J. Physiol. (1963), **166**, *pp.* 328–350
With 12 *text-figures*
Printed in Great Britain

PHARMACOLOGICAL PROPERTIES OF ACETYLCHOLINE-SENSITIVE CELLS IN THE CEREBRAL CORTEX

BY K. KRNJEVIĆ AND J. W. PHILLIS*
From the A.R.C. Institute of Animal Physiology, Babraham, Cambridge

(*Received* 7 *August* 1962)

In a previous paper (Krnjević & Phillis, 1963*a*) we gave a general description of the responses of mammalian cortical neurones to various chemical agents applied by iontophoresis. It was pointed out that apparently all neurones could be excited with L-glutamate and certain related dicarboxylic amino acids, and that some neurones were also excited strongly by acetylcholine (ACh). The distribution, identity and probable significance of these neurones were examined in a second paper (Krnjević & Phillis, 1963*b*); from various kinds of evidence, it was concluded that many cholinoceptive neurones are deep pyramidal cells, and that the large variations in ACh sensitivity between different cells may perhaps be ascribed to unequal concentrations of cholinergic synapses.

The only other neurones of the central nervous system shown convincingly to be cholinoceptive are the Renshaw cells of the spinal cord, which are supplied by recurrent collateral branches of motor axons (Eccles, Fatt & Koketsu, 1954; Eccles, Eccles & Fatt, 1956; Curtis & Eccles, 1958). Like skeletal muscle fibres, Renshaw cells have ACh receptors that are predominantly nicotinic in character; for instance, they are readily excited by nicotine itself and the action of ACh is effectively blocked by curariform antagonists, the most potent being dihydro-β-erythroidine (Curtis, Phillis & Watkins, 1961)

On account of these observations it has been assumed that nicotinic receptors are characteristic of central cholinergic synapses in general, and the presence of such receptors, as shown for example by a clear blocking action of dihydro-β-erythroidine, has been considered an important factor in the identification of cholinergic synapses (Curtis & Koizumi, 1961).

In the present paper we describe the pharmacological properties of the ACh receptors responsible for the activation of cholinoceptive neurones in the cortex. It will be shown that these receptors are of a clearly mus-

* Wellcome Fellow. Present address: Department of Physiology, Monash University, Melbourne, Australia.

carinic nature, and thus radically different from those of Renshaw cells. Short reports on this work have already appeared (Krnjević & Phillis, 1961, 1962).

METHODS

For most experiments we studied cells in the cerebral cortex of cats, rabbits or rhesus monkeys anaesthetized with intraperitoneal Dial compound (allobarbitone + urethane, Ciba Ltd.; 0·7 ml./kg). Other anaesthetics were also used: pentobarbitone sodium (30 mg/kg), chloralose (80 mg/kg) or ether. In several experiments we made observations on the '*cerveau isolé*' of cats after discontinuing ether anaesthesia; under these conditions the actions of ACh were not qualitatively different from those seen during anaesthesia.

The various substances were applied to single neurones by iontophoresis from five-barrelled micropipettes, recording at the same time the extracellular spikes. Interactions between substances were studied by their simultaneous release from different barrels, as first described by del Castillo & Katz (1957) and Curtis & Eccles (1958). Further details of the method have been given in an earlier paper (Krnjević & Phillis, 1963*a*).

Acetylcholine was released as a cation by outward currents from a 3M solution of AChCl. Quantitative aspects of ACh release have been examined and it has been shown that the mean transport number of ACh^+ under these conditions is approximately 0·4 (Krnjević, Mitchell & Szerb, 1963). Other solutions were made as concentrated as possible. Most of the compounds were ionized as cations and were therefore released by outward currents. Whenever necessary the acidity of the solution was adjusted to promote adequate ionization.

Because of possible variations in the properties of different micropipettes (Krnjević *et al.* 1963) all substances were tested by release from at least 2 pipettes and observations were usually repeated on at least 6–10 neurones, in more than one animal. Altogether we have examined about 500 cholinoceptive units.

When drugs were given intravenously, they were injected into the cephalic vein in the forearm.

The following is an alphabetical list of drugs obtained commercially for use in the present investigations: acetylcholine chloride (Roche Products Ltd.); acetyl-β-methylcholine chloride (L. Light & Co.); adrenaline acid tartrate (British Drug Houses); γ-aminobutyric acid (California Biochemical Foundation); arecoline hydrobromide (British Drug Houses); arfonad (Roche Products Ltd.); atropine sulphate (British Drug Houses); benzoylcholine chloride (Roche Products Ltd.); butyrylcholine chloride (L. Light & Co.); carbamyl-choline chloride (British Drug Houses); chloralose (Hopkin & Williams Ltd.); choline chloride (Roche Products Ltd.); decamethonium iodide (L. Light & Co.); dimethyltubocurarine bromide (Burroughs Wellcome & Co.); hyoscine hydrobromide (British Drug Houses); 5-hydroxytryptamine creatinine sulphate (May & Baker Ltd.); nicotine hydrogen tartrate (British Drug Houses); thiopentone sodium (Pentothal, Abbott Laboratories); physostigmine sulphate (eserine, British Drug Houses); pilocarpine hydrochloride (British Drug Houses); procaine hydrochloride (Imperial Chemical Industries); neostigmine methyl sulphate (prostigmine, L. Light & Co.); sodium L-glutamate (British Drug Houses); succinyl bis-choline chloride (L. Light & Co.); edrophonium chloride (tensilon, Roche Products Ltd.); tetramethyl ammonium bromide (L. Light & Co.); D-tubocurarine (Burroughs Wellcome & Co.).

In addition, very grateful acknowledgement is made to the following for gifts of these other compounds: Winthrop Laboratories Ltd., ambenonium chloride; Lederle Laboratories, Ltd., benzhexol hydrochloride (Artane); Geigy Pharmaceutical Co. Ltd., caramiphen hydrochloride (Parpanit) imipramine (Tofranil), muscarine and muscarone; Eli Lilly & Co., cycrimine hydrochloride (Pagitane); Parke, Davis & Co. Ltd., dimethylphenylpiperazinium (DMPP); May & Baker Ltd., gallamine triethiodide (Flaxedil), hexamethonium chloride; Merck, Sharp & Dohme, Ltd., dihydro-β-erythroidine, mecamylamine hydrochloride;

Burroughs Wellcome & Co., Ltd., procyclidine hydrochloride (Kemadrin), D-tubocurarine chloride, dimethyl-tubocurarine bromide; Roche Products Ltd., toxifrin (Ro4-2906); Ciba Laboratories Ltd., trasentine hydrochloride; Dr V. P. Whittaker (Agricultural Research Council, Babraham), acrylylcholine bromide, crotonylcholine iodide, β,β-dimethyl acrylylcholine iodide, iso-valerylcholine iodide, nicotinoylcholine iodide, palmitylcholine bromide, pentenylcholine iodide, propionylcholine *p*-toluenesulphonate, urocanylcholine chloride hydrochloride; Dr R. W. Brimblecombe (Chemical Defence Experimental Establishment, Porton Down, Wiltshire) oxo-tremorine; Dr E. A. Jacobsen (Danish Medicinal and Chemical Co., Copenhagen) benactyzine hydrochloride.

RESULTS

In the first section of the results we shall consider the effects produced by ACh and various cholinomimetics, certain potentiating (anticholinesterase) agents and several antagonists, as well as some other relevant compounds, when they are applied to cholinoceptive neurones by iontophoresis.

Excitation by cholinomimetics

Choline esters. The action of ACh has already been described in detail (Krnjević & Phillis, 1963*a*, *b*); it is therefore only necessary to mention its main characteristics, which are apparent in several of the figures (e.g. Figs. 1, 2, 6, 12). Unlike L-glutamate (e.g. Figs. 7, 8, 12) ACh excites comparatively slowly, after a latent period of 2–15 sec in most cases, and the discharge always continues for some 15–60 sec after the end of the application. There is no sign of desensitization if ACh is released for a long period,

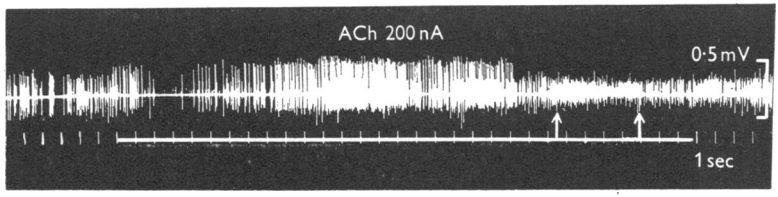

Fig. 1. Two cholinoceptive units in cortex of cat under Dial. White line indicates time during which large dose of ACh was applied iontophoretically. Both units were excited, but large spikes disappeared 21 sec after starting application. L-Glutamate released from another barrel between two arrows failed to excite this unit, showing that excess ACh probably caused a block by depolarization.

or by repeated applications. On the contrary repetitions within a few minutes of each other are associated with a reduction of latency and threshold, and a striking progressive increase in background 'spontaneous' activity. On the other hand, if ACh is applied too rapidly it is sometimes possible to cause a block of further discharge, presumably by an excess of depolarization. This phenomenon is illustrated in Fig. 1; two units were excited by a comparatively big release of ACh (200 nA). After 21 sec the

larger unit suddenly ceased to fire. Since ACh is released by an outward current, it was just possible (though very unlikely) that the unit had been blocked by anodal polarization (cf. Krnjević & Phillis, 1963*a*, Fig. 1*b*). To distinguish between the two types of block, L-glutamate was released from another barrel at the time indicated by the two arrows, causing a further acceleration of the small unit, but no discharge of the large unit, just as would be expected if the latter were already over-depolarized.

Other choline esters can be divided in two groups, one consisting of those with a substantial ACh-like excitant action, and the other of esters which are mainly inactive.

Other active esters. The most active of all choline esters tested, probably exceeding even ACh itself, was acetyl-β-methyl choline. Some neurones which only gave a rather doubtful response to ACh were excited very clearly by acetyl-β-methyl choline. In all respects the type of action was very similar to that seen with ACh, as can be seen in Fig. 11.

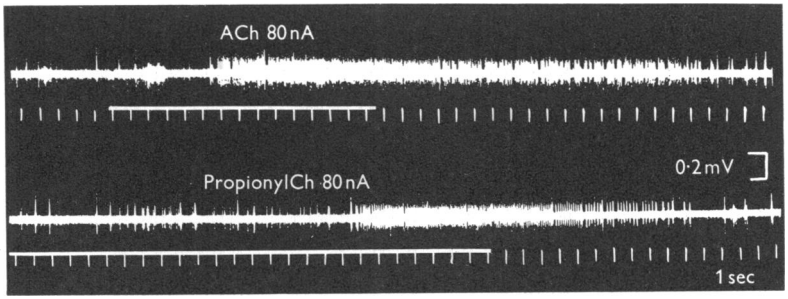

Fig. 2. Comparing the effects produced by ACh and propionylcholine, released by the same outward current, on cortical neurone in cat under Dial. Note much longer application of propionylcholine as shown by white line.

Other choline esters were consistently less effective than ACh. For instance propionylcholine regularly excited cholinoceptive cells, but it appeared to have only $\frac{1}{3}$–$\frac{1}{2}$ the potency of ACh. Thus the unit illustrated in Fig. 2 was excited less strongly and for a shorter period by propionylcholine than by ACh, even though the former was applied for a much longer time.

Carbaminoylcholine had a somewhat irregular action, always much weaker than that of ACh; its effect did not tend noticeably to outlast that of ACh.

The following esters as a rule also caused some excitation of cholinoceptive neurones; succinylcholine, nicotinoylcholine, acrylylcholine, palmitylcholine, urocanylcholine and benzoylcholine; though not strictly belonging to this group, choline itself and decamethonium may be mentioned here because of their weak excitant action. None of these

compounds had any special effect on cells that did not also respond to ACh.

Inactive esters. In this group we have butyrylcholine, crotonylcholine pentenylcholine, iso-valerylcholine and β-β-dimethylacrylylcholine. Of these only the first three appeared to have some genuine excitant properties when tested on a few cells that were particularly sensitive to ACh. The contrast between the typical effects seen when ACh and butyrylcholine were applied to the same cell is evident in the traces of Fig. 3. These related substances which were mainly inactive were also released in conjunction with ACh, but they did not cause any obvious interference with its action.

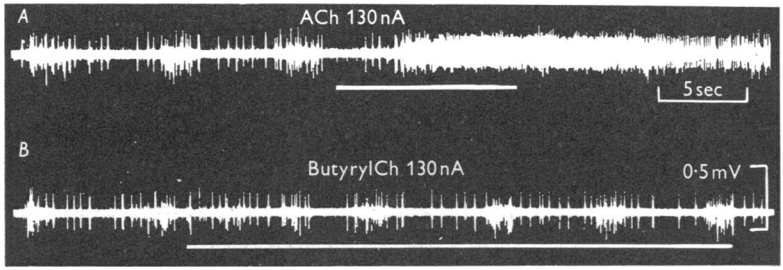

Fig. 3. Lack of any recognizable excitation of a cholinoceptive neurone by butyrylcholine. Cortical unit in cat under Dial.

Nicotine. We have already described the unspecific excitant action of nicotine on cortical neurones, irrespective of their ACh sensitivity (Krnjević & Phillis, 1963*a*). This excitation only occurs after a comparatively long application (10–60 sec); it is usually very abrupt in onset, giving a paroxysmal discharge which is quite different from the effects of ACh on cholinoceptive neurones (see Fig. 11 in Krnjević & Phillis, 1963*a*). In addition to this excitation, seen with about 1 in 5 of the neurones examined, nicotine tended to produce some mild depression of excitability of many cells (whether cholinoceptive or not) as tested by releasing L-glutamate or ACh. There was no specific antagonism to ACh itself.

Tetramethylammonium (Burn & Dale, 1915) and 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) (Chen, Portman & Wickel, 1951) which excite peripheral ganglia like nicotine, were also largely inactive.

Muscarinic agents. DL-muscarine and DL-muscarone particularly were very effective, their action being comparable with and probably somewhat greater than that of ACh. Muscarone excited strongly when released by smaller iontophoretic currents (Fig. 4); a similar rate of release usually caused the cells to discharge faster and after a shorter latency than with ACh, although the effect did not seem to persist much longer. A few units

which did not show a very definite response to ACh, were nevertheless excited by muscarone; these cells had all the usual characteristics of cholinoceptive cells with regard to distribution in depth, spontaneous firing, identification as Betz cells, etc. (Krnjević & Phillis, 1963*b*).

Both arecoline and pilocarpine have prominent though not exclusively muscarinic properties. They also excite cholinoceptive cortical neurones, in a rather similar manner. This action has a much slower onset than that of ACh, and it may therefore be overlooked unless the substances are applied for at least 45 sec. When the full effect is obtained after about 1 min, the fast rate of discharge may persist for several minutes, particularly with pilocarpine, as is shown in Fig. 5. The cholinoceptive unit illustrated there remained unresponsive to the pilocarpine released by iontophoresis for over 30 sec, but the subsequent excitation was clearly only just beginning to slow down at the end of the trace, and in fact it remained appreciable for several minutes.

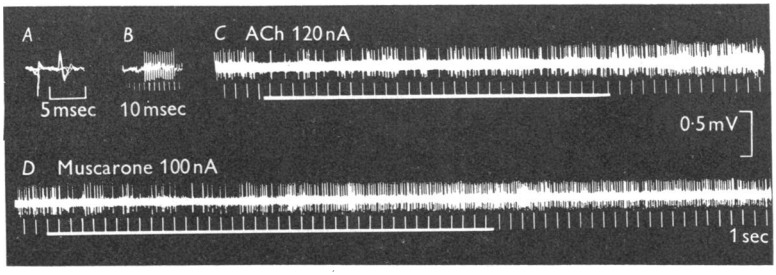

Fig. 4. Action of DL-muscarone on cholinoceptive unit in cat's cortex under Dial. *A* and *B* identification as Betz cell: (*A*) several superimposed traces of spike during threshold pyramidal stimulation, and (*B*) antidromic pyramidal activation at 200/sec. *C* and *D*, excitation by ACh and muscarone released iontophoretically during periods indicated by white lines.

Oxo-tremorine is another compound with substantial muscarinic properties (Haslett & Jenden, 1961); it is best known for its peculiarly potent action in causing tremor by a central mechanism when injected into animals. It was therefore of some interest to find that it was able to excite cholinoceptive neurones, including Betz cells identified by antidromic pyramidal stimulation. Its action was usually, but not always, comparatively slow, resembling that of pilocarpine rather than that of ACh.

Substances that potentiate ACh. Here we consider the effects of releasing some anticholinesterases before or together with ACh. It will have been noted that ACh causes rather prolonged excitation of cholinoceptive neurones, which does not seem consistent with a fast removal of ACh by the hydrolytic action of tissue cholinesterases, such as is observed at the neuromuscular junction. Further evidence in this respect is the relatively

weak action of carbaminoylcholine on cortical neurones when compared with its potency in peripheral organs; the latter is usually ascribed to its resistance to hydrolysis.

Accordingly anticholinesterases have not had any spectacular effect in potentiating ACh excitation of cholinoceptive cells. Micropipettes filled with solutions of eserine sulphate have not passed currents satisfactorily, so that we are not certain whether the observed lack of any action is really significant, but both prostigmine and tensilon have been released successfully, judging by their clear actions on a number of cells.

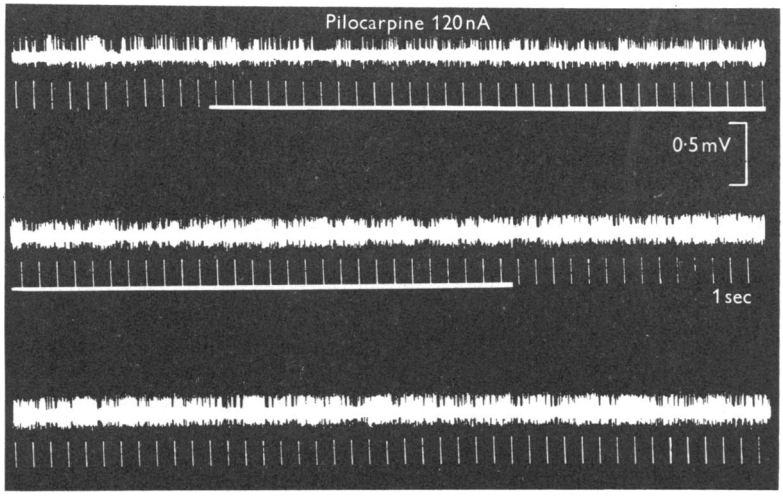

Fig. 5. Excitation of cholinoceptive unit, in cortex of cat under ether, by pilocarpine released for a period of 1 min, as shown by white line (three traces are continuous).

Of the two substances, tensilon gave the more consistent results. If large quantities were applied (with currents of 100 nA for > 30 sec) either agent sometimes initiated a discharge, especially of units which were already very excitable. More often, however, there were no observable changes unless ACh was applied as well. An appreciable potentiation was then revealed by a reduced latency of onset, faster firing at the peak, and a prolongation of the ACh effect. Some of these changes are visible in Fig. 6, the prolonged action of ACh after tensilon is particularly clear and the initial part of the trace in *B* (which starts 12 sec after the onset of the release of tensilon) shows that tensilon by itself could not initiate a discharge. No effect of this kind was observed when tensilon was given together with carbaminoylcholine. Ambenonium, another compound with anticholinesterase properties, had a rather potent excitatory action of its own.

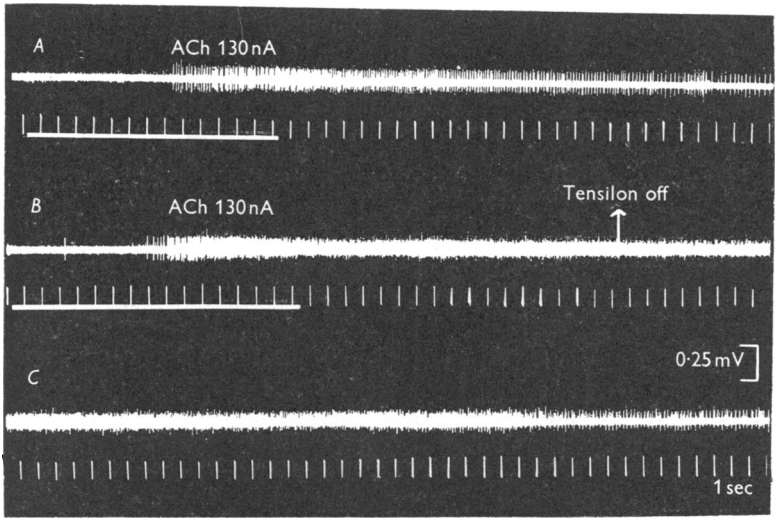

Fig. 6. Tensilon potentiating ACh action on cholinoceptive unit in rabbit under Dial. Trace *A* is a control. Trace *B* begins 12 sec after starting release of tensilon by outward current of 80 nA; ACh application again shown by white line: note that tensilon did not excite unit by itself, nor did it reduce latency of onset of ACh firing, but discharge was much accelerated and prolonged (*C* is continuous with *B*).

Antagonists of ACh

Curariform agents. Most of these proved incapable of blocking excitation by ACh and other cholinomimetics. Instead, some of them excited neurones in a non-selective manner, somewhat like nicotine; this only occurred after rather long applications (30–60 sec, or even more). The ineffective antagonists of this type were D-tubocurarine, dimethyl D-tubocurarine, toxiferin, dihydro-β-erythroidine and a sample of curare alkaloids described as 'curara, Schmiedeberg'. Excitation was only seen when testing the first 3 of these; the last 1 had a weak unspecific depressant effect on most neurones. Dihydro-β-erythroidine seemed to have a slight specific antagonistic action on ACh responses of a few cells in a monkey.

The only compound in this group capable of blocking excitation by ACh specifically and fairly consistently was gallamine, released from solutions of gallamine tri-ethiodide. Its action is shown in Fig. 7. As in all tests of this kind, the selective action against ACh was demonstrated by applying at regular intervals as a control small amounts of L-glutamate, which should give equal responses throughout, or at least remain much more effective than ACh. An outward current like that used to release gallamine tends to have some depressant effect on cells by anodal polarization; in addition, there may be some interference with an inward current passed

simultaneously through another barrel to release anions such as glutamate. These effects, which have been discussed previously (Krnjević & Phillis, 1963*a*), probably account for most of the slight depression of glutamate firing in Fig. 7*B*. The blocking action was fully reversible, normal ACh responses being again obtained within a few minutes, as in Fig. 7*C* where $2\frac{1}{2}$ min after the end of the application of gallamine ACh can be seen to excite almost as well as it did initially. About 20 % of cholinoceptive cells showed no definite blocking effect of gallamine. In view of this marked action on cortical cholinoceptive neurones, even though there is no evidence

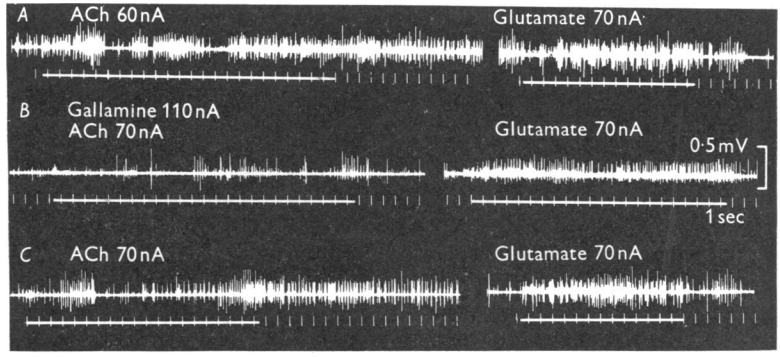

Fig. 7. Block of ACh responses by gallamine. *A*, control responses; *B*, 1 min after starting iontophoretic application of gallamine; *C*, $2\frac{1}{2}$ min after the end of gallamine release (which lasted $2\frac{3}{4}$ min). Betz cell in cat under Dial.

that gallamine crosses the blood–brain barrier at all readily, it is unsafe to assume that its synchronizing effect on the electrocorticogram, seen when it is given systemically, is solely due to a reduction in afferent input (cf. Hodes, 1962). It may be relevant that gallamine is known to have a weak atropine-like action on the heart (Riker & Wescoe, 1951; Roberts, Roy, Reilly, Garb, Riker & Hashimoto, 1955; Laity & Garg, 1962).

Procaine has a strong curare-like action at the neuromuscular junction (del Castillo & Katz, 1957; Furukawa, 1957), but this was not seen with cortical cells. There was a pronounced block of all neurones, presumably owing to procaine's local anaesthetic properties (cf. Krnjević & Phillis, 1963*a*, Fig. 19), but this was quite unspecific and rapidly reversible. It will be discussed in more detail below, in relation to the action of atropine. Decamethonium, which excites and then blocks the neuromuscular junction (Paton & Zaimis, 1952) seems to cause only a mild excitation in the cortex, as already mentioned above.

Some other ganglionic blocking agents. Hexamethonium was tested on many neurones, but only a slight antagonistic effect was observed in a few cases. Mecamylamine, on the other hand, had a strong depressant action

on many neurones, but this was quite unselective with no special block of ACh excitation (cf. the specific block of Renshaw cells observed by Ueki, Koketsu & Domino, 1961). A mild non-selective depression of cortical neurones by nicotine has already been mentioned.

Atropine and hyoscine. Practically all cortical neurones are strongly depressed by comparatively large amounts of these substances. Thus, release by a current of 100 nA leads in about 30 sec to a complete block of excitability as tested by repeated application of L-glutamate or by synaptic activation. This unspecific effect, which is fully reversible within about 1 min, is very similar to that produced by procaine (Krnjević & Phillis, 1963*a*).

The excitation of cholinoceptive neurones by glutamate is also blocked but these cells show an additional action of atropine and hyoscine, a very prolonged interference with ACh excitation. After a 30 sec application of atropine (as above), the hitherto cholinoceptive cells failed to show any further responses to ACh for periods which have varied between 25 and 50 min, whereas responses to L-glutamate reappeared within 10–30 sec. A sequence of changes actually observed in an experiment is illustrated in Fig. 8. There was a complete block of excitation by either L-glutamate or ACh towards the end of a 1 min application of atropine (Fig. 8*D*; before large arrow). However, responses were elicited by L-glutamate within 16 sec of the end of the release of atropine (signalled by the arrow in *D*), but ACh failed to excite for another 27 min (*E*, *F*).

If smaller quantities were released, there was no significant depression of general excitability, or at most a slight and transient reduction in glutamate discharges, but the responses to ACh were nevertheless completely blocked. However, the effect did not last so long and ACh again excited within a few minutes.

In all respects, hyoscine behaved like atropine, but it seemed even more effective in blocking ACh responses, and somewhat weaker as a non-selective general depressant.

Central cholinolytics. A number of other substances which are believed to have central anticholinergic actions and which have been used in the treatment of Parkinsonism were also tested on cholinoceptive neurones. It must be pointed out that all these drugs probably have complex actions, including a more or less pronounced non-selective blocking action, somewhat like that of atropine. They induce comparatively slow changes that are not very clear-cut and do not always lend themselves to a simple interpretation. Our observations to date must therefore be considered only as a preliminary report.

Of these drugs, only benactyzine proved in several cases to have a prolonged atropine-like action. In this respect it is weaker than atropine,

since the effect was not seen with all cholinoceptive cells. On the other hand, when benactyzine did cause a block it seemed just as effective as atropine would presumably have been.

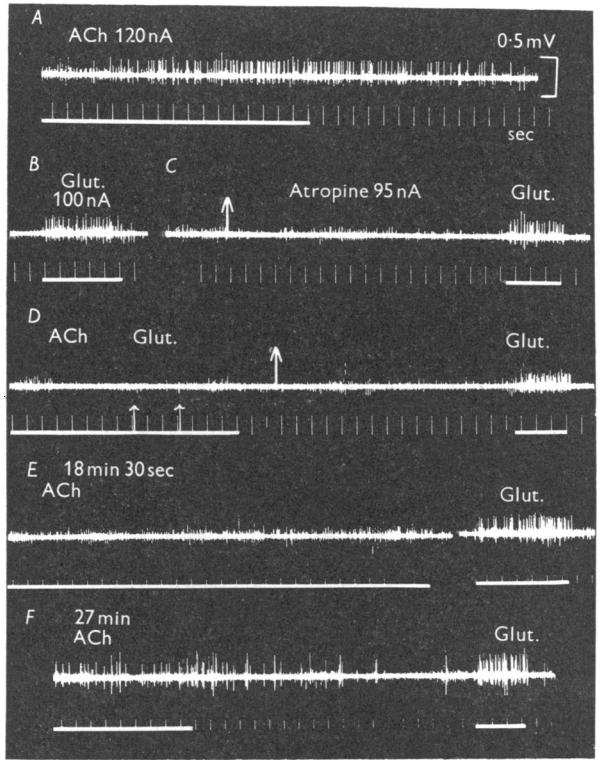

Fig. 8. Prolonged block of ACh sensitivity by atropine released iontophoretically. *A*, *B*, control records; note that in *A*, *D*, *E* and *F* ACh responses are shown 20 sec after starting release of ACh, last part of which is shown by white line. *C*, *D*, atropine was applied during 1 min period between two large arrows. Note block of ACh and L-glutamate responses in the first part of *D*, but glutamate was again effective 16 sec after end of atropine release. *E*, *F*, ACh gave no responses until 27 min later. There is some interference in record *F* by another unit which has now also started firing. Cat under Dial.

Caramiphen also caused a non-selective depression of excitability when applied by a current of 100 nA for about 1 min. Complete block was often preceded by a rapid discharge of the cell, a phenomenon that was seen sometimes with several other drugs, including occasionally atropine itself. Recovery of glutamate responses followed quite rapidly, usually within 1 min, whereas ACh remained ineffective for several minutes; however, there was never a really prolonged block like that produced by atropine.

Other drugs of this type, such as arfonad, cycrimine, benzhexol, tra-

sentin and procyclidine appeared to share some of the properties of caramiphen, though to a lesser degree.

Some other substances. As far as their responses to general excitatory and inhibitory agents are concerned, cholinoceptive neurones do not seem to differ qualitatively from other neurones in the cortex. Thus they can be excited by L-glutamate, although they seemed on the whole less easily excited in this way than typical non-cholinoceptive neurones. Many of them could not be made to discharge by L-glutamate released by a current of < 40–100 nA, whereas other cells would often fire with L-glutamate currents of only 10–20 nA.

Cholinoceptive neurones were readily inhibited by γ-aminobutyric acid (GABA) and other amino acids with similar actions (cf. Krnjević & Phillis, 1963*a*); GABA blocked with equal ease discharges initiated by glutamate or by ACh.

Various catechol and indole amines such as adrenaline and 5-hydroxytryptamine, which tend to depress cortical neurones (Krnjević & Phillis, 1963*c*), produced similar effects on cholinoceptive cells. Although it has been thought that adrenaline may potentiate the action of ACh at cholinergic synapses (Dale & Gaddum, 1930; Burn, 1945) no such effect was detected in the cortex, in agreement with comparable observations at the neuromuscular junction (Krnjević & Miledi, 1958). We have not observed any clear action of imipramine on cholinoceptive cells.

Actions of various drugs applied locally by iontophoresis on synaptic activity. A likely explanation for the cholinoceptive properties of some cortical neurones is that these neurones are naturally activated partly or exclusively by cholinergic synapses. In either case one may expect synaptic activity, such as 'spontaneous' discharges, or those evoked by remote stimulation to be affected to some extent by drugs that potentiate or antagonize ACh.

It has been possible substantially to increase with prostigmine and tensilon the characteristic repetitive discharge of cholinoceptive cells induced by synaptic activation (these substances have no comparable action on other cells). Thus in Fig. 9 the application of tensilon caused a temporary potentiation of the repetitive responses of cholinoceptive units evoked by stimulating the ventral posterolateral nucleus of the thalamus. Such effects were not usually very spectacular, possibly because cholinesterases play a relatively insignificant role in the removal of ACh from the synaptic region. Moreover, they do not tell us a great deal, since these drugs probably have a direct ACh-like action (Randall, 1951) even though it was clearly subthreshold in this case; they might therefore cause a general increase inexcitability, and thus potentiate non-selectively all subliminal synaptic effects.

Antagonists like atropine are capable of blocking all spontaneous and evoked activity simultaneously with the general blocking action previously described. However, spontaneous and evoked discharges reappear not long after glutamate responses, well before the return of the sensitivity to ACh. Only with caramiphen did we observe sometimes a recovery of glutamate responses which was not followed soon by synaptic activity. This phenomenon was particularly interesting because the synaptic activity returned after a successful application of ACh, as though caramiphen was driven off the appropriate receptors by a mass action. The more often ACh was applied, the quicker was the recovery of ACh responses and of synaptic activity.

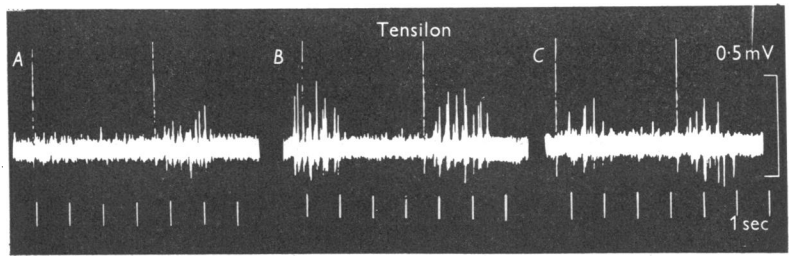

Fig. 9. Repetitive firing of cholinoceptive units evoked by single stimuli in ventral posterolateral nucleus of thalamus and potentiated by tensilon. *A*, 2 control responses; *B*, during iontophoretic release of tensilon (100 nA). *C*, 22 sec after stopping release. Cat under Dial.

Actions of drugs injected systemically. In view of the difficulties experienced in producing specific changes in synaptic activation by local iontophoresis, it seemed possible that only an insufficient part of the cell surface might be affected by this procedure, either because of the relatively large area involved (particularly if dendrites play an important role) or because diffusion barriers restrict the free movement of drugs. To overcome some of the difficulties several drugs were also administered by systemic injection which would permit a more diffuse application, admittedly at the risk of inducing changes that are only secondary to various actions in other parts of the brain or on peripheral structures. In addition, we examined the effects produced by some anaesthetics.

Eserine sulphate (0·2–1 mg/kg) given intravenously greatly increased the spontaneous activity of cholinoceptive neurones; but this may not have been very significant, because there were so many other obvious changes in the organism, including generalized twitching and alterations in heart rate, blood pressure and respiration, all of which may have excited the cortex indirectly.

Intravenous atropine sulphate was much more interesting as the animal's condition did not change appreciably. The injection was regularly

followed within a minute or two by a great reduction in spontaneous and evoked activity of cortical cholinoceptive neurones (Fig. 10) without much change in the activity or excitability of other cells. At the same time these neurones became either quite unresponsive or appreciably less responsive to local iontophoretic applications of ACh and related agents (Figs. 10 and 11). After doses of 1 mg/kg there was sometimes no recovery of ACh sensitivity for at least 30 min, the longest period for which we waited. However, as can be seen in the figures, the action of glutamate was not altered appreciably.

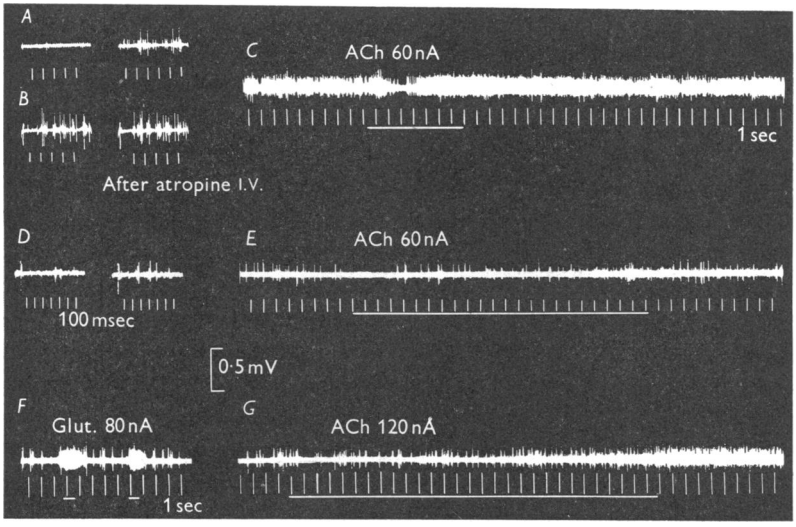

Fig. 10. Two cholinoceptive units discharging spontaneously (*A*, *C*) and giving late repetitive responses after single stimuli applied to internal capsule (*B*); note stimulus escape at beginning of each trace. 5 min after intravenous injection of atropine sulphate (1 mg/kg) spontaneous activity was very much reduced (*E*), practically no activity was evoked by stimulating internal capsule (*D*), and ACh applied by iontophoresis no longer caused any excitation (*E*), though L-glutamate was very effective (*F*). 10 min later units could be made to discharge by applying a much greater amount of ACh (*G*). Note shorter scales in *A*, *B* and *D*. Cat under Dial.

Smaller doses of atropine (*ca.* 0·2 mg/kg) had a milder action; although spontaneous activity was substantially reduced, applications of ACh were sometimes still effective, and they would reactivate spontaneous firing. Although systemic atropine tended to abolish the late repetitive responses evoked by stimulation of the specific afferent pathway (Fig. 10), which are characteristically associated with cholinoceptive neurones (Krnjević & Phillis, 1963*b*), there was no block of early discharges related to the primary evoked response (Fig. 11).

Some anaesthetics were also injected systemically while single cholino-

ceptive units were being observed. Barbiturates such as Dial and sodium pentobarbitone tended to produce an initial excitation, lasting for about 1 min, followed by a substantial and prolonged reduction in excitability. The cells could still be fired with L-glutamate, though less effectively than before; but some cholinoceptive cells now could no longer be made to discharge impulses with ACh, although it was possible to show a subthreshold excitant action by applying L-glutamate as well, which then triggered off a typical, prolonged ACh discharge (cf. Fig. 1 in Krnjević & Phillis, 1963*b*). This observation is of some importance, since it shows that cholinoceptive cells may not always be easily identified during barbiturate anaesthesia.

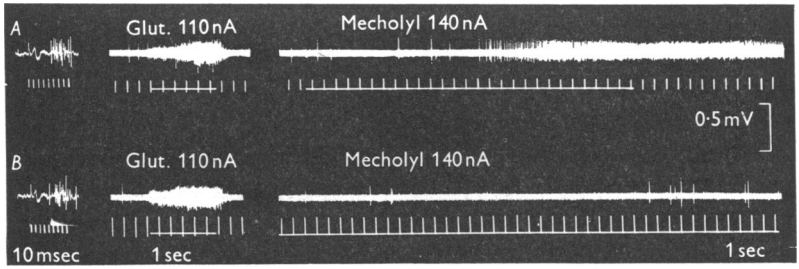

Fig. 11. Cholinoceptive unit giving responses to contralateral forepaw stimulation after comparatively short latency (*A*). 15 min after intravenous atropine sulphate (1 mg/kg) responses were still evoked strongly by peripheral stimulus or by L-glutamate, but unit was no longer sensitive to acetyl-β-methylcholine (mecholyl). Responses evoked by peripheral stimulation were enhanced by previous dose of chloralose, which reduced spontaneous activity but did not interfere with cholinoceptive property. Cat under Dial.

Chloralose was of particular interest because the spontaneous release of ACh in the cortex which is observed under many conditions cannot be detected if the animal is anaesthetized with this substance (MacIntosh & Oborin, 1953; Mitchell, 1963). It has also been shown that chloralose blocks the cholinergic synapses between motor axon collaterals and Renshaw cells in the spinal cord (Haase & van der Meulen, 1961). These observations suggest that one action of chloralose may be to interfere with the release of ACh at cholinergic synapses.

We have found that chloralose injected intravenously (20–50 mg/kg) sharply depressed the synaptic activity of cholinoceptive cells, inhibiting the characteristic spontaneous and evoked firing; this effect developed slowly over a period of about 10 min and persisted for as long as the observations could be continued (over 30 min). Thus the cholinoceptive unit shown in Fig. 12 initially gave clear repetitive discharges in response to single electrical stimuli applied to the ipsilateral ventral posterolateral nucleus of the thalamus (*A*) or the contralateral forepaw (*B*). Twenty

minutes after an intravenous dose of chloralose these repetitive responses could no longer be evoked (*E* and *F*), even though the unit had remained sensitive to L-glutamate and ACh (*G* and *H*).

It must be emphasized that chloralose did not block all the synaptic activity of these cells. On the contrary, it had a marked facilitatory effect on early discharges which tend to occur soon after the primary evoked responses (as in Fig. 11). Larger doses of chloralose caused a more pronounced diminution of general excitability, with a sharp reduction or even disappearance of ACh responses.

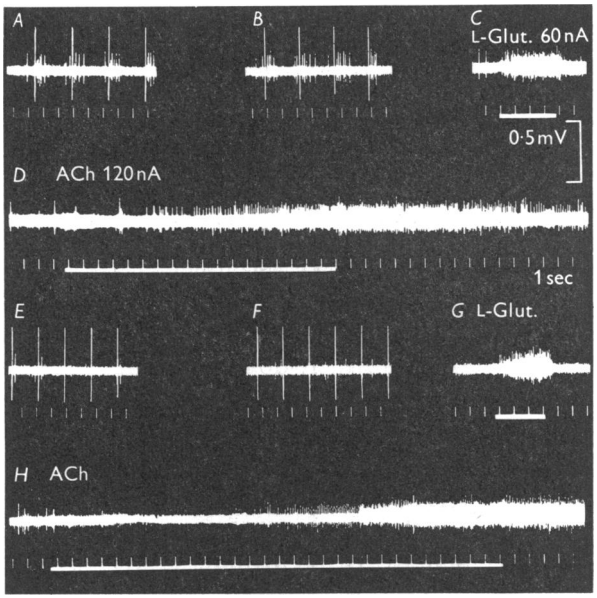

Fig. 12. Some effects of intravenous chloralose (20 mg/kg) on cholinoceptive unit in cortex of cat under Dial. *A–D*, control records, including repetitive responses evoked by electrical stimulation (with single pulses) of ventral posterolateral nucleus of thalamus (*A*) and contralateral forepaw (*B*). *E–G*, corresponding records taken 20 min after injection of chloralose. There was no return of either spontaneous activity or thalamic evoked responses in next 30 min.

DISCUSSION

The properties of cholinoceptive cells in the cerebral cortex are clearly very different from those of Renshaw cells in the spinal cord. The strong actions of acetyl-β-methylcholine, muscarine and muscarone, together with the ineffectiveness of nicotine, give unequivocal evidence of receptors with a remarkably well defined muscarinic character, apparently quite insensitive to nicotinic substances. Ever since the first studies of acetyl-β-methylcholine (Simonart, 1932; Hunt & Renshaw, 1934) it has been known

to have muscarinic actions comparable with those of acetylcholine, but practically none of the nicotinic actions. The most potent substance tested was muscarone, which has even stronger muscarinic properties than muscarine itself (Waser, 1961). The difference between the muscarine and nicotine sensitivity is also shown rather clearly by the contrasting actions of two other choline esters, propionyl- and butyrylcholine, both of which have nicotinic actions rather similar to those of ACh; their muscarinic actions, however, differ considerably, propionylcholine being about $\frac{1}{5}$th as active as ACh in this respect and butyrylcholine less than $\frac{1}{100}$ as active (Simonart, 1932).

Many previous authors have described various changes in cerebral function caused by the administration of ACh and it was generally believed that muscarinic effects play a predominant role (see reviews by Feldberg, 1945; Stone, 1957; Mikhelson, Savateev, Rozhkova & Lukomskaya, 1957). This has been the source of much difficulty in the interpretation of results, since any direct action on nerve cells could not be dissociated with certainty from secondary actions arising on account of vascular changes (Schlag, 1956). Muscarine and arecoline have been shown to have quite marked central actions (Riehl & Unna, 1960; Herz, 1962); although arecoline is much weaker than muscarine it is often used because of its relatively negligible peripheral actions, which do not interfere seriously with the observation of central changes.

The most important single criterion for identifying muscarinic actions is probably antagonism by atropine (Dale, 1914). With only a few exceptions (e.g. Brenner & Merrit, 1942) earlier studies of the effects of ACh on the brain have shown rather consistently a strong block by atropine (Miller, Stavraky & Woonton, 1940; Arduini & Machne, 1949; Bremer & Chatonnet, 1949; Chatfield & Purpura, 1954; Rinaldi & Himwich, 1955; Anichkov, 1959; Loeb, Magni & Rossi, 1960).

It has been suggested that many of the actions of atropine in the central nervous system may be unspecific, depending on its local anaesthetic properties, and therefore bearing little or no relation to the block of cholinergic synapses (Curtis & Phillis, 1960). Although atropine undoubtedly has an non-selective blocking action of this type in the cortex, such relatively large doses are required that it is unlikely that an adequate concentration would be achieved when it is given in the usual manner, i.e. by intravenous injections of about 1 mg/kg. However, this unspecific effect may account for some double actions of atropine (Chatfield & Purpura, 1954). On the other hand, we have shown that such amounts of atropine in the blood stream do markedly reduce the ACh sensitivity of cortical cholinoceptive neurones. At the same time, the normal spontaneous activity is much diminished, and the characteristic repetitive discharge elicited by electrical

stimulation of the afferent pathway at the periphery, in the thalamus or in the internal capsule is abolished (Figs. 10 and 11). It is therefore probable that most of the cortical changes usually produced by intravenous injections of atropine are due to a block of cholinoceptive neurones.

It should not be surprising that atropine and hyoscine released iontophoretically did not cause a prolonged block of synaptic activity as compared with the long-lasting depression in the responsiveness to local applications of ACh. The latter would be affected much more because the atropine and the ACh would have a similar distribution in the tissues, being applied in a similar way and would therefore react with the same receptors. Synapses are likely to be spread over a much wider area of the cell than can be reached by effective amounts of atropine especially if diffusion is slowed by various obstructions. Moreover, the concentration of the supposed cholinergic transmitter at the synapse would be much higher than that produced by iontophoretic release: this is probably an important factor, since the action of atropine is not 'all-or-none'; a substantial increase in the amount of ACh applied can overcome a partial block.

In this context it should be noted that the synaptic responses of Renshaw cells evoked by stimulating ventral roots cannot be blocked completely with dihydro-β-erythroidine applied by iontophoresis (Curtis & Eccles, 1958), and it is a general observation at many synapses that ACh responses are depressed much more readily than the effects produced by indirect stimulation (Dale & Gaddum, 1930; Dale, 1938; Ambache, 1955; Ursillo & Clark, 1956).

The prolonged effect of ACh, which is in marked contrast to the quickly reversible excitation by L-glutamate, together with the relatively unimportant actions of anticholinesterases, suggest that ACh remains attached to the receptors, out of reach of cholinesterases for a substantial time. It may be relevant that cholinesterase in the cortex has been seen mainly inside cells, in particular in glia (Koelle, 1950; Smusin, 1957). The apparent substantial binding between the receptors and ACh may account for the increase in the output of ACh from the cortex caused by atropine (MacIntosh & Oborin, 1953; Mitchell, 1963), just as sympathetic blocking agents increase the release of transmitter from the spleen (Brown & Gillespie, 1957).

The interesting actions of chloralose are consistent with the possibility that chloralose may interfere with the release of ACh (MacIntosh & Oborin, 1953; Mitchell, 1963). Like barbiturates, chloralose in relatively large doses reduces the excitability of the cells and thus the responses to ACh. In agreement with Adrian's (1941) finding that cortical repetitive responses are seen during light anaesthesia induced by various agents but not in the presence of chloralose, we have observed that spontaneous and evoked

activity of the supposed cholinergic pathway could be suppressed by chloralose without any marked change in responsiveness to ACh. This must be due to a reduced output of transmitter, but one cannot of course be sure that there has not been a block of transmission at an intermediate synapse in the cortex. A direct effect on ACh release is suggested by the blocking action of chloralose on the monosynaptic recurrent-collateral Renshaw-cell pathway (Haase & van der Meulen, 1961). On the other hand, chloralose greatly enhances transmission through a short-latency link between the afferent pathway and the Betz cells (as shown originally by Adrian & Moruzzi, 1939) presumably by increasing the cell's responses to dendritic activation at the level of the afferent arborization in layers III and IV. This synaptic link is apparently not cholinergic, to judge by its resistance to atropine (Fig. 11).

These results, therefore, seem to be clear evidence of the presence in the cortex of cholinoceptive cells with an undoubted muscarinic character and they are consistent with the possibility that these cells are associated with cholinergic synapses. These cortical neurones thus form a striking contrast to more peripheral ACh-sensitive neurones, such as Renshaw cells, autonomic ganglion cells and non-myelinated nerve fibres (Armett & Ritchie, 1961), all of which have predominantly nicotinic receptors. The rather widespread anticholinergic actions of atropine suggest that other cholinoceptive neurones in the brain may also tend to have muscarinic properties (such as those in the hypothalamus described recently by Stein & Seifter, 1962). The nicotinic responses of the Renshaw cells may in fact be rather exceptional in the central nervous system, though it is not at all clear how they have come to resemble peripheral neurones and muscle fibres in this respect rather than many other central cholinoceptive cells, unless one postulates that the presynaptic fibre can determine the pharmacological properties of the receptor in some way. However, it must be admitted that there is no conclusive evidence at present that the ACh-like substances released in muscle (Dale, Feldberg & Vogt, 1936; Emmelin & MacIntosh, 1956; Krnjević & Mitchell, 1961) and in the cerebral cortex (MacIntosh & Oborin 1953; Mitchell, 1963) are absolutely identical.

It is interesting that the action of ACh on cortical cells is thoroughly typical of muscarinic actions in general. According to Dale (1938), these are chiefly concerned with modifications of spontaneous activity; the effects have a long latency, rise slowly to a maximum and outlast the period of application. Whatever the possible function of ACh in prolonging and disseminating afferent activity in the cortex, this type of effect would clearly be of relatively little use in the Renshaw inhibitory loop, whose stabilizing action on muscular activity (Granit, Haase & Rutledge, 1960) must benefit from sharp timing.

SUMMARY

1. The pharmacological properties of ACh-sensitive cells in the cerebral cortex of cats (and also some rabbits and monkeys) mainly under Dial anaesthesia, have been investigated by the local iontophoretic release of various substances from multibarrelled micropipettes.

2. The most effective cholinomimetics were muscarone, muscarine and acetyl-β-methylcholine; substantial excitation was produced by several other choline esters, including propionylcholine, acrylylcholine, nicotinoylcholine, carbaminoylcholine and succinylcholine, but not butyrylcholine, and also by pilocarpine, arecoline and oxo-tremorine. Nicotine and some other ganglionic excitants had no specific action on these cells.

3. Tensilon and prostigmine potentiated the effects of ACh; in large doses they also excited.

4. Excitation by ACh was prevented very effectively by atropine, hyoscine and, to a lesser extent, by gallamine; several central anticholinergic drugs, such as benactyzine and caramiphen, also caused some depression. Tubocurarine, dihydro-β-erythroidine, toxiferin and hexamethonium were mostly ineffective.

5. From the evidence given in (2) and (4) it was concluded that cortical ACh receptors have a strongly muscarinic character.

6. Cholinoceptive cells are not markedly different from other cortical neurones with respect to the excitatory and inhibitory actions of various amino acids and catechol and indole amines.

7. The effects of potentiating and antagonistic drugs on spontaneous activity and on certain responses evoked indirectly are consistent with the possibility that cholinoceptive cells are supplied by cholinergic nerve fibres.

8. The activity and the ACh-sensitivity of these cells are strongly depressed by intravenous injections of atropine (1 mg/kg). Chloralose (20–50 mg/kg, I.V.) reduces their characteristic spontaneous and evoked activity without much change in ACh responses; this is in agreement with other evidence that chloralose may depress specifically the release of ACh at cholinergic synapses in the central nervous system.

REFERENCES

ADRIAN, E. D. (1941). Afferent discharges to the cerebral cortex from peripheral sense organs. *J. Physiol.* **100**, 159–191.

ADRIAN, E. D. & MORUZZI, G. (1939). Impulses in the pyramidal tract. *J. Physiol.* **97**, 153–199.

AMBACHE, N. (1955). The use and limitations of atropine for pharmacological studies on autonomic effectors. *Pharmacol. Rev.* **7**, 467–494.

ANICHKOV, S. V. (1959). Pharmacology of the central cholinergic synapses. Pp. 23–27 in Symposia and special lectures. *XXI int. physiol. Congr., Buenos Aires.*

ARDUINI, A. & MACHNE, X. (1949). Sul meccanismo e sul significato del azzione convulsivante dell'acetilcolina. *Arch. fisiol.* **48**, 152–167.

ARMETT, C. J. & RITCHIE, J. M. (1961). The action of acetylcholine and some related substances on conduction in mammalian non-myelinated nerve fibres. *J. Physiol.* **155**, 372–384.

BREMER, F. & CHATONNET, J. (1949). Acétylcholine et cortex cérébral. *Arch. int. Physiol.* **57**, 106–109.

BRENNER, C. & MERRITT, H. H. (1942). Effect of certain choline derivatives on electrical activity of the cortex. *Arch. Neurol. Psychiat., Chicago*, **42**, 382–395.

BROWN, G. J. & GILLESPIE, J. S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol.* **138**, 81–102.

BURN, J. H. (1945). The relation of adrenaline to acetylcholine in the nervous system. *Physiol. Rev.* **25**, 377–394.

BURN, J. H. & DALE, H. H. (1915). The action of certain quaternary ammonium bases. *J. Pharmacol.* **6**, 417–438.

CHATFIELD, P. O. & PURPURA, D. P. (1954). Augmentation of evoked cortical potentials by topical application of prostigmine and acetylcholine after atropinization of cortex. *Electroenceph. clin. Neurophysiol.* **6**, 287–298.

CHEN, G., PORTMAN, R. & WICKEL, A. (1951). Pharmacology of 1-1dimethyl-4-phenylpiperazinium iodide, a ganglion stimulating agent. *J. Pharmacol.* **103**, 330–336.

CURTIS, D. R. & ECCLES, R. M. (1958). The excitation of Renshaw cells by pharmacological agents applied electrophoretically. *J. Physiol.* **141**, 435–445.

CURTIS, D. R. & KOIZUMI, K. (1961). Chemical transmitter substances in brain stem of cat. *J. Neurophysiol.* **24**, 80–90.

CURTIS, D. R. & PHILLIS, J. W. (1960). The action of procaine and atropine on spinal neurones. *J. Physiol.* **153**, 17–34.

CURTIS, D. R., PHILLIS, J. W. & WATKINS, J. C. (1961). Cholinergic and non-cholinergic transmission in the mammalian spinal cord. *J. Physiol.* **158**, 296–323.

DALE, H. H. (1914). The action of certain esters and ethers of choline and their relation to muscarine. *J. Pharmacol.* **6**, 147–190.

DALE, H. H. (1938). Acetylcholine as a chemical transmitter of the effects of nerve impulses. *J. Mt. Sinai Hosp.* **4**, 401–429.

DALE, H. H., FELDBERG, W. & VOGT, M. (1936). Release of acetylcholine at voluntary motor nerve endings. *J. Physiol.* **86**, 353–380.

DALE, H. H. & GADDUM, J. H. (1930). Reactions of denervated voluntary muscle, and their bearing on the mode of action of parasympathetic and related nerves. *J. Physiol.* **70**, 109–144.

DEL CASTILLO, J. & KATZ, B. (1957). A study of curare action with an electrical method. *Proc. Roy. Soc.* B, **146**, 339–356.

ECCLES, J. E., ECCLES, R. M. & FATT, P. (1956). Pharmacological investigations on a central synapse operated by acetylcholine. *J. Physiol.* **131**, 154–169.

ECCLES, J. C., FATT, P. & KOKETSU, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor axon collaterals to motoneurones. *J. Physiol.* **126**, 524–562.

EMMELIN, N. & MACINTOSH, F. C. (1956). The release of acetylcholine from perfused sympathetic ganglia and skeletal muscles. *J. Physiol.* **131**, 477–496.

FELDBERG, W. (1945). Present views on the mode of action of acetylcholine in the central nervous system. *Physiol. Rev.* **25**, 596–642.

FURUKAWA, T. (1957). Properties of the procaine end-plate potential. *Jap. J. Physiol.* **7**, 199–212.

GRANIT, R., HAASE, J. & RUTLEDGE, L. T. (1960). Recurrent inhibition in relation to frequency of firing and limitation of discharge rate of extensor motoneurones. *J. Physiol.* **154**, 308–328.

HAASE, J. & VAN DER MEULEN, J. P. (1961). Die spezifische Wirkung der Chloralose auf die recurrente Inhibition tonischer Motoneurone. *Pflüg. Arch. ges. Physiol.* **274**, 272–280.

HASLETT, W. L. & JENDEN, D. J. (1961). Pharmacological properties of oxotremorine, an active metabolite of tremorine. *Pharmacologist*, **3**, 70.

HERZ, A. (1962). Wirkungen des Arecolins auf des Zentralnervensystem. *Arch. exp. Path. Pharmak.* **242**, 414–429.

HODES, R. (1962). Electrocortical synchronization resulting from reduced proprioceptive drive caused by neuromuscular blocking agents. *Electroenceph. clin. Neurophysiol.* **14**, 220–232.

HUNT, R. & RENSHAW, R. R. (1934). Further studies on the methyl cholines and analogous compounds. *J. Pharmacol.* **51**, 237–262.

KOELLE, G. B. (1950). The histochemical differentiation of types of cholinesterases and their localizations in tissues of the cat. *J. Pharmacol.* **100**, 158–179.

KRNJEVIĆ, K. & MILEDI, R. (1958). Some effects of adrenaline upon neuromuscular propagation in rats. *J. Physiol.* **141**, 291–304.

KRNJEVIĆ, K. & MITCHELL, J. F. (1961). The release of acetylcholine in the isolated rat diaphragm. *J. Physiol.* **155**, 246–262.

KRNJEVIC, K., MITCHELL, J. F. & SZERB, J. C. (1963). Determination of iontophoretic release of acetylcholine from micropipettes. *J. Physiol.* **165**, 421–436.

KRNJEVIĆ, K. & PHILLIS, J. W. (1961). Sensitivity of cortical neurones to acetylcholine. *Experientia*, **17**, 469.

KRNJEVIĆ, K. & PHILLIS, J. W. (1962). Excitation of Betz cells by acetylcholine. *Experientia*, **18**, 170–171.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*a*). Iontophoretic studies of neurones in the mammalian cerebral cortex. *J. Physiol.* **165**, 274–304.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*b*). Acetylcholine sensitive cells in the cerebral cortex. *J. Physiol.* **166**, 296–327.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963*c*). Actions of certain amines on cerebral cortical neurones. *Brit. J. Pharmacol.* (in the Press).

LAITY, J. R. L. & GARG, B. K. (1962). A note on the action of gallamine on isolated rabbit auricles. *J. Pharm. Lond.*, **13**, 93–97.

LOEB, C., MAGNI, F. & ROSSI, G. F. (1960). Electrophysiological analysis of the action of atropine on the central nervous system. *Arch. ital. Biol.* **98**, 293–307.

MACINTOSH, F. C. & OBORIN, P. E. (1953). Release of acetylcholine from intact cerebral cortex. *Abstr. XIX int. physiol. Congr.* 580–581.

MIKHELSON, M. Y., SAVATEEV, I. V., ROZHKOVA, E. K. & LUKOMSKAYA, I. Y. (1957). O roli atsetilkholine v osuschhestvlenii vysshei nervnoi deyatelnosti in '*Fiziologicheskaya rol' atsetilkholina i iziskanie novikh lekarstvenn'ikh veshchestv.*' P. 25–33. Ed. MIKHELSON, M. Y. Leningrad: Leningrad Medical Institute.

MILLER, F. R., STAVRAKY, G. W. & WOONTON, G. A. (1940). Effects of eserine, acetylcholine and atropine on the electrocorticogram. *J. Neurophysiol.* **3**, 131–138.

MITCHELL, J. F. (1963). The spontaneous and evoked release of acetylcholine from cerebral cortex. *J. Physiol.* **165**, 98–116.

PATON, W. D. M. & ZAIMIS, E. J. (1952). The methonium compounds. *Pharmacol. Rev.* **4**, 219–253.

RANDALL, L. O. (1951). Synthetic curare-like agents and their antagonists. *Ann. N.Y. Acad. Sci.* **54**, 460–474.

RIEHL, J. L. & UNNA, K. R. (1960). Effects of muscarine on the central nervous system. *Recent. Adv. biol. Psychiat.* **2**, 345–361.

RIKER, W. F. & WESCOE, W. C. (1951). The pharmacology of flaxedil, with observations on certain analogs. *Ann. N.Y. Acad. Sci.* **54**, 373–392.

RINALDI, F. & HIMWICH, H. E. (1955). Alerting responses and actions of atropine and cholinergic drugs. *Arch. Neurol. Psychiat., Chicago*, **73**, 387–395.

ROBERTS, J., ROY, B. B., REILLY, J., GARB, S., RIKER, W. F. & HASHIMOTO, K. (1955). The use of certain neuromuscular blocking drugs in an analysis of ventricular irregularities induced in the cat. *J. Pharmacol.* **114**, 279–288.

SCHLAG, J. D. A. (1956). Effect of drugs on pyramidal discharges evoked by cortical stimulation. *Electroenceph. clin. Neurophysiol.* **8**, 421–425.

SIMONART, A. (1932). On the action of certain derivatives of choline. *J. Pharmacol.* **46**, 157–193.

SMUSIN, Y. S. (1957). Gistokhimicheskoe opredelenie aktivnosti kholinesterazy pri eksperimental'nom otravlenii narkotikami in '*Fiziologischeskaya rol' atsetilkholina i iziskanie novikh lekarstvenn'ikh veshchestv*, p. 148–153. Ed. MIKHELSON, M. Y. Leningrad: Leningrad Medical Institute.

STEIN, L. & SEIFTER, J. (1962). Muscarinic synapses in the hypothalamus. *Amer. J. Physiol.* **202**, 751–756.

STONE, W. E. (1957). The role of acetylcholine in brain metabolism and function. *Amer. J. phys. Med.* **36**, 222–255.

UEKI, S., KOKETSU, K. & DOMINO, E. F. (1961). Effects of mecamylamine on the Golgi recurrent collateral—Renshaw cell synapse in the spinal cord. *Exper. Neurol.* **3**, 141–148.

URSILLO, R. C. & CLARK, B. C. (1956). The action of atropine on the urinary bladder of the dog and on the isolated nerve-bladder strip preparation of the rabbit. *J. Pharmacol.* **118**, 338–347.

WASER, P. G. (1961). Chemistry and pharmacology of muscarine, muscarone and some related compounds. *Pharmacol. Rev.* **13**, 465–515.