigmine or BW 284 C51 induced an increase in the firing frequency which commenced within a few seconds of application. The increased rate of discharge was maintained for several minutes after the applications had ceased.

On other cells it was possible to demonstrate a potentiation of acetylcholine excitation by the quaternary cholinesterase inhibitors in the absence of a direct change in neuronal excitability, as detected by the alterations in the responses to brief pulses of L-glutamate. Thus although the responses to L-glutamate were actually reduced in magnitude after an application of BW 284 C51 to the neurone in Fig. 3C, the action of acetylcholine was prolonged.

Acetylcholine antagonists

Atropine, hyoscine, dihydro-β-erythroidine (DHE), hexamethonium, mecamylamine and benzoquinonium (mytolon, Hoppe, 1950) were tested as acetylcholine antagonists on thalamic neurones.

Atropine and DHE were the most extensively tested antagonists, for one or the other compound was present in most electrodes used in this survey. Both compounds were tested on more than one hundred neurones in the thalamus.

Atropine had a dual effect on thalamic neurones. The excitability of most cells was depressed by large amounts of this compound regardless of whether they were cholinoceptive or not. This non-specific reduction in excitability, which has been observed in other areas of the central nervous system (Curtis & Phillis, 1960; Krnjević & Phillis, 1963; Phillis, Tebēcis & York, 1967), was of short duration and responses to L-glutamate had usually recovered within 60–90 sec of the end of the atropine application.

On most cholinoceptive neurones, atropine depressed or abolished the excitant actions of acetylcholine. This action had a duration of 25–30 min and could be clearly demonstrated once responses to L-glutamate had recovered. Illustrations of the depressant action of atropine on acetylcholine excitation have been presented in Figs. 3 and 9 of the preceding paper. Hyoscine had similar actions to atropine.

In a preliminary report on the pharmacology of thalamic neurones (McCance, Phillis & Westerman, 1966), DHE was considered to be relatively ineffective as an acetylcholine-antagonist in the thalamus. This was in contrast to the findings of Andersen & Curtis (1964), who reported that DHE depressed acetylcholine excitation of all the thalamic neurones on which it was tested.

Examples of the action of DHE are shown in Fig. 4. On both cells it had an excitant action: this was a frequent finding. The action of acetylcholine was abolished on one cell (Fig. 4A), and remained unaltered on the other (Fig. 4B). A similar failure of DHE to reduce or abolish the excitant actions of acetylcholine was frequently observed.

The ganglion blocking agents hexamethonium and mecamylamine depressed the acetylcholine-excitation of some neurones. Mecamylamine causes a specific block of acetylcholine excitation of Renshaw cells (Ueki, Koketsu & Domino, 1961) but it was

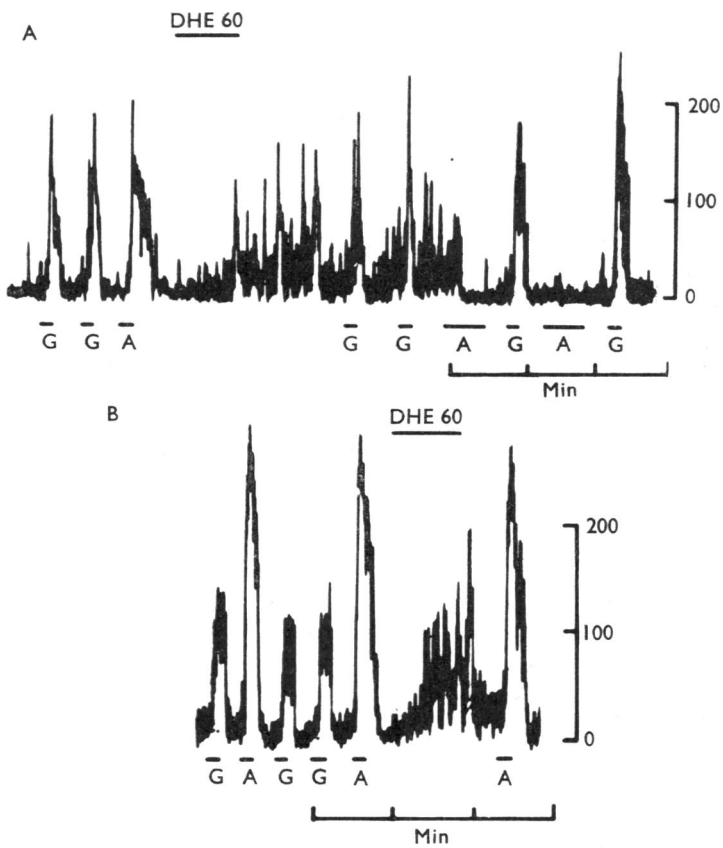

Fig. 4. Effects of DHE on neurones excited by acetylcholine. A (6,990 μ): DHE (60 nA) excited this cell directly and did not alter the response to L-glutamate (G, 20 nA). The response to acetylcholine (A, 40 nA) was abolished after DHE. B (8,660 μ): Acetylcholine (20 nA) excited the cell more powerfully than L-glutamate (30 nA). DHE (60 nA) also had an excitant effect but did not reduce firing induced by acetylcholine.

found to have a non-specific depressant action on all forms of activation of cerebral cortical neurones (Krnjević & Phillis, 1963). A specific acetylcholine-antagonism was also evident on some thalamic neurones, as well as a non-specific depressant action. Excitation of the thalamo-cortical unit in Fig. 5 induced by both acetylcholine and L-glutamate was depressed by mecamylamine (40 nA for 30 sec). The response to

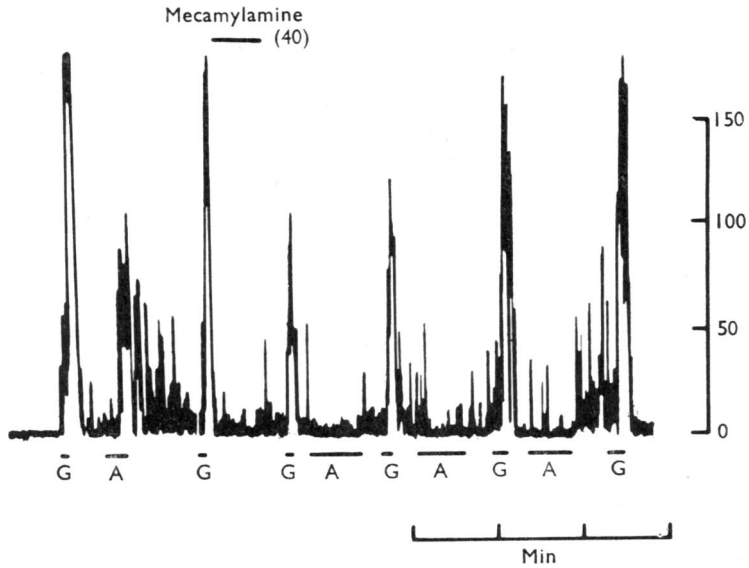

Fig. 5. Blocking action of mecamylamine on a VBC cell. Mecamylamine (40 nA) reduced the excitant effects of both L-glutamate (G, 40 nA) and acetylcholine (A, 60 nA), but whereas firing to glutamate returned to control level within 3 min, the response to acetylcholine remained blocked.

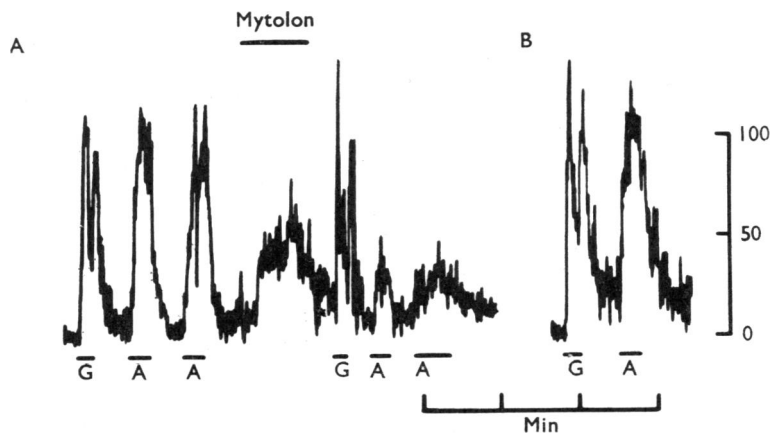

Fig. 6. Effects of mytolon on a cell recorded in the VBC. A: L-glutamate (G, 70 nA) and two applications of acetylcholine (A, 50 nA) induced comparable increases in cell firing frequency. Mytolon (60 nA) had a weaker excitant effect. Less than 1 min after mytolon, excitation induced by L-glutamate was virtually unimpaired but that caused by acetylcholine was markedly reduced. B: Same cell recorded 10 min later, showing complete recovery of response to acetylcholine.

L-glutamate had recovered fully 2.5 min later, while that to acetylcholine was still depressed. Acetylcholine-responses returned to control magnitude within 10 min of the application of mecamylamine.

Mytolon is a curariform agent that proved to be an effective acetylcholine-antagonist on cholinoceptive cells in the lateral geniculate nucleus (Phillis *et al.*, 1967). It was tested on nine cholinoceptive cells in the thalamus and depressed or abolished the action of acetylcholine on four of these. Mytolon frequently had an initial excitant action on cholinoceptive cells but not on cells insensitive to acetylcholine. An example of depression by mytolon of acetylcholine-excitation is shown in Fig. 6. This cell was initially excited by mytolon (60 nA) and although L-glutamate continued to induce firing after mytolon, the effects of acetylcholine were largely abolished.

Depression of acetylcholine-excitation by mytolon had a relatively short duration and when this cell was tested after a 10 min interval the response to acetylcholine had recovered.

Action of barbiturates

During the course of these experiments it was realized that acetylcholine excited cells in cats anaesthetized with gas more readily and with a considerably shorter latency than in animals anaesthetized with pentobarbitone.

The effect of this substance on acetylcholine excitation was therefore investigated by administering small doses intravenously to cats which were anaesthetized with nitrous oxide. The responses of several thalamic neurones were investigated in this manner and the results have been described in more detail elsewhere (Phillis & Tebēcis, 1967).

Administration of pentobarbitone was usually associated with the appearance of the " spindle " or burst type spontaneous discharges described in the preceding paper. The excitant actions of acetylcholine were reduced to a considerably greater extent than those of L-glutamate by small doses (2–4 mg/kg) of pentobarbitone sodium. Application of larger amounts of acetylcholine was usually effective in again evoking a discernible excitation.

Pentobarbitone also reduced the inhibitory actions of noradrenaline and 5-hydroxytryptamine on thalamic neurones. Although a more comprehensive study of the membrane excitability changes induced by pentobarbitone would be an essential prerequisite for a complete analysis of the effects of this compound, the results presented here suggest that it may depress the actions of some compounds to a greater extent than others.

DISCUSSION

The history of the development of the concepts of two kinds of receptor for acetylcholine has been reviewed by Curtis & Ryall (1966b). The terms " nicotinic " and " muscarinic " were originally introduced by Dale (1914) to describe the peripheral actions of various choline derivatives and have since been applied to the receptors with which these combine (Barlow, 1955 ; Waser, 1961, 1963). The receptors have been characterized on the basis of the activities of substances that either mimic or antagonize the actions of acetylcholine. Neuropharmacological investigators have considered that nicotinic

characteristics are exhibited when neurones are excited by carbachol (*inter alia*) and this excitation and that of acetylcholine can be antagonized by DHE (Unna, Kniazuk & Greslin, 1944). On the other hand, the possession of muscarinic characteristics implies that a neurone is susceptible to excitation by acetyl-β-methylcholine (*inter alia*) and excitation is antagonized by atropine (Dale, 1914; Henderson & Roepke, 1937; Ambache, 1955).

In the framework of this classification, receptors on deep pyramidal cells in the cerebral cortex have muscarinic properties (Krnjević & Phillis, 1963; Crawford & Curtis, 1966) while those on Renshaw cells in the spinal cord of the cat have been identified as nicotinic (Curtis & Ryall, 1966a, b). The functional significance of this latter finding is enhanced by the demonstration that the initial phase of orthodromic excitation of Renshaw cells by ventral root stimulation as well as excitation by carbachol is blocked by DHE but not by atropine (Eccles, Fatt & Koketsu, 1954; Eccles, Eccles & Fatt, 1956; Curtis, Phillis & Watkins, 1961; Curtis & Ryall, 1966c).

Further experiments on Renshaw cells have revealed the presence of two more types of receptors, one of which reacted with acetyl-β-methylcholine and *dl*-muscarine and was specifically blocked by intravenous atropine. This muscarinic receptor was also responsible for the late phase of orthodromic excitation of Renshaw cells by ventral root stimulation (Curtis & Ryall, 1966b, c). A third type of receptor was proposed to account for the finding that in the presence of DHE and atropine many cholinomimetics had a depressant action on Renshaw cells (Curtis & Ryall, 1966b).

A feature of the results presented in this and a previous report (Andersen & Curtis, 1964) is the difference between the receptors on thalamic neurones and either the nicotinic receptors on Renshaw cells or muscarinic receptors on deep pyramidal cells.

The thalamic acetylcholine receptors seem to be of an intermediate type with properties similar to receptors in the lateral geniculate nucleus (Curtis & Davis, 1963; Phillis *et al.*, 1967) and brain stem (Salmoiraghi & Steiner, 1963; Bradley & Wolstencroft, 1965). This conclusion is derived from the results with various cholinomimetics, such as carbachol and acetyl-β-methylcholine, which had comparable actions on thalamic neurones, and the finding that both muscarinic and nicotinic acetylcholine-antagonists were effective on many thalamic neurones.

The nicotinic action of acetylcholine on Renshaw cells has a rapid onset and is of brief duration after the cessation of acetylcholine ejection. In contrast, on deep pyramidal cells of the cortex, or on Renshaw cells that have been blocked with DHE, the excitant action of acetylcholine is of slow onset and prolonged duration. The time course of acetylcholine excitation of thalamic neurones was intermediate between those recorded on Renshaw cells and deep pyramidal cells.

Thus, although the excitant effects of acetylcholine on thalamic neurones may ultimately prove to be a result of interactions with several different types of receptor, it seems that the properties of the dominant receptor can be distinguished from those of both typical nicotinic and typical muscarinic types.

Attempts to analyse the nature of acetylcholine receptors on thalamic neurones in greater detail are currently in progress. Unlike receptors on the Renshaw cell population in the spinal cord, however, the receptors in different thalamic neurones exhibit a variability that has hindered detailed evaluation.

The depression of responses to cholinomimetics following the use of carbachol and muscarone is being investigated. The effects could result from either desensitization of receptors or a depressant action proper. Indications are that both of these phenomena are present.

SUMMARY

1. The nature of acetylcholine receptors on feline thalamic neurones was studied using the technique of iontophoresis. The receptors appeared to have properties intermediate between classical nicotinic and muscarinic types.

2. Excitant potencies of a variety of cholinomimetic substances relative to that of acetylcholine were compared using nine-barrelled electrodes. Carbachol, acetyl-β-methylcholine and acetylcholine were the most active compounds tested.

3. Anticholinesterases excited some cells and potentiated the actions of acetylcholine.

4. Atropine and dihydro-β-erythroidine were extensively tested as acetylcholine antagonists as well as various other compounds. Atropine and hyoscine were the most effective antagonists.

5. Depression of some cholinoceptive cells was observed to follow the application of carbachol and muscarone.

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