REVIEW ARTICLE

THE CHOLINERGIC RECEPTOR*

BY PETER G. WASER, Prof.Dr.Med., Dr.Phil.

Department of Pharmacology, University of Zürich

SINCE the time when a knowledge of anatomical structure first offered a contribution to the understanding of the functioning of the living body, advances in physiology, and later in biochemistry, helped to further our understanding of the basic principles of life. This approach in biological research has remained the same in modern times. As we try to solve more and more problems of ultrastructural dimensions we still follow the same road that leads from morphology to function. Perhaps one day we will come to the point where we can see molecules and ions in action in a living tissue or organ. Then the two routes will meet and we will have complete understanding of a biological phenomenon. The final goal of all our endeavours in research will therefore be to link morphology with function.

Today good tools have been developed with which to attack the basic problems of this sort. Microscopy has been surpassed by electron microscopy; simple electrodes for measuring action potentials by micro-electrodes, which can be placed inside single cells. Chemical compounds can be labelled with radioactive atoms and followed in the body to their site of action. Enzymes can be measured in single cells or even in microsomes. These are only a few of the many ingenious methods used in modern laboratories for biological research. In the instance I propose to discuss we have applied some of these methods to the elucidation of the nature of the cholinergic receptor.

Acetylcholine as a Neurohormone

A main problem of biology is the phenomenon of nerve activity, nerve conduction and synaptic transmission. In the last century anatomy described different nervous systems: the central nervous system, the peripheral motor and sensory nerves and the autonomic nervous system. But soon they were shown to be similar in many physiological and biochemical properties. One of these properties is the action of acetylcholine as a vital part of their functional mechanism. Early in this century it was found that choline and muscarine have many properties in common, notably the lowering of blood pressure and pulse rate, and the increase of intestinal peristalsis and glandular secretion. As different derivatives of choline were studied, Reid Hunt[1] reported in 1906 that the acetyl ester of choline has an activity many thousand times greater than choline. After the work of Sir Henry Dale[2] on the blocking of the muscarinic action of choline esters by atropine, Otto Loewi in 1921[3],

* Based on a Special London University Lecture. Lecture given at King's College, London, on 26th May, 1960.

demonstrated the liberation of a chemical substance which caused bradycardia after stimulation of the mixed vagus nerve, with his famous experiment using the crossed circulation of two frog hearts. This neurohormone was later shown to be acetylcholine. It was found in many other synapses, where it transmits the nerve impulse: in the superior ganglion cervical, the adrenal medulla, at the effector cells of the sweat glands, the myoneural junction and in motor and sensory neurones of the central nervous system.

The Cholinergic Transmitter

Acheson in 1948[4], in his review of the chemical aspects of neuromuscular junctions, summarised the quantitative results of different research workers who had determined the output of acetylcholine per nerve impulse for each nerve ending (Table I). The amount of acetylcholine

TABLE I

CALCULATION OF OUTPUT OF ACETYLCHOLINE PER NERVE IMPULSE FOR EACH NERVE END (AFTER ACHESON[4])

	Acetylcholine released per volley	Estimated number of endings of fibres	Acetylcholine released per nerve ending per impulse
Perfused organs:			
Cat tongue[35]	10^{-4} µg.	720,000	$1{\cdot}4 \times 10^{-10}$ µg./ endplate
Cat cervical ganglion[36]	10^{-4} µg.	1,000,000	1×10^{-10} µg./ synapse
Cut end of nerve trunk:			
Frog sciatic[37,38]	2×10^{-7} µg.	1,000	2×10^{-10} µg./ fibre
Quick frozen nerve trunk:			
Frog sciatic[39]	10^{-8} µg. per µ length		$1{\cdot}5 \times 10^{-10}$ µg./ endplate

released by one impulse is $1{\cdot}5 \times 10^{-10}$ µg., and what seems to be very important, it is independent of the substrate, endplate or ganglionic synapse. When we calculate the number of molecules acting in a free state at this moment, we find the relatively small number of 6×10^5. As part of the acetylcholine might be destroyed by cholinesterase in spite of neostigmine, present during the experiments, the real number would be likely to be larger, perhaps 10^6 molecules of acetylcholine.

With another method Buchtal and Lindhard, in 1942[5], found a much higher minimal dose, 5 ng., of acetylcholine applied to the endplate of a single muscle fibre of the lizard was needed to produce a contraction. But as the volume added to the endplate was a droplet of about 50 times the volume of the endplate, the need for the large amount may be explained by diffusion effects and immediate enzymatic breakdown of most of the acetylcholine. The same must be said for the results from close arterial injection. The best results, so far, have been obtained by del Castillo and Katz[6] with their elegant technique of electrophoretic application of acetylcholine to the external surface of a frog muscle endplate at a distance of $10 - 20\,\mu$. With $10^{-15} - 10^{-16}$ moles of acetylcholine, or $6 \times 10^7 - 10^8$ molecules; under these conditions depolarisation was obtained. But

acetylcholine release into the interior of the muscle fibre failed to depolarise the endplate and to excite the muscle. Here the effective number of externally applied molecules might be smaller, as the distance between nerve ending and subneural membrane is less than 0·1 μ, and therefore fewer molecules may suffice for an immediate contact with the membrane. I believe an average minimal number of 10^6 molecules of acetylcholine is required for depolarisation of the membrane. To my knowledge no measurements of this kind have been made at ganglionic synapses.

Indirect proof for the vital action of acetylcholine in the endplate was given by Nachmansohn in 1939[7] in his investigation of acetylcholinesterase in muscle. He found a very high concentration of this enzyme right in the endplate, whereas the muscle itself had a low enzymatic activity. The endplate of frog sartorius contains enough activity to split $1{\cdot}6 \times 10^9$ molecules of acetylcholine in 1 millisecond, or a thousand times more than that needed for depolarisation. The cholinesterase in the endplates may be stained histochemically (Koelle)[8]. The same proof is possible with ganglionic cells of the ciliary ganglion (Fig. 1), or of some regions in the brain and in many other places, as for instance in Pacinian corpuscles[9].

In addition to the splitting enzyme, the synthesising enzyme, choline acetylase, was detected at the same site[10]. Its distribution in the central nervous system was studied extensively by Feldberg and Vogt[11]. Beside acetylcholine other cholinergic transmitters, propionylcholine, butyrylcholine and murexine, are present in different animals and organs, but their action is not yet understood.

We must therefore conclude that acetylcholine plays an important role in the synaptic transmission in various anatomical substrates. With the exception of sensory fibres and postganglionic sympathetic or adrenergic neurones, all the nerves of the mammalian peripheral nervous system are probably cholinergic, liberating acetylcholine. Just as in the peripheral nervous system, the concentrations of acetylcholine, cholinesterase and cholineacetylase run nearly parallel to each other in different regions of the brain and spinal cord.

Even in primitive animals acetylcholine plays an important role. The heart of the mollusc, murex brandaris, octopus, venus mercenaria, mytilus, or helix pomatia, is slowed by its action and the amplitude is diminished. In annelids, lumbricus terrestris, it shows an exciting and tachycardic effect. In insects the important action of organophosphorus insecticides may be explained by irreversible blocking of cholinesterase and death by maximum synaptic stimulation by acetylcholine.

The reason for the importance of acetylcholine in synaptic transmission is that this neurotransmitter depolarises the synaptic membrane. This causes physical events which may be measured in various ways. These include the measurement of action potentials (Fig. 2), ion movements through the membrane, especially of radioactive potassium and sodium, and even heat production and temperature changes. The activating mechanism, may be described as a rise in permeability of the membrane. Due to this change, ions may pass freely from inside the cell to the outside and the reverse. This happens in cholinergic neurons having synapses

with other ganglionic cells, muscle fibres, striated, smooth or cardiac muscle, or even with secretory cells. By far the largest number of the synapses in the mammalian body use this mechanism and only a small number work with other neurotransmitters like noradrenaline, adrenaline and probably other unknown neurohormones.

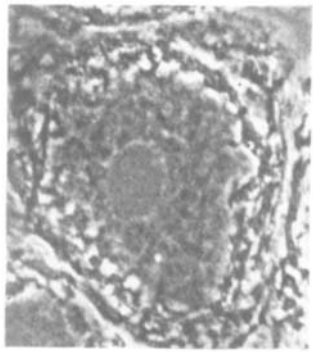


FIG. 1. Ganglion-cell with acetylcholinesterase (phase contrast) of ciliary ganglion of cat[40].

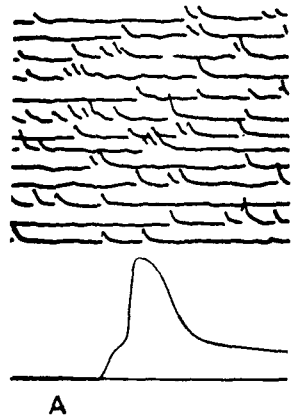


FIG. 2. Spontaneous subthreshold activity at motor nerve-ending (above) and action potential after stimulation of nerve (below)[41].

The main questions to be discussed are:

(i) what is the substrate on which acetylcholine acts? and

(ii) what is the change of this substrate by depolarisation?

For our problem it is very important that a ganglionic synapse or an endplate are representative for other cholinergic synapses, even central ones. Investigations are much easier with peripheral organs which may be isolated and studied under controlled conditions.

There is excellent evidence for a specialised function of the endplate region. Langley demonstrated its stimulation by nicotine, which curare prevented. He proposed the existence of a specialised material, which he designated the "receptive substance". The same region in an isolated fibre is depolarised by very small concentrations of acetylcholine or nicotine, the rest of the surface of the muscle being at least a thousand times less sensitive to these substances.

The Use of Labelled Curarine

We are interested in the action of curare and depolarising drugs on endplates, and we hope to get more information on the cholinergic receptor substance by labelling different molecules and by tracing them to their place of action[13–15]. ^{14}C-calabash curarine (Fig. 3) was first synthesised and extensive studies of its metabolism in cats were made[16]. This alkaloid has a strictly selective action on muscle, yet the concentration in muscle of cats paralysed by the minimal effective dose was below 0·2 μg./g.

Furthermore, curarine was metabolised slowly in the animal body, one-third being excreted unchanged in the urine within 3 hours. The paralysing action of the low concentration in the skeletal muscle was traced by autoradiographic analysis.

Mouse Diaphragm Autoradiographs

The biological assay system used was simple. We injected the minimal lethal dose of an aqueous solution into the tail veins of mice, which died

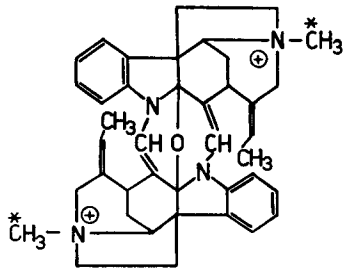


FIG. 3. Formula of ^{14}C-calabash curarine.

of asphyxia within one to two minutes. Immediately after respiration had stopped they were decapitated and bled to diminish the interfering radioactive blood volume as far as possible. Then the diaphragms were isolated and stretched over steel rings of appropriate diameter (Fig. 4).

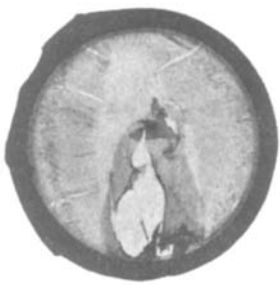


FIG. 4. Mouse-diaphragm mounted on steel ring. Band of endplates with staining of cholinesterase[8] around centrum tendineum.

FIG. 5. Autoradiograph with ^{14}C-curarine localised in the endplates, in the central tendon and in some central vessels. (Exposure: 5 months, Ilford film PM2.)

The 100 μ thick diaphragms were then dried in air for a few hours, cut away from the steel rings and placed on X-ray film or mounted on microscope slides and covered by stripping film. Two to six months later the contact films were developed under controlled conditions. In addition, the endplates in the diaphragms were separately stained for cholinesterase by Koelle's method[8].

The endplates are seen to be arranged in a circular band around the central tendon (Fig. 5). The details of this band, which is often split in

different parts, especially on the right side, show up beautifully in the autoradiographs. We can distinguish the individual endplates, the average diameter being only 8–18 μ. There is always some background fog on the film which gets stronger with long exposure. In the middle of the diaphragms blackening by radioactive blood within the vessels and in the tendon is seen. Increased radioactivity at the periphery is due to small vessels reaching the endplate band from the periphery.

The stripping film showed an accumulation of silver grains directly over the endplates (Fig. 6). Because of the heavy cross-fire from deeply situated radioactive endplates in the diaphragm there was a large number of grains in the muscle tissue between them. The radioactivity responsible for this originated mainly in the synaptic structures, because on the stripping film, four times as many silver grains were counted over the endplate band than over the endplate free central part of the muscle.

To get an idea of the distribution in a cross-section through the diaphragm, diagrams of grain density were made (Fig. 7). Approaching the endplate region from the outside there is first some radioactivity caused by small peripheral vessels originating from the ribs. Then follows the main activity due to the endplate region, and finally a third peak due to the blood in the large vessels around and in the central tendon. The total amount of silver grains in the endplate region was integrated and the grains due to radioactivity in the muscle tissue, in the blood of capillaries and due to background fog were subtracted. The average radioactivity of one endplate was calculated by dividing this remaining activity by the number of endplates in the region considered. For this one must use an empirical formula. The radioactivity of one endplate corresponds to the number of curarine molecules reacting with the receptor surface. We have estimated in this way that 8×10^6 molecules of curarine are bound to one endplate.

With a later and much better method we have now determined this number again. Comparing for calibration purpose the autoradiographs of diaphragms with artificial discs made of gelatine of the same weight and thickness and varying concentrations of radiocurarine, the concentration of curarine in one endplate can be directly measured by densitometer recordings of the film. The number of molecules may then be calculated without empirical factors as 3×10^6. This approximate calculated figure agrees fairly well with the number of acetylcholine molecules needed for synaptic transmission. Since at most one receptor can be stimulated by one molecule of acetylcholine, with the minimal paralytic dose of curarine, less than 10^6 receptors will remain free. Therefore the total number of cholinergic receptors in one endplate is probably a little larger. It might well be that one endplate has a reserve of receptors not all of which are usually needed, like the large reserve of cholinesterase for the destruction of the liberated acetylcholine. Our experiments with neostigmine may later confirm this point.

The figure of $3 - 4 \times 10^6$ for the cholinergic receptors at one endplate is in fact small when the molecular volume of curarine (mol. wt. 853) is compared with the large surface of the subneural membrane. With the

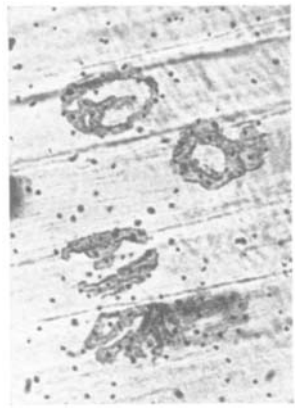


FIG. 6. Endplates of diaphragm with stripping film × 1,000 (Kodak AR10). (Exposure: 60 days.)

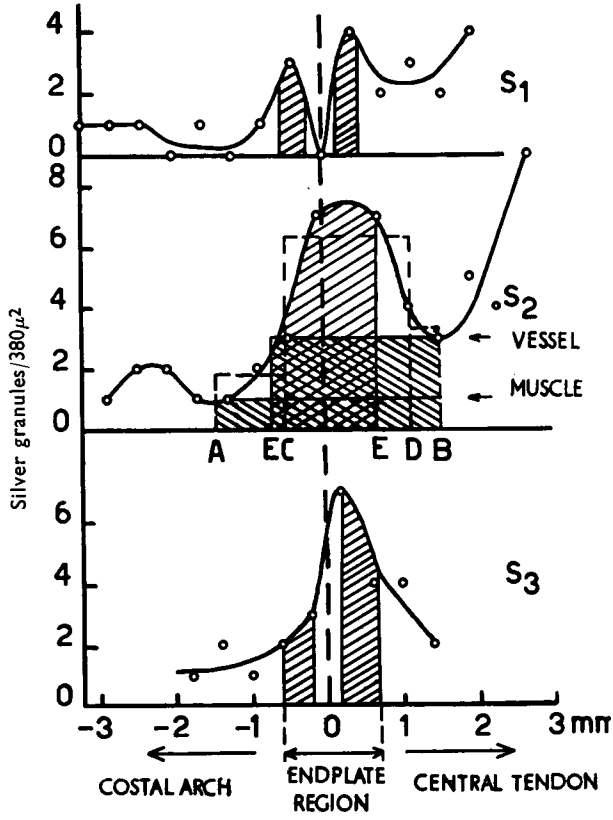


FIG. 7. Diagram of grain density at three different cross-sections through diaphragm. ^{14}C-curarine.

help of the electron microscope it was found by various authors[17] that the palisade structure of Couteaux[18] consists of many folds (Fig. 8). 6×10^{11} molecules of curarine would be present in 1 cm.^{2}, if we consider only the surface of the endplate without any folds. But on the same surface we could place up to 10^{14} molecules side by side in a monolayer. Therefore the coverage of the fold entrance area must be less than 1 per cent, and of the whole postsynaptic surface area considerably less than 1 per cent. Andersson-Cedergren[19] determined the percentage of fold entrance area

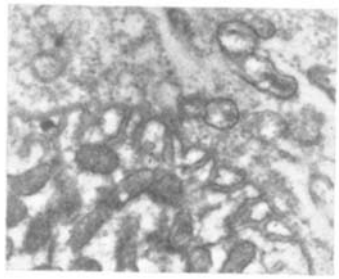


FIG. 8. Electron micrograph of endplate in mouse-diaphragm. Nerve axon above with mitochondria and plenty of granules, sarcoplasma of muscle with many mitochondria below, folds of postsynaptic membrane between[42].

to whole postsynaptic surface area as 10 per cent. As we assume the subneural membrane to have a regular structure with evenly distributed receptors, this consideration shows that only a few highly differentiated and widely distributed receptors are occupied by the curarine molecules. They might be at the bottom of the folds.

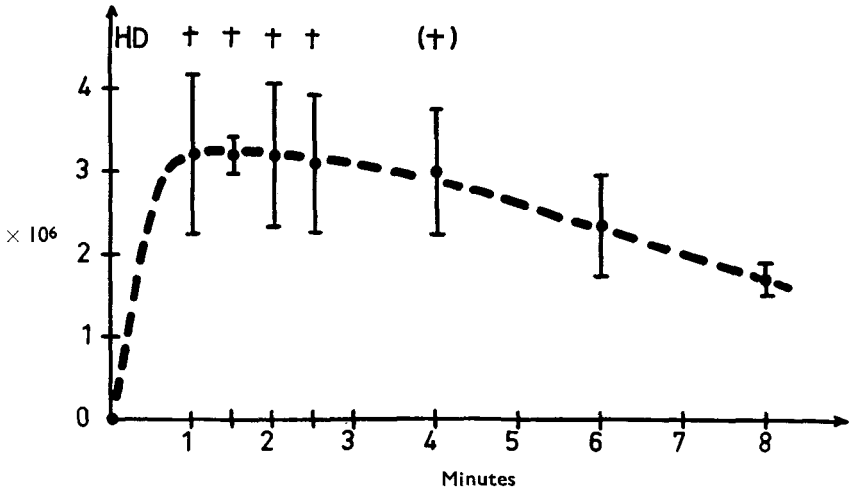


FIG. 9. Number of curarine molecules per endplate of different mouse-diaphragms receiving the minimal lethal dose of 0·1 μg./g. intravenously. HD: head drop, +: death by asphyxia.

The radioactivity in the endplates of animals surviving in spite of being given the minimal lethal dose, decreases slowly although respiration is resumed at a higher than normal rate. Autoradiographs of the diaphragms made two or four minutes after intravenous injection look nearly the same as those obtained at the moment of death in the more sensitive animals. Four minutes later the radioactivity is diminished and 12 minutes after the injection traces of radiocurarine still remain. Measurements with the densitometer show the time course of curarisation at the endplate (Fig. 9). As curarine seems to be fixed at the endplate for quite some time and metabolism of this compound is very slow, synaptic transmission must be possible with a large proportion of the receptors occupied by curarine, leaving a relatively small number of receptors free

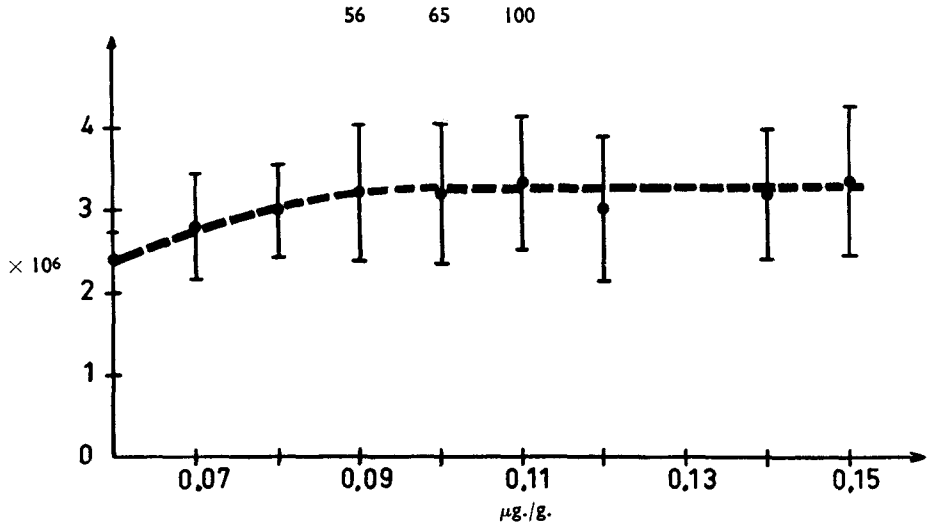


FIG. 10. Number of curarine molecules per endplate in diaphragms of mice receiving increasing doses of curarine. Saturation is reached with 0·1 μg./g. i.v. Figures above graph are per cent death by curarisation.

for acetylcholine. Curarine might on the other hand be inactivated by binding to some unspecific macromolecular structure and later slowly eliminated.

When we injected a higher than the minimal lethal dose, the mice died within 1–2 minutes. The number of curarine molecules in one endplate determined by densitometer measurements of the films does not surpass 3×10^6. With lower doses this figure is proportionally diminished, and the mice may survive the curarisation. Near the minimal lethal dose saturation of all receptor groups is reached (Fig. 10). This shows again their number to be finite. The autoradiographs after high doses are darker, due to more radioactivity in the muscle.

Anatgonism of neostigmine to curarine at the level of the endplates was shown by simultaneous injection of both substances[20]. The minimal lethal dose of radiocurarine was mixed with different doses of the

antagonist. Although nervous transmission through the endplate was restored within a minute, radioactivity in the endplates was not noticeably diminished by low doses of neostigmine when compared with the same dose of curarine alone (Fig. 11). Radiocurarine was lost by the endplates only when a lethal, ten times normal dose of neostigmine was given.

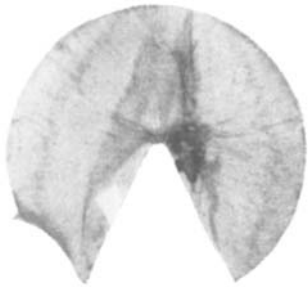


FIG. 11. Radiocurarine (0·15 μg./g. i.v.) in endplates in spite of antagonising neostigmine (2·5 μg. i.v.).

This may indicate that the cholinergic receptors blocked by curarine and the cholinesterase blocked by neostigmine are located at different sites, since both molecules cannot be at the same place. Competitive antagonism between acetylcholine and the curarising drug likewise does not

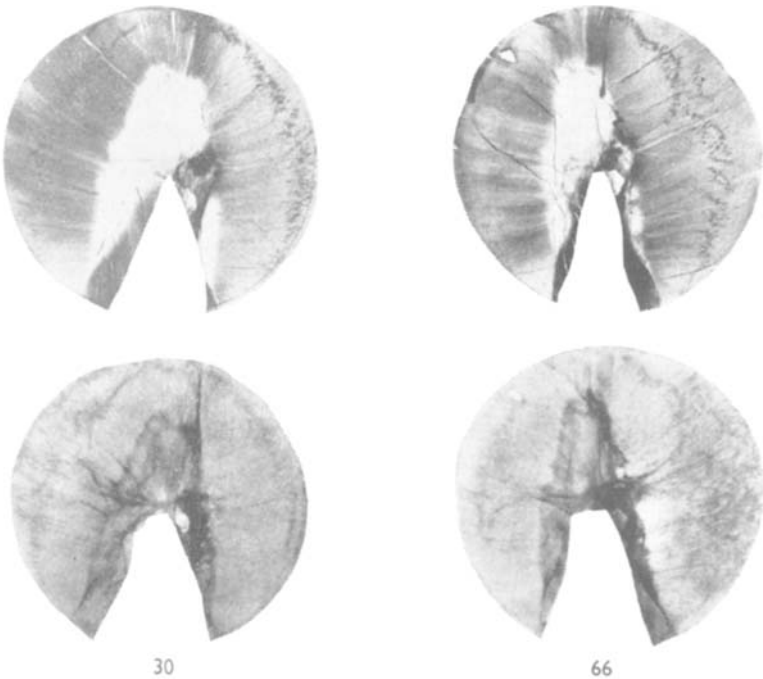


FIG. 12. Effect of phrenicotomy on left side on acetylcholinesterase (above) and curarine fixation (below) after 30 and 66 days.

explain why the radioactivity should remain in the endplates after neostigmine. If, however, there is a large reserve of cholinergic receptors for neuromuscular transmission, the reactivation of a few of them will not be easily detectable.

To show competitive antagonism between acetylcholine and curarine at the same receptor, we injected into isolated diaphragms of the living mouse by the so called close arterial injection technique through the abdominal aorta. With different combinations of both drugs or potassium chloride and curarine this antagonism is apparent by quenching the radioactivity in the endplates.

The Relation of the Receptor and Cholinesterase

What is the relationship between the cholinergic receptor and cholinesterase? To answer this question we studied the influence of denervation on binding of ^{14}C-curarine by cutting the left phrenic nerve as previously

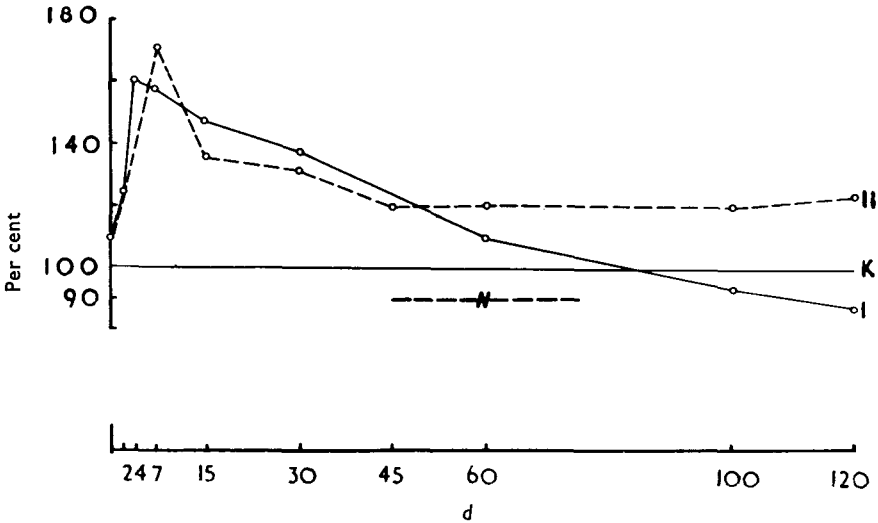


FIG. 13. Radioactivity by ^{14}C-curarine fixation after total phrenicotomy (I) and coagulation of phrenic nerve with regeneration (II). K: normal control side (right part of diaphragm), N: radioactivity of muscle.

described[15,20] Different time intervals after phrenicotomy the endplates on the severed side were compared with the endplates on the intact right side of the diaphragms (Fig. 12). In a similar group the phrenic nerve was only coagulated with dry ice (CO_2) so that regeneration was possible. In both groups an increase of curarine fixation up to the 45th–60th day, with a maximum after 4–7 days, after the operation was seen. In the denervated group the radioactivity then disappeared completely within 60–120 days (Fig. 13). In the other group regeneration set in immediately and after 45 days curarine fixation was normal. Cholinesterase, determined histochemically with Koelle's method[8], was slightly augmented in the first 7 days and then decreased continually to zero

120 days after denervation (Fig. 14). However, 30 days after regeneration cholinesterase was normal again. We must conclude from this corresponding behaviour that there is a close connection between the amount of active cholinesterase and the radioactivity due to bound curarine in the endplate. Differences in their concentrations may be partly due to different techniques in their determination.

Localisation of Depolarising Drugs

To study the localisation of depolarising drugs we synthesised decamethonium with six radioactive methyl groups (Fig. 15). On a molar basis the radioactivity thus obtained was 3·5 times higher than that of the labelled curarine. Decamethonium behaved similarly to curarine, and

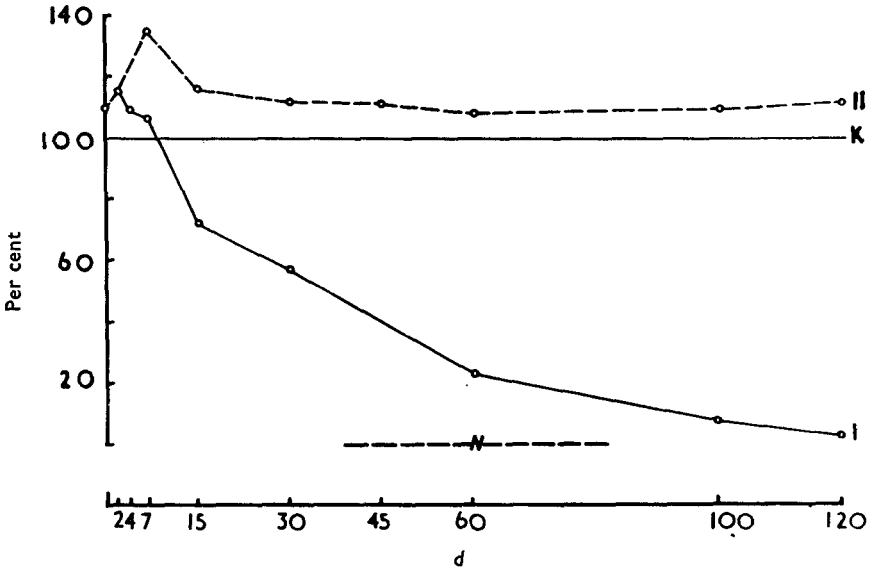


FIG. 14. Cholinesterase activity determined by histochemical staining and measurement with densitometer (I, II, K and N same as Fig. 13).

after an exposure time of only 3 days the contact films showed selective fixation in the endplates. Again the distribution of endplates identified in this way was similar to the Koelle stained diaphragm. The similarity between the autoradiographs of the two types of curarising drugs demonstrates the direct action of curarising and depolarising drugs at the endplate. But there are some important differences. On stripping films over diaphragms with decamethonium we found 10 times more grains than in films over diaphragms with curarine. On the contact films the endplate band always has a blurred appearance and the resolution of the endplates was not good (Fig. 16). We never obtained a clear picture showing individual endplates as we did with curarine. Comparing the autoradiographs with artificial diaphragms made of gelatine with different concentrations of decamethonium we found $1{\cdot}4 \times 10^8$ molecules in one endplate

after the minimal paralytic dose. This amount is 50 times higher than with curarine. The diffuse appearance of the endplate band may be explained by fixation of decamethonium not only in the receptor area but farther out on the muscle membrane around the endplate. Perhaps the very different chemical structure enables decamethonium in contrast to curarine to diffuse from the post synaptic space into the muscle, which would explain the extended region of depolarisation[21].

It would be most difficult to use radioactive acetylcholine for our purpose, as this neurotransmitter acts in a very short time and is

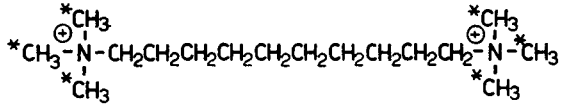


FIG. 15. Formula of ^{14}C-decamethonium.

immediately destroyed by cholinesterase. Instead of acetylcholine we have to use different cholinergic molecules resembling acetylcholine but being more stable. In the last years we have been interested in muscarine. We were able to isolate this alkaloid for the first time in a pure crystalline form from *Amanita muscaria* and to determine its pharmacological action[22,23]. Muscarine occupies the same receptors as acetylcholine but

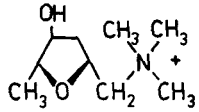
FIG. 16. Autoradiograph of diaphragm with 0·08 μg. decamethonium/g. i.v. Note diffuse fixation in endplate band.

preferably in the periphery of the autonomic nervous system[24]. Nevertheless there are marked differences in action. Muscarine is stronger than acetylcholine but much slower to act. It sensitises synapses to acetylcholine and has a prolonged action because it cannot be destroyed by cholinesterase. Endplates are not affected and ganglionic synapses are depolarised only with a high dose. Very little is known about the central effects.

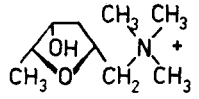
The Structure of Muscarine

The structure of muscarine was found to be very similar to acetylcholine, the only difference being a tetrahydrofurane ring with a hydroxy group instead of the carbonyl in the ester group[25,26] (Fig. 17). The different isomers of muscarine are all at least 100 times less potent in action on

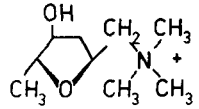
blood pressure of cats and on other cholinergic effects than the natural alkaloid[27]. The activity of the isomers depends on the position of the constituents on the tetrahydrofurane ring. Steric hindrance of the hydroxy group and of ether oxygen plays an important part in the contact



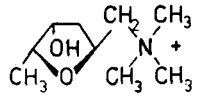
(+)—Muscarine-I (natural form)



(±)—Epimuscarine-I

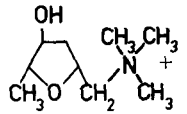


(±)—Allomuscarine-I

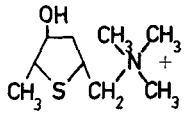


(±)—Epiallomuscarine-I

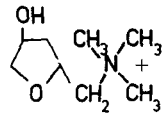
FIG. 17. Formulae of stereoisomers of muscarine.



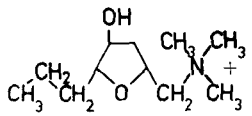
(±)—Muscarine-I



(±)—Thiomuscarine-I



(±)—Desmethylmuscarine-I



(±)—Propylmuscarine-I

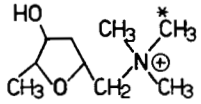
FIG. 18. Formulae of derivatives of muscarine.

with the cholinergic receptor. As nor-muscarine without the quaternary nitrogen is inactive, at least three different points of chemical interaction have to be considered. This is stressed by the fact that muscarine is highly stereospecific in its action. Muscarine with a thiophene ring instead of the tetrahydrofurane loses much of its activity probably because

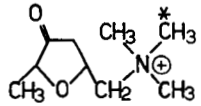
no hydrogen bridge may be formed with the sulphur atom in the ring[28] (Fig. 18). Three such pharmophore groups can be fixed only in one way with the corresponding receptor. Because of the tetrahydrofurane ring the positions of the hydroxy—and the methyl groups are absolutely fixed and only the trimethylammonium side chain can move. With many other synthetic muscarine-like molecules we found that the volume of the ring and the length of the side chains play important roles, as has been suggested by Ing[29] and by Pfeiffer[30].

The Application of Radioactive Muscarone

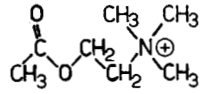
Recently muscarone with a carbonyl instead of the hydroxy group was synthesised (Fig. 19). This molecule resembles acetylcholine very closely but again it has no ester group that can be hydrolysed by cholinesterase. Its pharmacological action is astonishing[27]: in the cat it is 10 times as



(+)—Muscarine



(−)—Muscarone



Acetylcholine

FIG. 19. Formulae of ^{14}C-muscarine and ^{14}C-muscarone.

active as acetylcholine and on intravenous injection after atropinisation it even depolarised ganglionic and neuromuscular synapses. While muscarine is highly stereospecific in its action, muscarone as compared to its isomers, is not. Therefore the configuration of this stable and rigid molecule must closely resemble the active form of the flexible acetylcholine molecule at the receptor site (Fig. 20). Hence we felt justified in using radioactive muscarone instead of acetylcholine for our investigations of endplates.

When we injected ^{14}C-muscarone with a high specific activity intravenously into mice, they were immediately killed by its strong parasympathomimetic action. The endplates of the diaphragms did not show any accumulation of radioactivity. But when we injected the labelled alkaloid into atropinised mice, they were completely paralysed and died of asphyxia in the same manner as with decamethonium. An obscure blackening of the endplate region was visible similar to the decamethonium type, but unfortunately the muscle fibres also contained much radioactive muscarone (Fig. 21).

The conclusions we can draw from these results are: The receptor sites cannot be in one plane but must have a definite three dimensional structure. The cholinergic molecules have to fit into them. For depolarisation, their pharmophore groups (quaternary nitrogen, carbonyl and ether

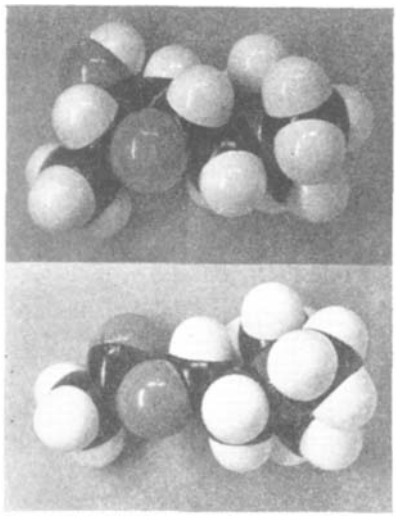


FIG. 20. Comparison of molecular models of muscarine and acetylcholine.

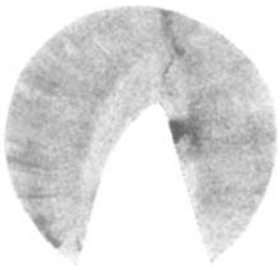


FIG. 21. Autoradiograph of mouse-diaphragm with 0·8 μg./g. ^{14}C-muscarone i.v. after being atropinised (50 μg. i.v.).

oxygen) have to form bonds (polar, covalent, hydrogen and van der Waalsbonds) with corresponding groups of the receptor substance.

The Nature of the Receptor Site

These and many other observations suggest that the receptor site might be a pore in the postsynaptic membrane[31,32]. This membrane pore should have an anionic wall, to which the quaternary *N*-groups are fixed, and an esteratic site, perhaps an imidazole group, nearby, to which the ester groups of acetylcholine becomes attached. This arrangement might possibly cause a change in the macromolecular configuration of the wall, such as the dislocation or folding of the protein and might result in the

development of permeability for potassium and sodium ions through the membrane pore during the short excitation period.

If these assumptions are correct, the neuromuscular blocking action of curare substances could be visualised as the covering of the pores of the endplates by the large molecules of C-curarine, tubocurarine, or gallamine thus inhibiting the access of acetylcholine to the receptors and preventing the flow of Na and K ions through the membrane. On the other hand depolarising drugs should not block this exchange of ions, but even enhance it.

The Nature of the Receptor Substance

Finally, what is the receptor substance? We know it to be located in the postsynaptic space, forming the few receptor sites responsible for synaptic transmission. It will be very difficult to extract the small quantity present in some scarcely distributed endplates in the muscle or from ganglionic synapses. Here the electric eel, *Electrophorus electricus* comes to our help, as it has transformed many of its endplates and muscles into a powerful electric organ, which is used for hunting and self defence in his native Amazon river. The electric organ is comparable to endplates because it is full of acetylcholine, cholinesterase and has the same mechanism of depolarisation. It can be blocked by curare drugs or depolarised by decamethonium or suxamethonium.

Chagas and colleagues[33] first tried to bind radioactive gallamine to a component in an extract of electric tissue. They found strong but probably nonspecific binding to a polysaccharide. Lately Ehrenpreis[34] and Nachmansohn[7] isolated a protein from the electric tissue, which showed many characteristics of the receptor substance *in vivo*. There is striking parallelism between the ability of this protein to bind acetylcholine analogues or curare drugs in equilibrium dialysis and their effect on electrical manifestations of intact electroplates.

It will be very difficult to prove that this *in vitro* protein is the receptor substance and corresponds to the cholinergic receptor in the tissue.

At the end of my review I therefore have to admit, that we know and can prove only the existence of the cholinergic receptor. But we do not know its nature, nor do we understand its functioning.

The use of labelled curarising and depolarising drugs has perhaps permitted a new look at an old problem, which is still far from being completely solved.

References

1. Hunt and Taveau, *Brit. med. J.*, 1906, **2**, 1788.
2. Dale, *J. Pharmacol.*, 1914, **6**, 147.
3. Loewi, *Pflügers Archiv Physiol.*, 1921, **189**, 239.
4. Acheson, *Fed. Proc.*, 1948, **7**, 447.
5. Buchtal and Lindhart, *Acta physiol.*, 1942, **4**, 136.
6. del Castillo and Katz, *J. Physiol.*, 1955, **128**, 157.
7. Nachmansohn, *Chemical and Molecular Basis of Nerve Activity*, Academic Press, 1959.
8. Koelle and Friedenwald, *Proc. Soc. exp. Biol., N.Y.*, 1949, **70**, 617.
9. Loewenstein and Mollins, *Science*, 1958, **128**, 1284.
10. Nachmansohn and Machado, *J. Neurophysiol.*, 1943, **6**, 397.
11. Feldberg and Vogt, *J. Physiol.*, 1948, **107**, 372.

12. Langley, *ibid.*, 1905, **33**, 374.
13. Waser and Lüthi, *Nature, Lond.*, 1956, **178**, 981.
14. Waser and Lüthi, *Arch. int. Pharmacodyn.*, 1957, **112**, 272.
15. Hadorn, Thesis, Zürich, 1960.
16. Waser, Schmid and Schmid, *Arch. int. Pharmacodyn.*, 1954, **96**, 386.
17. Robertson, *J. Biophys. Biochem. Cytol.*, 1956, **2**, 381.
18. Couteaux, *Int. Rev. Cyrol.*, 1955, **4**, 335.
19. Andersson-Cedergreen, *J. Ultrastructure Research*, 1959, Suppl. 1.
20. Waser, *Helv. physiol. Acta*, 1958, **16**, 171.
21. Paton and Zaimis, *Pharm, Rev.*, 1952, **4**, 219.
22. Eugster and Waser, *Experientia*, 1954, **10**, 298.
23. Waser, *ibid.*, 1955, **11**, 462.
24. Kuenzle and Waser, *Helv. physiol. Acta*, 1958, **16**, 44.
25. Kögl, Salemink, Schouten and Jellinek, *Rec. Trav. chim. Pays-Bas.*, 1957, **76**, 109.
26. Eugster and Waser, *Helv. chim. Acta*, 1957, **40**, 888.
27. Waser, *Experientia*, 1958, **14**, 356.
28. Waser, *ibid.*, 1960, **16**, in press.
29. Ing, *Science*, 1949, **109**, 264.
30. Pfeiffer, *ibid.*, 1948, **107**, 94.
31. Waser, *Schweiz. Med. Wschr.*, 1957, **87**, 577.
32. Waser, *Curare and Curare-like Agents*, Elsevier, 1959, p. 229.
33. Chagas, *ibid.*, p. 327.
34. Ehrenpreis, *Science*, 1959, **129**, 1613.
35. Dale, Feldberg and Vogt, *J. Physiol.*, 1936, **86**, 353.
36. Feldberg and Vartiainen, *J. Physiol.*, 1934, **83**, 103.
37. Lissak, *Amer. J. Physiol.*, 1939, **127**, 263.
38. Scheinfinkel, *Helv. physiol. Acta*, 1943, **1**, 149.
39. von Muralt, *Pflügers Archiv ges. Physiol.*, 1942, **245**, 607.
40. Fukuda and Koelle, *J. Biophys. Biochem. Cytol.*, 1959, **5**, 433.
41. Fatt and Katz, *J. Physiol.*, 1952, **118**, 73.
42. Vogel and Waser, unpublished.