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The concept of transmitter receptors: 100 years on

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Abstract

It is nearly one hundred years since John Langley of Cambridge developed the idea of the 'receptive substance' or 'receptors' as we now call them. This historical review traces the background to his introduction of this concept of the transmitter receptor and of how succeeding generations built on his ideas to generalise the applicability of this concept to synapses in general. It starts with a consideration of the discovery by Bernard (1844) that curare could paralyse rabbits without affecting their hearts because, as Vulpian (1866) suggested, curare acts on some intermediate zone between nerve and muscle. No further progress could be made without establishing the idea of chemical transmission, which Elliott (1904) then achieved, building on observations concerning sympathetic transmission to smooth muscle made previously by his mentor Langley (1901). Then between 1905 and 1907 Langley, in a wonderful act of creative ability, carried out a series of experiments on the somatic neuromuscular junction which established the idea of transmitter receptors. This review gives details of the experiments which persuaded both Langley and a recalcitrant Ehrlich that pharmacological substances could possess the necessary structure for them to combine with appropriate molecules on cells. The subsequent identification by Dale and his colleagues (1936) of acetylcholine as the transmitter acting on the receptors first discovered by Langley at the somatic neuromuscular junction as well as of acetylcholine on receptors in the heart by Loewi (1921) is then detailed. The review concludes with the triumph of the first recordings of the electrical signs of single channel openings by Neher and Sakmann (1976) at the receptors which Langley had first described.

Keywords: Receptors; Transmitter; Synapses; Neuromuscular junction

1. Introduction

The idea of the 'receptive substance', or receptors as we now call them, was developed by John Langley of Cambridge 90 years ago (Fig. 1A). Between 1901 and 1905 Langley laid the foundations for the idea of chemical transmission with his student Thomas Elliott (Fig. 1B) through their investigations on sympathetic neuroeffector transmission. In an extraordinary act of creative ability, Langley then carried out a series of investigations between 1905 and 1907 on the somatic neuromuscular junction that established the idea of transmitter receptors. This historical review traces the development of Langley's ideas over this period, especially in relation to the concept of the 'chemoreceptor' developed by Paul Ehrlich. The review then examines how this work was applied by a number of investigators to place the concept of transmitter substances and their receptors on a firm foundation for the modern molecular approaches to the delineation of receptor types and their function. In order to assist the reader, a chronological table of significant experiments in the history of receptors is provided (Table 1), together with a list of the major contributors to these experiments (Table 2) and the agents they used to delineate the receptor concept (Table 3).

2. Claude Bernard and curarization: the notion of an intermediate zone between nerve and muscle

In June of 1844 Claude Bernard wrote in his experimental note book that:

A poisoned arrow obtained from a friend who had connections with South American natives was thrust

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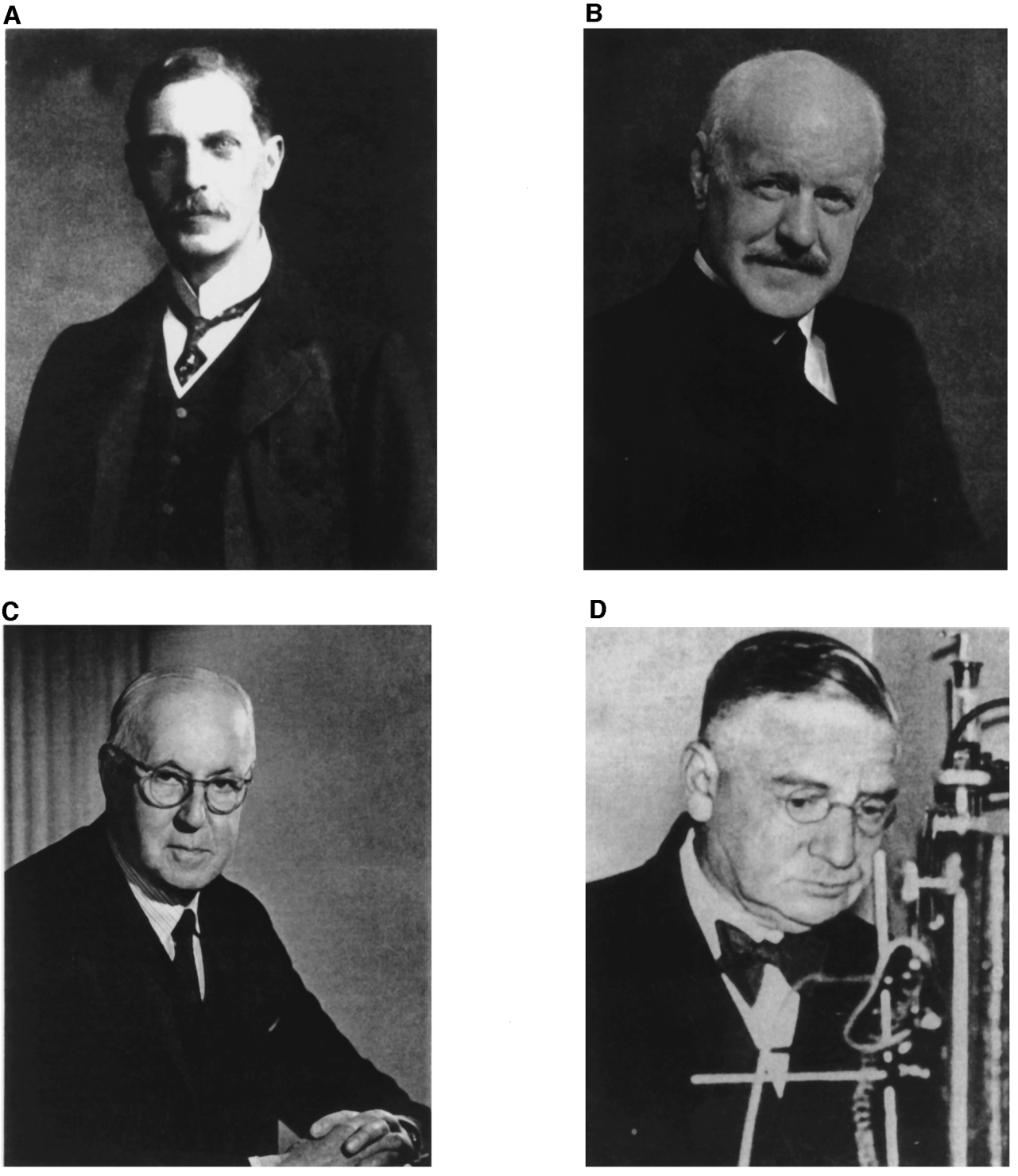

Fig. 1. The founding fathers of chemical transmission at synapses. (A) J.N. Langley (1852–1925), Fig 20.4 in Finger (1994). (B) T.R. Elliott (1877–1961), portrait facing p. 53 in Dale (1961). (C) H.H. Dale (1875–1968), portrait facing p. 77 in Feldberg (1970). (D) O. Loewi (1873–1961), Fig. 20.5 in Finger (1994).

into the subcutaneous tissue of a rabbit at the internal part of the thigh and maintained there for 30 seconds. The animal was then observed. At first, nothing happened. But after six minutes it became totally paralysed: no reflex movements were observed on pinching the rabbit, although the heart continued to beat. The animal subsequently died and at autopsy it was not possible to find any lesion capable of explaining paralysis and death (Fessard, 1967).

Although this observation had been made in 1811 by

Table 1
Chronological table of significant events in the history of receptors

1844	Curare paralyses rabbits without affecting the heart	Bernard
1866	Curare acts on an intermediate zone between nerve and muscle	Vulpian (1866)
1899	Supra-renal extract (adrenaline) contracts and relaxes different smooth muscles	Lewandowsky (1899)
1901	Supra-renal extract (adrenaline) contracts or relaxes different smooth muscles as does stimulation of their sympathetic nerve supply	Langley (1901)
1901	Nicotine stimulates sympathetic ganglion cells directly	Langley (1901)
1904	Adrenaline acts at the junction between nerves and smooth muscle cells not on nerve terminals	Elliott (1904a)
1905	Nicotine stimulates skeletal muscles directly and this is blocked by curare	Langley (1905)
1905	The concept of a 'receptive substance' on skeletal muscles first described	Langley (1905)
1906	The 'receptive substance' shown to provide the receptor for alkaloids such as nicotine and curare	Langley (1906)
1906	Acetylcholine synthesised and shown to have powerful effects on the circulation	Hunt and Taveau (1906)
1914	Acetylcholine has similar actions on smooth muscles and cardiac muscle as stimulating the vagus nerve	Dale (1914a)
1921	'Vagusstoff' is released by the vagus nerve and controls the heart beat	Loewi (1921)
1926	Physostigmine potentiates the effects of applied acetylcholine on the heart	Loewi and Navratil (1926b)
1929	Acetylcholine shown to be a natural constituent of horse and ox spleen; likely to be 'Vagusstoff'	Dale and Dudley (1929)
1934	Acetylcholine is released in autonomic ganglia on nerve stimulation; likely to be the transmitter	Feldberg and Gaddum (1934)
1936	Acetylcholine collected in venous fluid from skeletal muscles on nerve stimulation	Dale et al. (1936)
1936	Acetylcholine injected into the arteries of skeletal muscles initiates contraction	Brown et al. (1936)
1970	Acetylcholine applied at the endplate gives membrane noise due to the opening of channels	Katz and Miledi (1970)
1976	Acetylcholine receptor channels give electrical signal that may be recorded directly	Neher and Sakmann (1976)

Table 2
Scientists who contributed significantly to the idea of the receptor

Name	Location	Dates of research	Mentor
C.Bernard	Paris	1844–83	
J.Langley	Cambridge	1874–1908	M. Forster
T.Elliott	Cambridge	1904–05	J. Langley
H.Dale	London	1914–36	
O.Loewi	Austria	1921–26	
W.Feldberg	London	1934–36	H. Dale
G.Brown	London	1936–37	H. Dale

Table 3
Definition of some of the agents used to delineate the receptor concept

Agent	Site of action	Muscle type	Antagonist
Curare	Nicotinic receptors	Skeletal	
Pilocarpine	Muscarinic receptors	Smooth and cardiac	Atropine
Supra-renal extract		Smooth and cardiac	
Adrenaline		Smooth and cardiac	
Nicotine	Nicotinic receptors	Skeletal	Curare
Acetylcholine	Muscarinic receptors	Smooth and cardiac	Atropine
Vagusstoff	Muscarinic receptors	Cardiac	
Eserine	Cholinesterase		
Acetylcholine	Nicotinic receptors	Skeletal muscle	Curare

Brodie, who later went on to show that curarized animals could be maintained alive on an artificial respirator, the advent of Bernard into this area of research brought a keen experimental mind to bear on the problem of the action of curare. Bernard constructed a galvanic stimulator for exciting either nerves or muscle fibres which allowed him to carry out investigations on curare that led him to report that:

> Electrical stimulation of a motor nerve in a curarized frog has no effect, whereas its muscles contract when directly stimulated.

This pointed to curare acting on nerves rather than generally acting as some kind of anaesthetic. In order to test whether both the motor and sensory nerves were affected by curare, Bernard designed an experiment in which a ligature was passed around the waist of a frog, so that the lower limbs were isolated from the rest of the body, except for the sciatic nerve, as shown in Fig. 2A. Bernard then reported the following observations:

> Curare is introduced under the skin of the back. It poisons the anterior part of the body and prevents movement there; but sensation in this part is conserved, for stimuli applied to this paralysed portion cause energetic reflex movements in the isolated posterior half. Curare is thus a poison which not only produces physiological separation of nerves and muscles, but also separation of two major kinds of nervous manifestations. It suppresses movement but has no action on sensation; so that in a way it dissects out the neuromotor system and separates it from the muscular system, the sensory nervous system, and other tissues.

He then designed an experiment that is illustrated in Fig. 2B: here electrical stimulation was applied to the sciatic nerve lying in a bath of curare, as shown in V, and contraction of the muscle outside the curare bath was present. On the other hand, when the muscle was placed in the curare bath as shown in V′, stimulation of the nerve outside the bath did not give rise to contraction. The obvious conclusion to this experiment would seem to be that some junctional structure between the nerve and the muscle had been affected by curare. However, Bernard did not reach this conclusion. Fessard (1967) has conjectured as to why Bernard did not follow the appropriate deduction from his observations. Perhaps Bernard was concerned that he was dealing with organs that were separated from the body and not subjected to the 'milieu interieur' and circulation of the blood? It seems that Bernard was persuaded of the idea that the action of curare should be related to the circulation of the blood, perhaps as Bernard himself suggested through an alteration of the gas exchange between the blood and the air in the lungs or the tissues of the capillaries.

Bernard then turned to the experiment illustrated in Fig. 2C. The technique involves a kind of close arterial injection, as illustrated by the insert. Curare is injected into the artery supplying a muscle, so that it does not come into contact with the nerve trunk; furthermore there is an outlet in the vein which prevents the curare containing blood from reaching the central nervous system. This beautiful experiment seemed to Bernard to show that curare acted on the nerve terminals within the muscle. There is no mention in his books of the notion that curare might work at a junction formed between the nerve and the muscle although in his notebooks there is mention that "Curare must act on the terminal plates of motor nerves" and that "Curare does no more than interrupt something motor which puts the nerve and the muscle into electrical relationship for movement" (Fessard, 1967). These quotes suggest that he had envisaged the notion of a neuromuscular junction, although this was never pursued in his formal statements to be found in his books concerning these experiments. The explicit claim that curare does not act on motor–nerve terminals, but rather on some intermediate zone between nerve and muscle was left to Vulpian (1866, p. 920) in his 'Lecons sur la Physiologie Generale et Comparee du Systeme Nerveux'. The nature of this intermediate zone was next investigated by histologists, seeking to find the site at which curare works. Chief amongst these at this time was Bernard's student Kuhne, however it is now clear in retrospect that the histological approach to this problem had to await the advent of ultrastructural techniques, nearly a century later. As to the proper development of the functional approach, that might have been pursued immediately. Although there were no technical limitations to such an approach, another 40 years had to pass before the problem was elucidated by Langley.

3. Paul Ehrlich and the idea of the 'receptive side chains' of cells

In 1885, Ehrlich presented his thesis to the University of Leipzig in which he described for the first time his 'side chain theory' of cellular action. The protoplasm of a cell was considered to be a giant molecule incorporating a central structure responsible for the specific activity of a particular cell type (such as a muscle cell or a neurone), which possessed chemical side chains. The side chains were envisaged as carrying out processes common to all cells. For example, one such side chain might be involved in the process of oxidation, following which the chain had to be regenerated by the cell. Two years later, in 1897, Ehrlich elaborated this idea into his influential side chain theory of immunity. He postulated that a 'receptive side chain' of a particular cell, for

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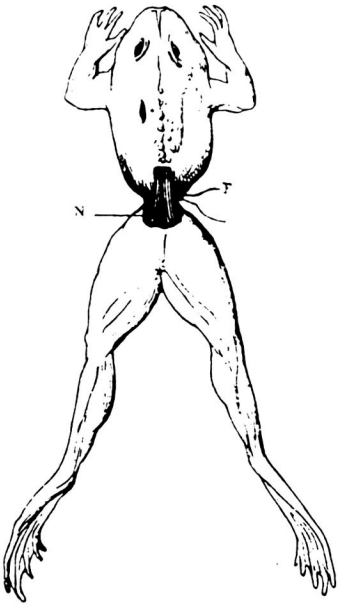

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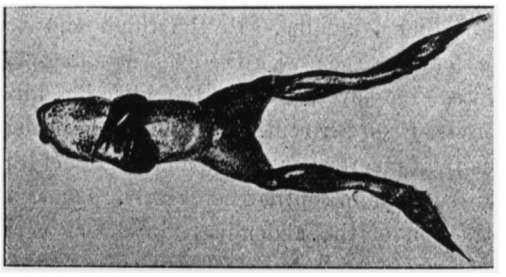

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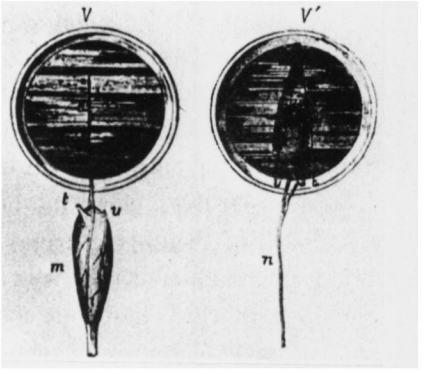

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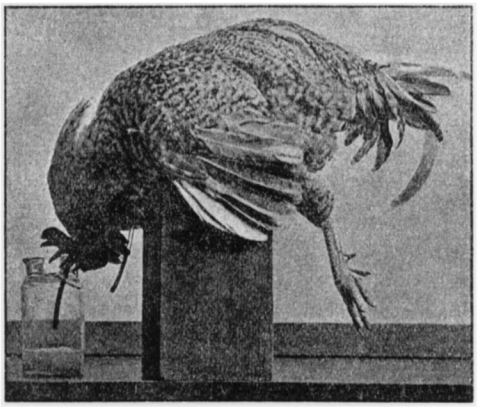

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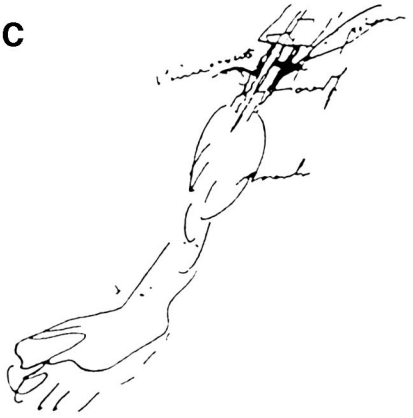

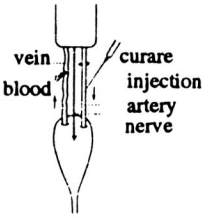

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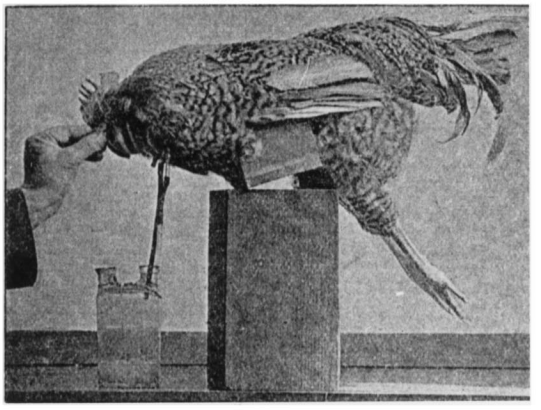


Fig. 2. The experiments of Claude Bernard and John Langley establishing the concept of 'receptive substances' at somatic neuromuscular junctions. (A) A frog preparation illustrating an experiment by Claude Bernard. A ligature passed around the waist of the frog isolated the lower-limbs from the rest of the body, except for the sciatic nerve trunk. The experiment is described in the text (reproduced from Lecons, 1883 edition, Fig. 26, p. 345; from Fig. 26 in Bernard, 1883; reproduced by Fessard as Fig. 2, p. 111 in Grande and Visscher, 1967). (B) Illustration of an experiment with curare carried out by Claude Bernard (reproduced from Lecons, 1883 ed., Fig. 23, p. 329; from Fig. 23 in Bernard, 1883; reproduced by Fessard as Fig. 3, p. 112 in Grande and Visscher, 1967). (C) A drawing made by Claude Bernard to illustrate one of his experiments on neuromuscular curarization (see text). Reproduced from the original note book 'Cahier rouge' (also recently reproduced in Cahier de Notes, 1965, p. 76). Inset: an explanatory scheme of the drawing that is described in the text (from p. 76 in Cahier de Notes, 1965; reproduced by Fessard as Fig. 4, p. 113 in Grande and Visscher, 1967). (D) Frog killed by destroying the whole of the central nervous system. Contraction of the muscles of the forelimbs caused by nicotine (from Fig. 7 in Langley, 1906). (E) Nicotine injected into the abdominal cavity of a frog, whose spinal cord and brain had been destroyed. For details of the experiment see the text (from Fig. 9 in Langley, 1906). (F) Fowl, anaesthetised with morphia and A.C.E. mixture, balanced on its thorax in a V-shaped piece of wood. The neck and legs hang down and are flaccid, the eyes are shut (from Fig. 1 in Langley, 1906). (G) The same fowl as in (F), 2 min after injection of 5 mg of nicotine into the jugular vein. The injection caused a gradual and fairly quick extension of the legs, retraction and twisting of the neck, and opening of the eyes. In order to show the eyes, the beak was held when taking the photograph. The fowl was unfastened throughout, and the injection caused no general movement nor any decrease of the anaesthesia (from Fig. 2 in Langley, 1906).

example one involved in nutrition, has an atom group which by mere coincidence possessed specific combining properties for a particular toxin, such as tetanus toxin. The normal function of the side chain is lost once the toxin binds to the group, triggering the cell to produce a large number of such side chains. Many of these excess side chains then break off from the cell and are so released into the blood stream. Here they act as antibodies or antitoxins, combining with the toxin in the blood stream and so preventing it from combining with cells. Ehrlich in this work likened the relation between toxin and receptive side chain, which by 1900 he referred to simply as 'receptor', to that between a 'lock and a key'.

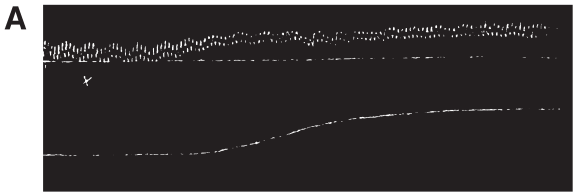
In his Croonian Lecture to the Royal Society of London in 1900, Ehrlich specifically excluded his receptor theory for the actions of toxins as being applicable to the action of drugs on cells. He came to the conclusion that drugs are not bound firmly to cells like toxins as most of the former are easily extracted from tissues by solvents. Thus toxins are bound to the protoplasmic molecule by chemical union, whereas pharmacological drugs are not as they do not possess appropriate groups. It follows that they are not capable of eliciting the production of antibodies.

> If alkaloids, aromatic amines, antipyretics, or aniline dyes be introduced into the animal body it is a very easy matter, by means of water, alcohol, or acetone, according to the nature of the substance, to remove all these things quickly and easily from the tissues …. We are therefore obliged to conclude that none of the foreign bodies just mentioned enter synthetically into the cell complex; but are merely contained in the cells in their free state. The combinations into which they enter with the cells, and notably with the not really living parts of them are very unstable, and usually correspond only to the conditions in solid solutions, while in other cases only a feeble salt — like formation takes place.

The conclusion reached by Ehrlich then in 1900, and reiterated in 1902, was that pharmacological substances do not possess the necessary atomic groups which would allow them to combine with the appropriate groups of the cell protoplasm (Ehrlich and Morgenroth, 1900). The 'lock and key' concept did not then apply to the interaction of drugs with cells, so that the 'receptor' concept did not apply in this instance. However, by 1907 Ehrlich had completely changed his mind on this issue, and even introduced the word 'chemoreceptor' to describe the interaction of drugs with cells. What had happened in the 5 years between 1902 and 1907 to change his mind on this issue was largely due to the work during this period of the laboratory of Langley, which will now be described.

4. Langley and Elliott: the emergence of the concept of chemical transmission between sympathetic nerves and smooth muscle

In 1899 Lewandowsky observed that supra-renal extract in cats causes dilation of the pupil, withdrawal of the nictitating membrane (Fig. 3A), separation of the eyelids and protrusion of the eyeball. Lewandowsky suggested that the extract acted directly on the smooth muscle and not on the nerve endings in the muscle as he obtained the same results with the extract after excision of the superior cervical ganglion and degeneration of the postganglionic nerves as in the normal animal. This was an extraordinary insightful interpretation, which formed the basis for the subsequent comprehensive study of the effects of supra-renal extract by Langley. In 1901 he inquired into the effects produced by supra-renal extract in the cat and rabbit on different organs, and arranged them in order as regards the amount of extract required per body weight to produce an obvious effect, as shown in the table of Fig. 3B. This table shows that the extract in some cases contracts smooth muscles of a particular organ and in other cases relaxes the muscle. Langley had already, in 1898, defined the autonomic nervous system which he divided into sympathetic, cranial, sacral and



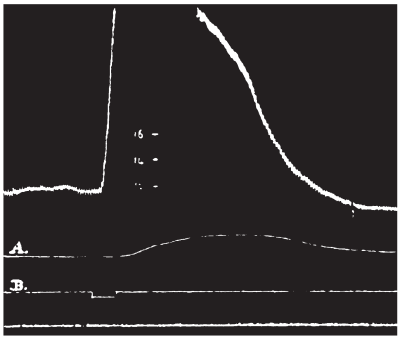
B

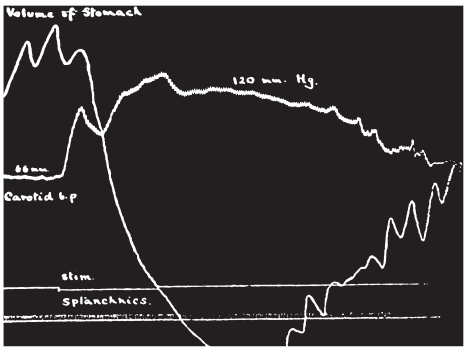
Inhibition of the sphincter of the stomach and of the intestine (rabbit).
Inhibition of the bladder.
Dilation of the pupil (cat).
Withdrawal of nictitating membrane (cat) } slightly less readily
Separation of the eyelids (cat) } than the foregoing.

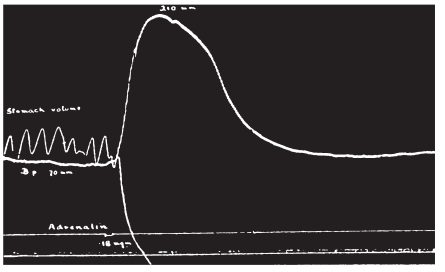
Contraction of uterus, vas deferens, seminal vesicles, etc. (rabbit).
Salivary and lachrymal secretion.
Inhibition of the stomach.
Inhibition of the gall-bladder and increased bile secretion.
Dilation of pupil (rabbit).
Inhibition of internal anal sphincter (rabbit).
Contraction of internal anal sphincter (cat) } effects relatively
Contraction of internal generative organs (cat) } slight.

Contraction of the muscles of the hairs.

Contraction of tunica dartos of scrotum } no certain effect.
Secretion of sweat }







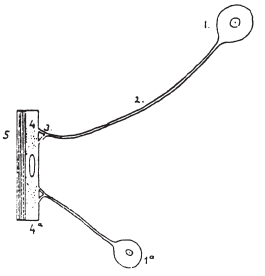


Fig. 3. Elliott and Langley establish the concept of adrenaline as a transmitter at the autonomic neuromuscular junction. (A) Blutdruck und Membrana nictitans. Injection of 1 c.c. of extract of the adrenal bodies (from Fig. 2 in Lewandowsky, 1899). (B) The effects produced by suprarenal extract in the cat and rabbit may be arranged roughly in the order shown as regards the amount of extract required per body weight to produce an obvious effect (from the table in Langley, 1901; note 'Rise of blood pressure should head this list'). (C) Cat. Vagi cut. Injection of 0.3 mg adrenaline into external jugular vein. A is the record of the ileo-colic sphincter under pressure of 15 cm. B gives the period of injection. The figures 12, 14, 16 indicate the blood-pressure in cm, given in the upper trace. Bottom trace gives time marker in seconds (not detectable in the original figure) (from Fig. 6 in Elliott, 1904b). (D) Inhibition of cat's stomach by splanchnic nerves. Ether. Vagi and splanchnic cut within thorax and placed on shielded electrodes. Artificial respiration. Record of volume change of stomach under constant pressure of 7 cm water. Stimulation of splanchnics caused rise of blood pressure and relaxation of stomach by 30 c.c. Period of stimulation of the splanchnics is given by the second trace from the bottom. Bottom trace gives time marker in seconds (barely detectable in original figure) (from Fig. 6 in Elliott, 1905). (E) From same experiment as (D). Injection of 18 mg adrenaline completely relaxed the stomach (second trace). Tone did not return until the vagi were again stimulated. Period of application of adrenaline given by the second trace from the bottom. The upper-most trace gives the blood pressure. The numbers on the traces are almost illegible: 210 mmHg is indicated from above a base of 70 mmHg. Bottom trace gives time marker in seconds (not clear in original record) (from Fig. 8 in Elliott, 1905). (F) Diagram of doubly innervated muscle–nerve system: (1) the sympathetic motor ganglion cell, (2) its axon, and (3) the nerve ending; (4) the myoneural junction; (5) the contractile muscle fibre; (1a) and (4a) the corresponding parts of the inhibitor mechanism. To simplify the diagram the motor myoneural junction (4) is represented as spatially separated from the inhibitor (4a) (from Fig. 10 in Elliott, 1905).

enteric components. In his 1901 paper he makes the historic remarks:

> It is a noteworthy fact that the effect of supra-renal extract in no case corresponds to that which is produced by stimulation in normal conditions of a cranial autonomic or of a sacral autonomic nerve. It does not produce the effect of stimulating the third nerve on the eye, nor of the vagus on the stomach or the heart, nor the effect of stimulating the pelvic nerve on the bladder, the rectum, the anus, or the generative organs. It is true that it causes a free secretion of saliva, but the secretion is not accompanied in its first stages by increased vascularity such as is caused by stimulation of the chorda tympani of Jacobson's nerve. It is equally noteworthy that the effects produced by supra-renal extract are almost all such as are produced by stimulation of some one or other sympathetic nerve. In many cases the effects produced by the extract and by electrical stimulation of the sympathetic nerve correspond exactly.

Having made these observations, and the fact that the effects of the extract persist after denervation of the organs, as Lewandowsky had first observed, Langley reached the conclusion that:

> the difference in action on different autonomic tissues must depend upon their intrinsic differences.

However, at this time Langley did not comment on the possiblity that the sympathetic nerves exerted their effects by the release of a substance equivalent to supra-renal extract.

In 1904, Langley's student Elliott reported experiments that showed even further the parallel effects of sympathetic nerve stimulation and of supra-renal extract (now identified as adrenaline by Takamine, 1901) on autonomic effectors. He showed that stimulation of the sympathetic nerves causes the sphincter at the junction between the small and large intestine to contract, at the same time inhibiting the circular muscle in the wall of the ileum and colon adjoining the sphincter. Adrenaline produced the same effect as sympathetic nerve stimulation, thus contracting the sphincter (Fig. 3C) and relaxing the circular muscle of the surrounding ileum. These observations emphasised the parallel actions of adrenaline and of sympathetic nerve stimulation on the smooth muscle of different organs, and in this case within an organ. In that same year, Elliott carried out an extensive study of the parallel actions of sympathetic nerve stimulation to the smooth muscles of different organs and that of the action of adrenaline on these, such as inhibition of the stomach by the splanchnic nerves (Fig. 3D and E), examining all apparent exceptions to this rule by previous investigators (not including Langley) and came to the conclusion, published in 1905, that:

> In all vertebrates the reaction of any plain muscle to adrenalin is of similar character to that following excitation of the sympathetic (thoracico-lumbar) visceral nerves supplying that muscle. The change may be either to contraction or relaxation. In default of sympathetic innervation plain muscle is indifferent to adrenalin. A positive reaction to adrenalin is a trustworthy proof of the existence and nature of sympathetic nerves in any organ. Sympathetic nerve cells with their fibres, and the contractile muscle fibres are not irritated by adrenalin.

Elliott was therefore led to conclude that since some plain muscles that do not receive a sympathetic innervation are not affected by adrenaline, then the contractile apparatus cannot be the site of action of this substance. Furthermore, as Lewandowsky, Langley and Elliott himself had shown that the actions of adrenaline were not dependent on an intact sympathetic nerve supply, then it was concluded that adrenaline did not exert its effects through the nerve supply. Elliott was then led to the important conclusion that:

> The stimulation takes place at the junction of muscle and nerve (Fig. 3F). The irritable substance at the myoneural junction depends for continuance of life on the nucleoplasm of the muscle cell, not of the nerve cell.

However, nowhere in this classic paper of 1905 is there any mention that stimulation of the muscle by the nerve involves the release of a chemical substance, let alone that this substance in the case of sympathetic nerves is adrenaline. Yet in a proceedings note to the Physiological Society of 1904 Elliott makes his claim for chemical transmission at sympathetic nerve terminals and that this might be adrenaline. In that famous note he presents the evidence in favour of his two hypotheses as follows (Elliott, 1904a):

1. "the effect of adrenalin upon plain muscle is the same as the effect of exciting the sympathetic nerves supplying that particular tissue"
2. the "medulla and the sympathetic ganglia have a common parentage" (see Kohn, 1903a,b);
3. "the facts suggest that the sympathetic axons cannot excite the peripheral tissues except in the presence and perhaps through the agency, of the adrenalin or its immediate precursor secreted by the sympathetic paraganglia";
4. "Adrenalin does not excite sympathetic ganglia when applied to them directly, as does nicotine. Its effective action is localised to the periphery";

5. “even after such complete denervation, whether of three days’ or ten months’ duration, the plain muscle of the dilatator pupillae will respond to adrenalin”.

In summary then:

> Therefore it cannot be that adrenalin excites any structure derived from, and dependent for its persistence on, the peripheral neurone. But since adrenalin does not evoke any reaction from muscle that has at no time of its life been innervated by the sympathetic (for example the absence of action on the muscle of the bronchioles and of the pulmonary blood vessels, as shown by Brodie and Dixon, 1904), the point at which the stimulus of the chemical excitant is received, and transformed into what may cause the change of tension of the muscle fibre, is perhaps a mechanism developed out of the muscle cell in response to its union with the synapsing sympathetic fibre, the function of which is to receive and transform the nervous impulse. Adrenalin might then be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery.

We therefore have for the first time a succinct statement of the concept of chemical transmission but also identification of a transmitter substance. Although Langley undoubtedly supplied the intellectual environment in the laboratory for the development of these hypotheses, there is no sign in his papers to this time (1904) that he had joined together the set of numbered observations indicated above to arrive at the conclusion that chemical transmission is most likely to occur at sympathetic nerve terminals and that the transmitter is adrenaline. Langley was always loathe to speculate and develop hypotheses. His great experimental career consists of generating a formidable set of facts that lead inexorably to a conclusion. On no occasion did he draw a diagram of the kind shown in Fig. 3F from Elliott (1905) that so brilliantly concentrates one’s interest on the neuromuscular junction. Indeed this figure may be compared for its fruitful prescience with that of Sherrington’s figure of 1906 in the ‘Integrative Action of the Nervous System’, showing the monosynaptic connections of the motor and sensory nerves in the spinal cord. Langley’s reticence meant that he missed out generating the brilliantly fruitful hypotheses of Elliott. It may also be that Elliott himself was dissuaded by Langley from continuing down the path of elaborating his ideas further, as following the note of 1904 there is no mention by Elliott in the very substantial paper of 1905 of either the chemical transmission hypothesis or the possibility that adrenaline is a transmitter.

5. The action of curare and John Langley’s development of the idea of transmitter receptors

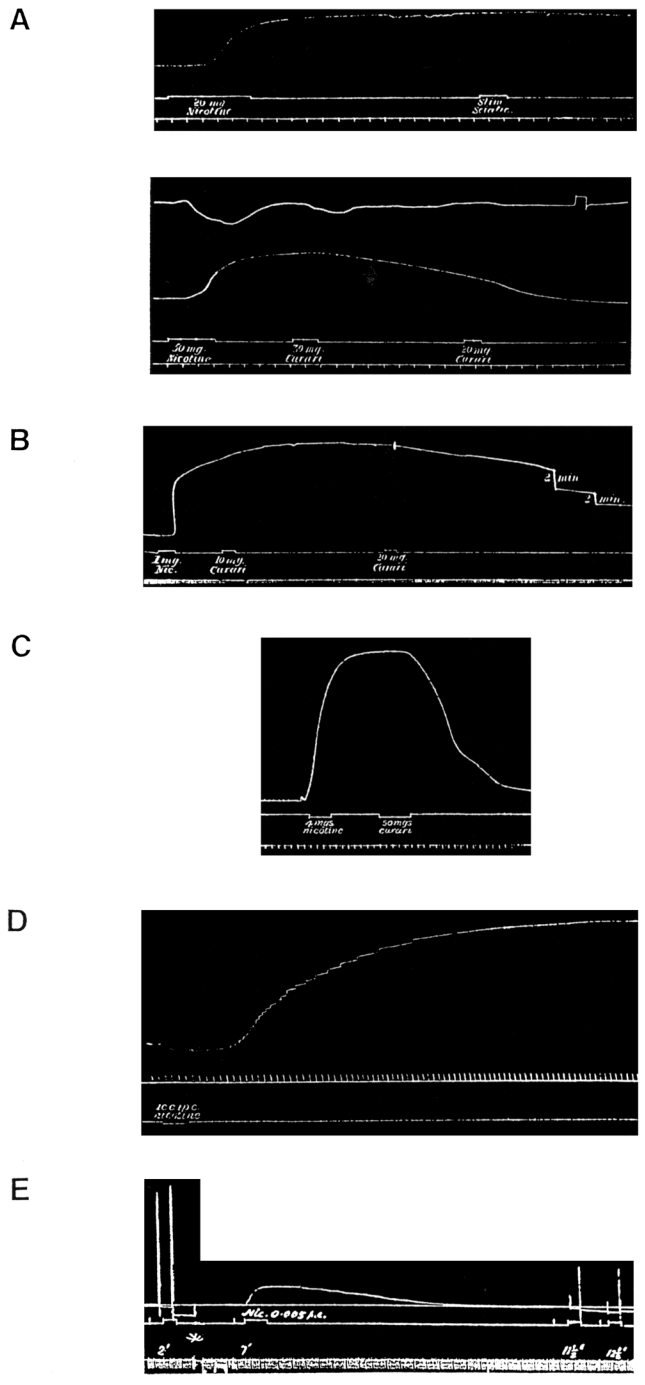
By 1904 it was clear that adrenaline acted on those smooth muscles that received a sympathetic innervation and that this action was independent of the nerve supply to the muscles. Elliott did not elaborate further on his concept of chemical transmission in his 1905 paper that there is a:

> mechanism developed out of the muscle cell in response to its union with the synapsing sympathetic fibre, the function of which is to receive and transform the nervous impulse (Fig. 3F)

However, there were undoubtedly discussions in the Cambridge Physiological Laboratory concerning his hypotheses. This is made to some extent explicit by Langley in his first paper on the actions of curare on striated muscle in 1905 in which he says in the introduction:

> Elliott brings forward further and most striking evidence that adrenalin stimulates tissues which are stimulated by sympathetic nerves and these only. This leads him to look on adrenalin as acting on some substance common to sympathetic nerves. He finds, however, that degeneration of the nerves does not diminish the action of adrenalin, and as he considers that the axon endings degenerate, the substance affected by adrenalin must be in trophic connection with the muscle. This as I have pointed out above is, I think, the same as saying that it is part of the muscle. But in view of the close relation of adrenalin to sympathetic nerves, and because he considers it improbable that the varying action of adrenalin can be due to intrinsic differences in the muscle, he concludes that when sympathetic nerves unite with unstriated muscle they cause the formation in it of a new substance, the myo-neural junction, and it is this which is acted upon by adrenalin. Now supposing that nervous connection does cause in the muscle the formation of a new substance, this does not make the new substance any the less part of the muscle. The fundamental fact of Elliott’s view is then, I think, the same as mine, viz. that adrenalin acts directly on muscle.

The concept of Elliott’s ‘new substance’ therefore had a major influence on how Langley designed his experiments concerning the manner by which curare acted. These were not only based on the conceptual framework of Elliott but also on Langley’s own discovery that nicotine stimulates sympathetic nerve cells by a ‘direct action upon them’ (Langley, 1901). Furthermore, it must not be forgotten that Langley’s first experiments in 1874, while still a student at Cambridge under the guidance of Michael Foster, involved an investigation into the



actions of atropine and pilocarpine (muscarine like) on the secretion of saliva by the submaxillary gland. He found that these had the opposite effects and in his full paper on this antagonism, published in 1878, there is the comment that:

> we may, I think, without much rashness, assume that there is a substance or substances in the nerve endings or gland cells with which both atropine and pilocarpine are capable of forming compounds. On this assumption then the atropine or pilocarpine compounds are formed according to some law of which their relative mass and chemical affinity for the substance are factors.

So Langley, some 30 years or more before the experiments of Elliott or for that matter of his own on curare, was already developing the idea of pharmacological agents forming compounds with the substances in cells. The concept of the receptor is clearly present in these early formulations, which are in contrast to those of Ehrlich in 1900 mentioned above. Furthermore, the observations upon which these conjectures were developed were made well before Ehrlich began his research in 1878.

In 1905, Langley showed that injection of nicotine into the vein of an anaesthetised fowl led to gradual stiffening and extension of the hindlimbs over a couple of minutes due to tonic contraction of the red muscles (Fig. 2F and G). This effect still occurred after section of the sciatic and crural nerves, so that it did not involve the nerve supply. In order to provide quantitative details of this effect, Langley took measurements of the gastrocnemius muscle in the fowl following injection of nicotine into the vein, without interfering with the muscle's blood supply and after cutting the sciatic and crural

Fig. 4. The experiments of John Langley that established the existence of 'receptive substances' at the somatic neuromuscular junction. (A) Effects of curari and nicotine. Upper panel shows a contraction produced by a large dose of nicotine, applied for a time given by the second trace from the bottom. The bottom trace gives a time marker in 10-s intervals. There is no calibration of the size of the contractions. Lower panel shows a similar contraction annulled by curari; the upper curve gives the blood pressure (from Figs. 4 and 5 in Langley, 1905). (B) Denervated muscle. Effect of nicotine and of curari after nicotine applied at times indicated in the trace, second from the bottom. Time in seconds, omitting every tenth, is shown in the bottom trace, but is not clear in the original figure. There is no calibration of the size of the contraction (from Fig. 7 in Langley, 1905). (C) Abolition by curari of the contraction in the gastrocnemius muscle of the fowl caused by nicotine. The line second from the bottom gives the period of application of the drugs. The lowest line marks intervals of 10 s. No calibration is given of the size of the contraction (from Fig. 4 in Langley, 1906). (D) Frog. Brain and spinal cord destroyed. A thread was tied to the manus and connected with an unweighted lever, so that the flexion of the arm caused a rise of the lever; 1 c.c. of 1% nicotine was injected into the abdominal cavity at the time shown by the signal in the bottom trace (the '1cc1pc nicotine' is printed on the trace). Time marked in 10-s intervals. No calibration is given of the size of the upper trace (from Fig. 8 in Langley, 1906). (E) Contraction of sartorius with nicotine compared with that due to direct stimulation (top trace). Effect of stimulating with make and break induction shocks before and after nicotine: 0′ — muscle in Ringer's fluid (this is not shown in the original figure); 2′ — stimulate first with make and then with break shock, raise lever to base line; 7′ — pour 0.005% nicotine into muscle chamber, beginning and end of pouring is marked; 11′ — stop drum, and run off nicotine; 11 ½′ and 12 ½′ — stimulate with make and break shocks. Bottom trace, time in 1-s intervals. Period of application of make and then break shocks and of nicotine indicated in second bottom trace. No calibration is given of the size of the contraction in the upper trace (from Fig. 1 in Langley, 1907).

nerves. The results showed that the muscle contracted for several minutes (Fig. 4A, upper). Langley then injected curare about 1 min after the beginning of the nicotine-induced contraction: the muscle then relaxed (Fig. 4A, lower). Repeating this experiment after cutting the sciatic and crural nerves several weeks previously so as to allow their peripheral extensions to fully degenerate, did not alter the results of injecting nicotine and curari, as shown in Fig. 4B. Langley (1905) commented on these experiments that:

> I conclude then that nicotine acts upon the muscle substance, and not upon the axon-endings. It has been shown above that curari acts upon the same substance as nicotine. It follows then that curari acts upon the muscle substance and not upon the axon-endings. Since, in the normal state, both nicotine and curari abolish the effect of nerve stimulation, but do not prevent contraction from being obtained by direct stimulation of the muscle or by a further adequate injection of nicotine, it may be inferred that neither the poisons nor the nervous impulse act directly on the contractile substance of the muscle but on some accessory substance. Since this accessory substance is the recipient of stimuli which it transfers to the contractile material, we may speak of it as the receptive substance of the muscle.

This is the first occasion on which the phrase 'receptive substance' or as we now simply call it 'receptor' was used. In developing this concept, Langley (1905) makes his indebtedness to Elliott quite clear when he comments that:

> The subsequent work mentioned in the Introduction, and especially that of Elliott on the action of adrenalin, made the issues clearer.

He goes on to say that:

> In my view, the myo-neural junction is a part of the receptive substance localized in the neighbourhood of the axon-ending.

Although Langley (1907) went on to give detailed descriptions of how nicotine acts to block transmission at the myo-neural junction, especially at high concentrations (Fig. 4E), and of the fact that the effects of nicotine are only found in those parts of individual muscles where nerve endings are found, his concept of the receptive substance was fully matured by the time he gave a Croonian lecture to the Royal Society of London in 1906. In that lecture he presented most elegant tracings of how contractions of the fowl's gastrocnemius due to nicotine are blocked by curari (Fig. 4C). In addition, he gave graphic demonstrations of the effects of nicotine when injected into the abdominal cavity of frogs whose brain and spinal cord were destroyed. These passed from a state in which the muscles are flaccid, so that when the limbs are raised they at once fall, to a condition in which there is maximum flexion of the forelimbs, as shown in Fig. 2D (see also Fig. 4D). The same experiment when performed on toads gives rise to a cataleptic condition in both the forelimbs and the hindlimbs. The contraction of the flexors and extensors of the arm are about equal so that there is little movement or no movement.

> The forelimbs can then be moved about almost as if made of lead, and stay with but slight return movement in any position in which they are placed consistent with the arrangement of the joints and ligaments (Fig. 2E).

These cataleptic conditions are completely abolished by sufficient doses of curari. The conclusion is reached that:

> The mutual antagonism of nicotine and curari on muscle can only satisfactorily be explained by supposing that both combine with the same radicle of the muscle, so that nicotine-muscle compounds and curari-muscle compounds are formed. … Since neither curari nor nicotine, even in large doses, prevents direct stimulation of muscle from causing contraction, it is obvious that the muscle substance which combines with nicotine or curari is not identical with the substance which contracts. It is convenient to have a term for the specially excitable constituent, and I have called it the receptive substance.

Langley (1906) concludes his lecture by drawing attention to the fact that if the set of experiments on muscle with nicotine and curari are carried out on the excitability of nerve cells in sympathetic ganglia, then analogous results are obtained (Langley, 1901). Thus: "I conclude that the substance affected by the poisons is a special receptive substance and not the fundamental substance of the cell". As to transmission between nerve endings and smooth muscle as well as glands, the arguments outlined above concerning the action of adrenaline that have been developed in particular by Elliott indicate that:

> The legitimate statement from the premises is that it does not act on any muscle substance or on any nerve substance outside the limits of the myoneural junction. … As regards the localisation of the receptive substance, strong evidence that this occurs to a considerable extent is afforded by the action both of adrenalin and of chrysotoxin on tissues which have a double nerve supply, but the evidence cannot be regarded as conclusive.

It seems likely, despite some of Langley's arguments to the contrary (viz. that Elliott adopted the theory that nerve and unstriated muscle are continuous), that Elliott had conceived of Langley's receptive substance, but without giving it a name he comments:

> But since adrenalin does not evoke any reaction from muscle that has at no time of its life been innervated by the sympathetic, the point at which the stimulus of the chemical excitant is received, and transformed into what may cause the change of tension of the muscle fibre, is perhaps a mechanism developed out of the muscle cell in response to its union with the synapsing sympathetic fibre, the function of which it is to receive and transform the nervous impulse.

One cannot do better in this early part of the history of the receptor than to quote the conclusion of Langley's great Croonian lecture:

> In the foregoing account we have seen reason to believe that in each of the three great types of connection of the peripheral end of an efferent nerve with a cell it is some constituent of the cell substance which is stimulated or paralysed by poisons ordinarily taken as stimulating or paralysing nerve endings. This theory adds to the complexity of the cell. It necessitates the presence in it of one or more substances (receptive substances) which are capable of receiving and transmitting stimuli, and capable of isolated paralysis, and also of a substance or substances concerned with the main function of the cell (contraction or secretion, or, in the case of nerve cells, of discharging nerve impulses).

The Croonian lecture of 1906 on the antagonism between curare and nicotine giving rise to the concept of the transmitter receptor brings to a conclusion the research program embarked on by Langley some 30 years earlier while still a student at Cambridge working on the antagonism between atropine and pilocarpine. The antagonism of this set of drugs is seen as acting on the protoplasmic substance or substances in the muscle or effector organ, and does involve the combination of an alkaloid with protoplasm, in contradistinction to the suggestions of Ehrlich. Following these experiments of Langley, Ehrlich accepted the idea of the receptor for alkaloids such as nicotine and curare.

6. The Langley–Ehrlich receptor theory

It is fascinating to trace the productive interaction of the ideas of Ehrlich and Langley over this period from 1878 to 1908, beginning as each did from quite different research programs. In the case of Ehrlich, his research was concerned with drug resistance as a consequence of studies on the chemotherapy of trypanosomes. The receptive side chain concept was developed in order to give a theoretical underpinning to his work on the chemotherapy of such micro-organisms, in particular in the use of substances that act in a manner that is largely irreversible, such as the arsenicals. For Langley, the starting point involved his research on the effects on muscle and nerve of alkaloids such as nicotine, curare, atropine, pilocarpine, strychnine and adrenaline, all of which produce an action that is relatively reversible compared with the actions studied by Ehrlich. Nevertheless by 1908 Langley could say:

> My theory of the action is in general on the lines of Ehrlich's theory of immunity. I take it that the contractile molecule has a number of 'receptive' or side-chain radicles and the nicotine by combining with one of these causes contraction and by combining with another causes twitching ….

To which Ehrlich commented in 1914:

> For many reasons I had hesitated to apply these ideas about receptors to chemical substances in general, and in this connection it was, in particular, the brilliant investigations by Langley, on the effects of alkaloids, which caused my doubts to disappear and made the existence of chemoreceptors seem probable to me.

Thus was conceived the tremendously fruitful concept of the receptor.

7. The discovery of acetylcholine and its physiological action at autonomic neuroeffector junctions

In 1906 Hunt and Taveau synthesized acetylcholine and reported that:

> as regards its effect upon the circulation, it is the most powerful substance known.

They went on to say:

> we have not determined the cause of the fall of blood pressure from acetyl-cholin, but from the fact that it can be prevented entirely by atropine, I am inclined to think that it is due to an effect upon the terminations of the vagus in the heart.

In the same year, Dixon (1906) gave a description of his experiments on the vagus inhibition of the heart, and stated that he was of the opinion that:

> the heart contains a substance — 'pro-inhibitin', which, as a result of vagus excitation, is converted into a chemical body — 'inhibitin'. This substance, combining with the heart muscle, results in cardiac standstill.

These suggestions were extraordinarily prescient of Loewi's experiments on the identity of acetylcholine as the inhibitory transmitter released by the vagus in the heart subsequently carried out in the early 1920s (see below).

In 1914, Ewins showed that extracts of certain specimens of the fungus ergot contained an active principle, which he identified as acetylcholine. Much of this work of Ewins was done at the instigation of Dale (Fig. 1C), as a consequence of his knowledge of the work of Hunt and Taveau (1906) who had already shown that synthesized acetylcholine had powerful depressor activity, indeed Dale reported to Elliott:

> We got that thing out of our silly ergot extract. It is acetylcholine and an most interesting substance. It is much more active than muscarine, though so easily hydrolysed that its action, when it is injected into the blood-stream, is remarkably evanescent, so that it can be given over and over again with exactly similar effects, like adrenaline. Here is a good candidate for the role of a hormone related to the rest of the autonomic nervous system, I am perilously near wild theorising … I shall be surprised, however, if this principle, once identified, does not turn up in all sorts of tissue-extracts (see Letter, Dale to Elliott, 11 December 1913, Contemporary Medical Archives Centre, Wellcome Institute, GC/42 'T.R. Elliott'; quoted in Tansey, 1991).

It was then of considerable interest to see if the depressor effect of acetylcholine was its principal effect. In 1914, Dale was determined to carry out a systematic study of the effects of acetylcholine on various organs of the autonomic and somatic nervous systems (Dale, 1914a,b). In that work he showed that following the injection of acetylcholine into an animal, there were considerable responses elicited in a number of organs: cat's blood pressure declined (Fig. 5A); there was complete cessation of the heart beat in frogs (Fig. 5B) and the small intestine of the rabbit contracted vigorously (Fig. 5C). Dale was alert to the fact that these effects are those produced by stimulation of the vagus nerve, and in a sense constitute a complementary set of effects to those observed by Langley and Elliott with respect to adrenaline and sympathetic nerve stimulation. He comments that:

> The question of a possible physiological significance, in the resemblance between the action of choline esters and the effects of certain divisions of the involuntary nervous system, is one of great interest, but one for the discussion of which little evidence is available. Acetylcholine is, of all the substances examined, the one whose action is most suggestive in this direction. The fact that its action surpasses even that of adrenine both in intensity and evanescence, when considered in conjunction with the fact that each of these two bases reproduces those effects of involuntary nerves which are absent from the action of the other, so that the two actions are in many directions at once complementary and antagonistic, gives plenty of scope for speculation.

The main problem at this time in making a physiological claim for acetylcholine was:

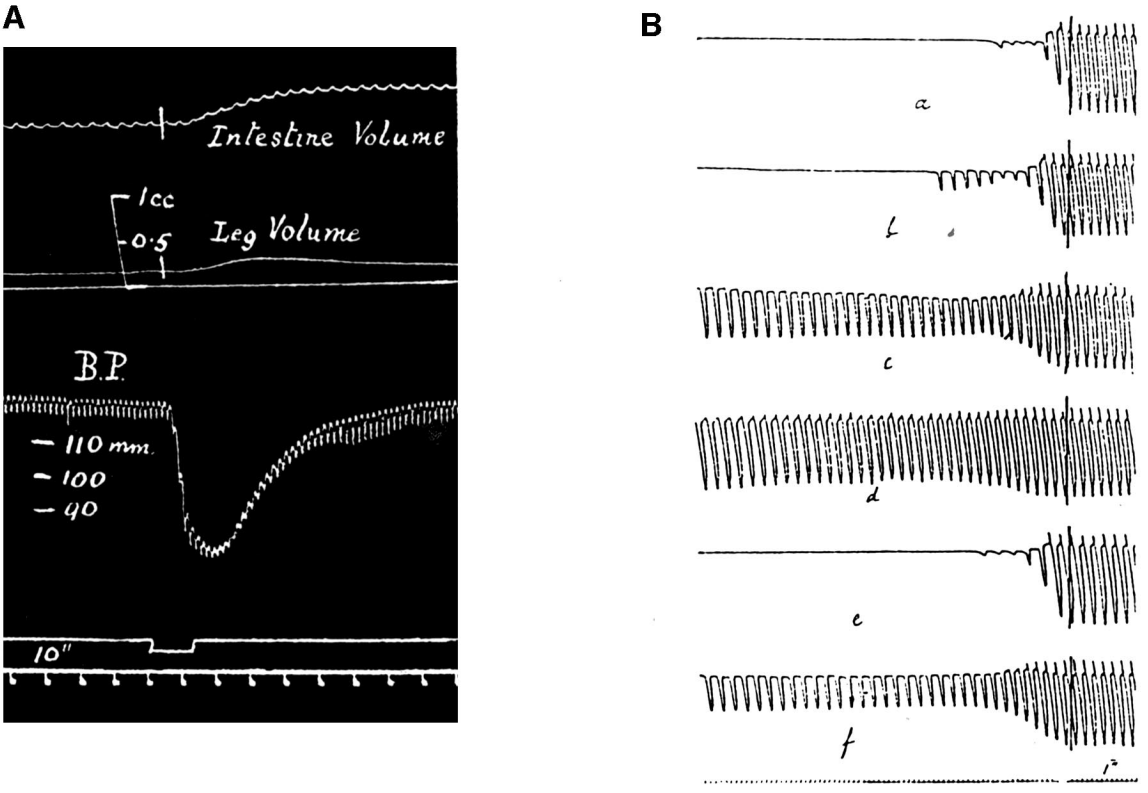
> On the other hand, there is no known depot of choline derivatives, corresponding to the adrenine depot in the adrenal medulla, nor, indeed, any evidence that a substance resembling acetylcholine exists in the body at all.

So Dale concludes in this paper which gives the first systematic account of the actions of acetylcholine in the peripheral nervous system that:

> Acetylcholine occurs occasionally in ergot, but its instability renders it improbable that its occurrence has any therapeutic significance.

There the matter rested until after the First World War.

In 1921 Loewi (Fig. 1D) reported from Austria his experiments on the heart indicating that chemical transmission occurred between the vagus nerve and the heart, mediated by a substance which he called 'Vagusstoff'. Fig. 5D shows his original record, in which the heart beat in 1 is in normal Ringer, whereas the subsequent decline in the heart beat in 2 is due to the addition of Ringer that has been in contact with another heart whose vagus had been stimulated for 15 min; at 3 the heart beat returns to normal as a consequence of it being exposed to a Ringer that had been in contact with another heart for which the vagus was not stimulated; at 4 atropine was added to the normal Ringer and this increases the heart beat. By 1926 Loewi and Navratil had produced sufficient evidence to mount a persuasive case that Vagusstoff was acetylcholine: application of very low concentrations of acetylcholine to the heart greatly decreased the heart beat (Loewi and Navratil, 1926a; Fig. 5E and F). These authors went on to show that inactivation of esterases in the heart for acetylcholine, using eserine (physostigmine; Fig. 5G–I; Loewi and Navratil, 1926b; Engelhart and Loewi, 1930) greatly potentiated the decrease in the heart beat brought about by the exogenous application of acetylcholine. This at last pro-



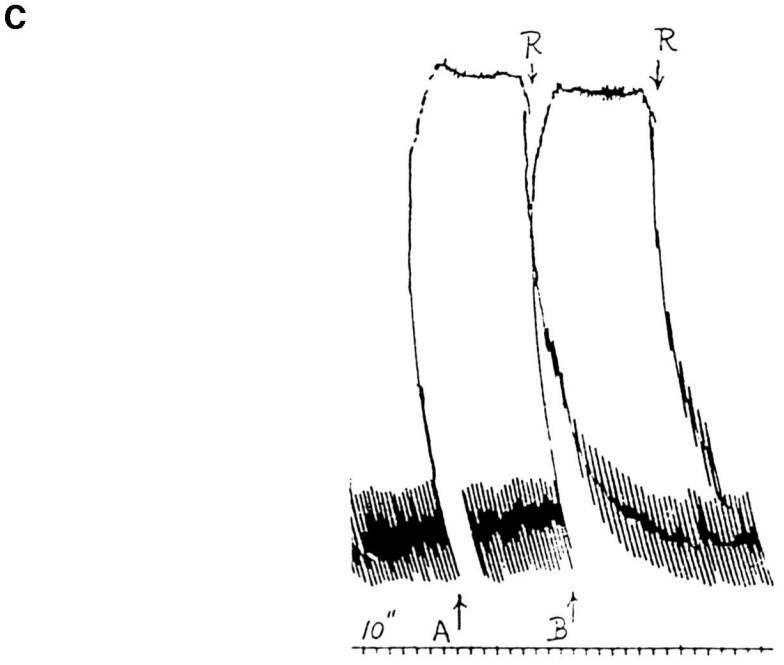


Fig. 5. (*continued on page 537*)

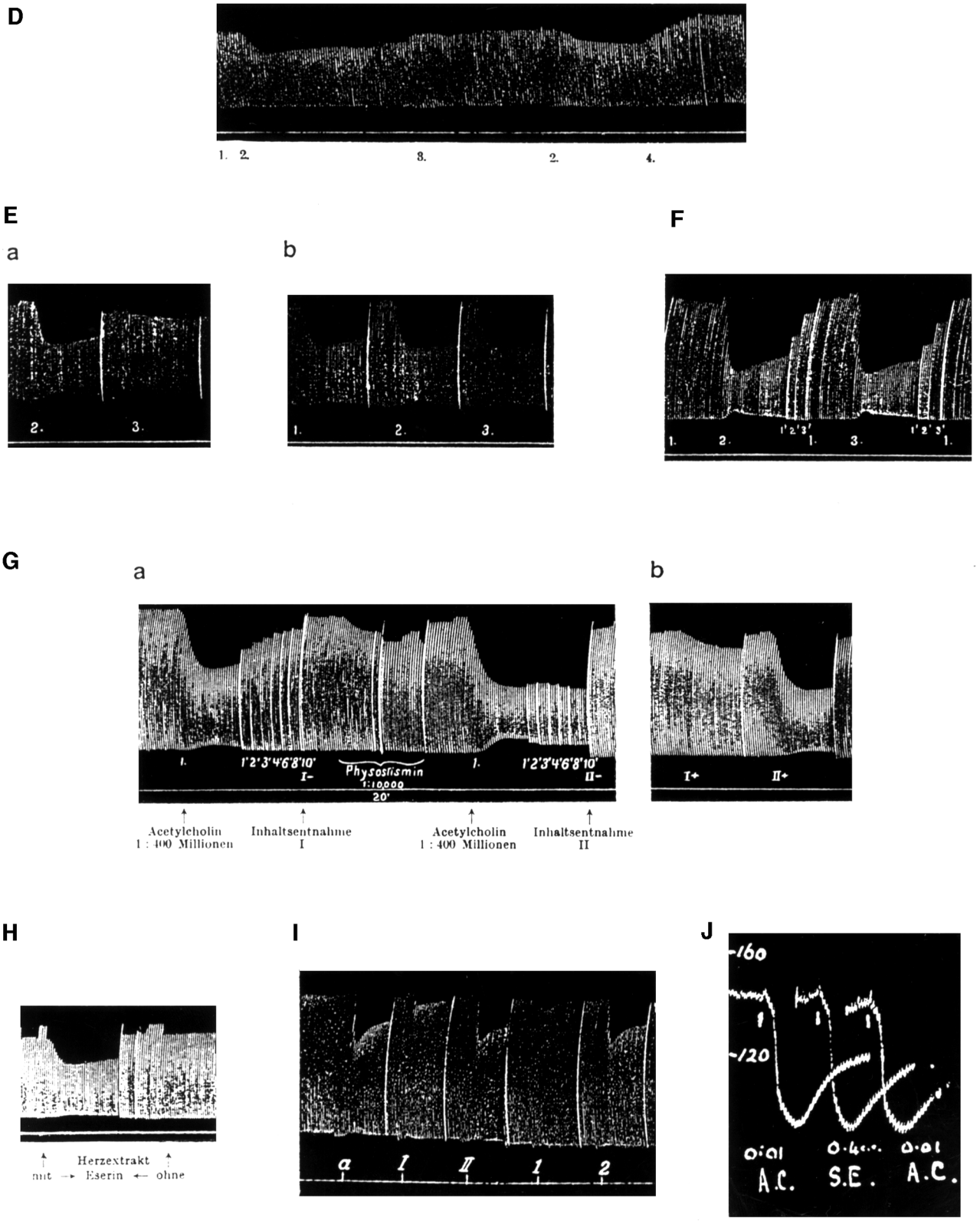


Fig. 5. (*continued*)

Fig. 5. Henry Dale and Otto Loewi establish acetylcholine as a transmitter substance at autonomic neuromuscular junctions. (A) Cat: ether. Plethysmograph records from intestine and limb with calibration in c.c. Carotid blood-pressure shown with calibration in mmHg. Injection of 0.001 mg acetyl-choline indicated by the second trace from the bottom. Bottom trace is time marker in 10-s intervals. Third trace from the top is not specified (from Fig. 3 in Dale, 1914a). (B) Perfused heart of frog, recorded by suspension-lever. Tracings read from right to left, the vertical line in each case indicating change from pure Ringer's solution to a similar solution containing a choline-ester (the vertical line is about 7 beats from the right in each case): (a) acetylcholine, 1 in 100 million; (b) acetylcholine, 1 in 200 million; (c) acetylcholine, 1 in 500 million; (d) acetylcholine, 1 in 1000 million; (e) nitroso-choline, 1 in 100,000; (f) nitroso-choline, 1 in 1 million. Bottom trace is time in seconds. There is no calibration of the size of the contraction (from Fig. 11 in Dale, 1914a). (C) Loop of rabbit's small intestine in 50 cc of Tyrode's solution at A, 0.01 mg synthetic acetyl-choline; B, 0.01 mg of acetyl-choline from ergot, added to the bath; and R, R, fresh Tyrode's solution. Bottom trace is time marker in 10 s intervals. No calibration is given for the size of the contraction (from Fig. 13 in Dale, 1914a). (D) Frog heart contractions. 1, Ringer; 2, Ringer from a heart that received 15 min of vagal stimulation; 3, Ringer from a heart that did not receive stimulation; 4, addition of 0.1 mg atropine. Bottom trace is not specified and there is no time or contraction calibration given (from Fig. 1 in Loewi, 1921). (E) Frog heart contractions. (a) The testing material is heart extract (1:20 dilution) plus acetylcholine (dilution 1:100,000); 2 is inactivated heart extract plus acetylcholine (dilution 1:1000); 3 is heart extract plus acetylcholine again. (b) The starting material is again heart extract (dilution 1:20) plus acetylcholine (dilution 1:100,000); 1 is heart extract inactivated by standing for 30 min plus acetylcholine (dilution 1:750); 2 is heart extract inactivated by standing for 90 min plus acetylcholine (dilution 1:1000); 3 is heart extract inactivated by standing for 90 min plus acetylcholine (dilution 1:150). Bottom trace is not specified and no time or contraction calibrations are given (from Fig. 5 in Loewi and Navratil, 1926a). (F) Frog heart contractions. 1, Ringer; 2, Vagusstoff (alcoholic extract from the heart); 3, acetylcholine (dilution 1: 100 million). Bottom trace not specified and no time or contraction calibrations given (from Fig. 4 in Loewi and Navratil, 1926a). (G) Effect of stimulating the heart nerve on contraction of the frog's heart in relation to the effects of applied acetylcholine and physostigmine given at the times indicated. In the left-hand panel, samples were taken from the heart at the time indicated by I and another taken at the time indicated by II (Inhaltsentnahme). In the right-hand panel, the effects of applying these samples (I and II) to another heart are shown. Bottom trace is not specified and no time or contraction calibration is given (from Fig. 3 in Loewi and Navratil, 1926b). (H) Effect of eserine on the frog's heart. The caption reads: "Heart extract with Eserine without. Left standing for 2 hours". Bottom trace not specified and no calibrations given for the time of contraction (from Fig. 5 in Loewi and Navratil, 1926b). (I) Frog heart: a, acetylcholine (dilution 1:million); I, bovine blood plus acetylcholine; II, heated bovine blood plus acetylcholine; 1, inactivated bovine blood plus acetylcholine; 2, heated bovine blood plus acetylcholine (from Fig. 7 in Engelhart and Loewi, 1930). (J) Comparison of purified spleen extract (S.E.) and acetylcholine (A.C.) solution on the blood-pressure of a cat under ether. Calibration of the blood pressure is given in mmHg. The amount of A.C. given is 0.01 mg. About 0.4 cc of the spleen extract gave the same result as that of 0.01 mg of A.C. (from Fig. 1 in Dale and Dudley, 1929)

vided an explanation for the problem that Dale had referred to with regards to acetylcholine, that:

> when it is injected into the blood-stream, it is remarkably evanescent.

Although it had been known for some time that physostigmine 'sensitized' the heart to stimulation of the vagus, this demonstration by Loewi and Navratil that physostigmine acted to potentiate the actions of exogenous acetylcholine led directly to the concept by these authors that the heart possesses an endogenous esterase for acetylcholine, for which they later coined the term 'cholinesterase'.

Dale continued in the late 1920s to try and find a natural source of acetylcholine in the body like that provided for adrenaline in the adrenal medulla. He commented to a friend in 1929:

> We are still struggling with the acetylcholine problem, which I mentioned to you when I saw you in the autumn. I am more and more convinced that the thing is there to be found, if only we can overcome the technical difficulties (Letter, Dale to Richards, 22 March 1929, Archives of the National Institute for Medical Research, File 647; quoted in Tansey, 1991).

The breakthrough occurred in 1929, when Dale and a chemist Dudley discovered that acetylcholine was a natural constituent of both horse and ox spleens (Fig. 5J), thus giving the long sought after 'depot of choline derivatives' in the body. This then provided the necessary impetus for once more examining the role of acetylcholine as the mediator of the effects of transmission from parasympathetic nerves to the effectors of the autonomic nervous system as well as at other sites of transmission in the body. For as they state in their paper (Dale and Dudley, 1929):

> But there has been a natural and proper reluctance to assume, in default of chemical evidence, that the chemical agent concerned in these effects, or in the humoral transmission of vagus action, was a substance known, hitherto, only as a synthetic curiosity, or as an occasional constituent of certain plant extracts….
>
> It appears to us that the case for acetylcholine as a physiological agent is now materially strengthened by the fact that we have now been able to isolate it from an animal organ and thus to show that it is a natural constituent of the body.

They go on to say:

> We feel, however, that its definite isolation from one organ has so far altered the position that, when an extract from, or a fluid in contact with the cells of, an animal organ can be shown to contain a principle having the actions, and the peculiar instability, of acetylcholine, it will be reasonable in future to assume the identification. On such lines a physiological sur-

> vey of its distribution should be practicable. Similarly, when there is evidence associating some physiological event with the liberation of a substance indistinguishable from acetylcholine by its action, the presumption that it is, indeed, that ester will be strengthened by the knowledge that acetylcholine occurs in the normal body.

By 1930, then, three decades of research had shown firstly that acetylcholine, either synthesized or extracted from ergot, had dramatic depressor effects (Ewins, 1914; Hunt and Taveau, 1906; Dale, 1914b); secondly, that stimulation of the vagus to the heart released a substance that seemed to mediate transmission and which had properties remarkably similar to that of acetylcholine (Loewi, 1921), and finally that acetylcholine occurred naturally in mammals (Dale and Dudley, 1929). The question that came to dominate the 1930s was whether acetylcholine could be detected in the overflow from other organs than the heart during nerve stimulation, and also whether the effects of such stimulation could be mimicked by the close arterial injection of acetylcholine into these organs. Loewi had already shown that the effects of exogenous acetylcholine on the heart or of stimulation of the vagus could be greatly enhanced if esterases for acetylcholine in this organ were first inhibited using physostigmine or eserine (Fig. 5G–I; Engelhart and Loewi, 1930). In 1930, Matthes, working in Dale's laboratory, showed that the destruction of acetylcholine in the blood was due to the action of an esterase and that the action of this enzyme could be inhibited by eserine. Thus, inhibition of acetylcholinesterases was a necessary requirement of any attempt to show that acetylcholine was released from a particular nerve ending, either by using an intravenous injection of eserine or by adding it to the organ bath. Shortly after this work of Matthes, Feldberg arrived in Dale's laboratories as a refugee from Germany to Great Britain. He introduced the eserinized leech muscle preparation which had previously been shown to be exquisitely sensitive to applied acetylcholine by the German pharmacologist Fuhner (1918). Feldberg was inspired to use this approach because of the experiments of Loewi and Navratil (1926a,b) on the actions of physostigmine in potentiating the effects of applied acetylcholine on the frog's heart. In the hands of Dale and Feldberg (1934) the eserinized leech muscle was first used to show that acetylcholine appeared in the venous blood after stimulation of the vagus nerve to the stomach. This was followed by experiments that showed acetylcholine also appeared after stimulation of the splanchnic nerves to the suprarenal glands (adrenal medulla; Feldberg et al., 1934).

8. The physiological action of acetylcholine in autonomic ganglia

In 1934 experiments were also begun to see if acetylcholine could be detected at neuronal synapses in addition to neuroeffector junctions. To this end Feldberg and Vartiainen (1934) were able to show that:

> when the superior cervical ganglion of the cat is perfused with warm, oxygenated Locke's solution containing a small proportion of eserine, acetylcholine appears in the venous effluent whenever the cervical sympathetic nerve is effectively stimulated, and only then.

The assay for this acetylcholine was either the frog's heart (Fig. 6A, upper panel) or the leech muscle treated with eserine (Fig. 6A, lower panel). They regarded these observations as (Feldberg and Gaddum, 1934):

> support for the theory that the mechanism by which each impulse normally passes the synapse consists in the liberation of a small quantity of acetylcholine.
> This discovery of the release of acetylcholine in autonomic ganglia then raised the possibility that the 'Vagusstoff' of Loewi was in fact liberated from ganglia in the heart rather than at the neuroeffector junction. Whilst this would certainly be the case, Feldberg and Gaddum (1934) were persuaded of the view that most likely the Vagustoff collected by Loewi came from both the synapses in the intramural ganglia as well as the neuroeffector junction, although no very effective argument was offered in defence of this proposition.

One caveat in these experiments on the ganglia was that Eccles had, in the same year, shown that the electrical signs of the action potential set up in the postganglionic nerve trunk of the superior cervical ganglion by volleys in the preganglionic trunk were depressed by eserine rather than potentiated, as would be expected as a consequence of an enhanced transmission through the ganglion on inactivation of acetylcholinesterase with eserine (Eccles, 1934). Later in 1934, Feldberg and Vartiainen offered a vigorous defense of the idea that acetylcholine is the transmitter substance in autonomic ganglia. Using the nictitating membrane of the cat as a measure of the postganglionic volleys in response to stimulation of the preganglionic supply to the ganglion, they were able to show that 18 impulses delivered in 10 s gave rise to a greatly potentiated response of the membrane after the ganglion had been perfused with eserine (Fig. 6B (c) and (d)) compared with that in the absence of eserine (Fig. 6B (a) and (b)), leading them to conclude that:

> these new items of evidence entirely support the conception of transmission at ganglionic synapses by liberation of acetylcholine.

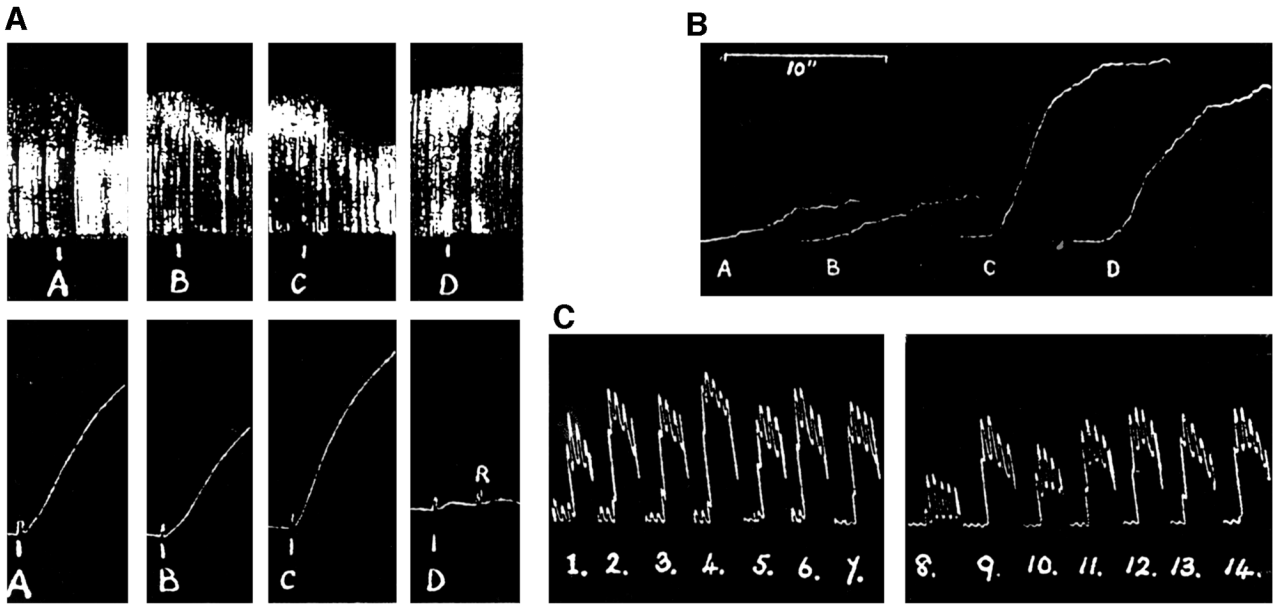


Fig. 6. The identification of acetylcholine as a transmitter substance in autonomic ganglia. (A) Effect of fluid collected from stimulated cat sympathetic ganglia on contraction of the frog's heart and of eserinised leech muscle. Upper panel: frog's heart (Straub's method). Lower panel: leech muscle treated with eserine. A, Fluid collected from the ganglion during the stimulation. D, Control fluid. B, C, Acetylcholine (15 and 30 μg per litre respectively). No time base or contraction calibration is given in the original figure (from Fig. 6 in Feldberg and Gaddum, 1934). (B) Effect of eserine on nictitating membrane contraction due to stimulation of the cervical sympathetic. Responses to equal groups of submaximal shocks to cervical sympathetic; A and B, as before; C and D, during perfusion of eserine 1 in 10^6. Time calibration is 10 ms; no contraction calibration is given (from Fig. 6 in Feldberg and Vartiainen, 1934). (C) The evaluation of esterase activity in normal and decentralized sympathetic ganglia using rabbit's jejunum contraction as test. Numbers give the contractions to the following amounts of acetylcholine: 1, 1.5 μg; 2, 2 μg; 7, 1.8 μg; 8, 1 μg; 10, 1.5 μg; 11, 1.7 μg; 12, 1.9 μg; 14, 2 μg. Numbers 9 and 13 give the contraction in the presence of 10 μg of acetylcholine plus an extract of normal sympathetic ganglia. Numbers 4 and 5 give the contraction in the presence of 3 μg and 2 μg of acetylcholine respectively in the presence of extract of denervated sympathetic ganglia. No time or contraction calibration is given (from Fig. 1 in Brucke, 1937).

The idea that eserine has a depressant action on cholinesterase, which may be located at nerve terminals in ganglia, so that eserine potentiates the effects of endogenously released acetylcholine, was shown to be very likely when Brucke (1937) found high concentrations of cholinesterase in the superior cervical ganglion. He showed that this mostly disappeared on section and degeneration of the preganglionic nerves to the ganglion. These results are illustrated in Fig. 6C, where the contractile responses of the rabbit's jejunum to different concentrations (in gamma units) of acetylcholine alone (1=1.5; 2=2; 6=2; 7=1.8; 8=1; 10=1.5; 11=1.7; 12=1.9; 14=2), or acetylcholine together with extract of normal ganglia that therefore contains cholinesterase (9 and 13=10), or acetylcholine plus extract of denervated ganglion plus eserine (4=3; 5=2) are shown. It will be noted that much higher concentrations of acetylcholine and extract of normal ganglion had to be used in order to get responses comparable to that of acetylcholine alone, indicating the effects of cholinesterase in this case (Fig. 6C). The consensus of opinion at the end of the 1930s was that acetylcholine acted as the transmitter of impulses in autonomic ganglia. Eccles provided the main continuing resistance to this idea with some persuasive arguments that are detailed elsewhere (Bennett, 1994).

9. The identity of acetylcholine as the transmitter substance at somatic neuromuscular junctions

Although the concept of the transmitter receptor was developed primarily in relation to striated muscle, as detailed above, identification of acetylcholine as the transmitter substance that acts on these receptors came relatively late, well after the establishment of acetylcholine as the transmitter from the vagus nerve to the heart. In the late 1920s and early 1930s, the problem of the relationship between motor nerves and muscle revolved around questions relating to their relative excitability and the actions of agents thought to exert effects at the junction between nerve and muscle, such as curare, had on this excitability. Lapicque had at the beginning of the century carried out a series of experiments on the excitability of nerve and muscle in which he had defined the chronaxie and rheobase of the strength–duration curve for setting up excitation in these tissues, as are now described in many text books (Lapicque and Lapicque, 1906; Lapicque, 1926). In these works he developed the concept that nerve and muscle possessed the same chronaxie which he defined as isochronism. This was challenged by Rushton (1930) who showed, following the work of Lucas (Lucas and Mines, 1907), that in general,

muscle possessed two different excitabilities, one associated with the intramuscular nerves and the other with the muscle fibres themselves (Rushton 1930, 1932), so that nerve and muscle did not possess isochronism. The possibility that nerve terminals and the muscle or endplate could be brought into isochronism by the action of acetylcholine was then entertained as involved in the direct transmission of the nerve impulse into the muscle. This proposition was put forward as agents, such as acetylcholine, could shorten the chronaxie (Fredericq, 1924) whereas curare lengthened the chronaxie (Fig. 7A; Lapicque, 1934). The heated arguments used in the considerable controversy between Lapicque and Rushton outlined in their papers in the Journal of Physiology seemed to offer at this time the possibility for an esoteric interaction between the action of agents known to affect transmission at the endplate and the electrical properties of this region of the muscle.

These electrical controversies concerning isochronism in relation to the mechanism of transmission between nerve and muscle declined in the second half of the 1930s. First, using the eserinized leech preparation, Dale et al. (1936) showed that considerable quantities of acetylcholine could be collected in venous fluids from the cat gastrocnemius muscle following nerve stimulation (Fig. 7C). This also occurred if the muscle was directly stimulated, even in the presence of curare, but not if the muscle had previously been denervated. Dale and his colleagues were careful at this time not to claim this showed acetylcholine, released at nerve terminals, transmitted the impulse from the terminals to muscle. They were still open to an interpretation:

> That the propagated disturbance in the nerve fibre is directly transmitted to the effector cell, but that the latter cannot accept it for further propagation unless sensitized by the action of the acetylcholine, which appears with its arrival at the nerve ending. Such an hypothesis might be stated in terms of Lapicque's well-known conception, by supposing that the action of acetylcholine shortens the chronaxie of the nerve cell, or of the motor end-plate of the muscle fibre, so that it is momentarily attuned to that of the nerve.

Later in 1936, Brown et al. showed that injection of acetylcholine directly into the empty arteries of a normal mammalian muscle could, if given with adequate rapidity, cause contraction of the muscle at not less that half the speed of a maximal motor nerve twitch (Fig. 7B). Furthermore, eserine caused the response to stimulation of the nerve supply to be converted from a simple twitch to a repetitive response with a maximum tension twice that of the normal twitch, a result reproduced and examined further by Bacq and Brown (1937); Fig. 7F). No mention is made any longer of Lapicque's theories, especially given the effect of close intrarterial injection of acetylcholine mimicking the normal twitch response of the muscle to nerve stimulation. Rather they suggested that:

> acetylcholine, is liberated by arrival of the nerve impulses at the nerve ending, and destroyed during the refractory period by a local concentration of cholinesterase.

The facts supporting this hypothesis are:

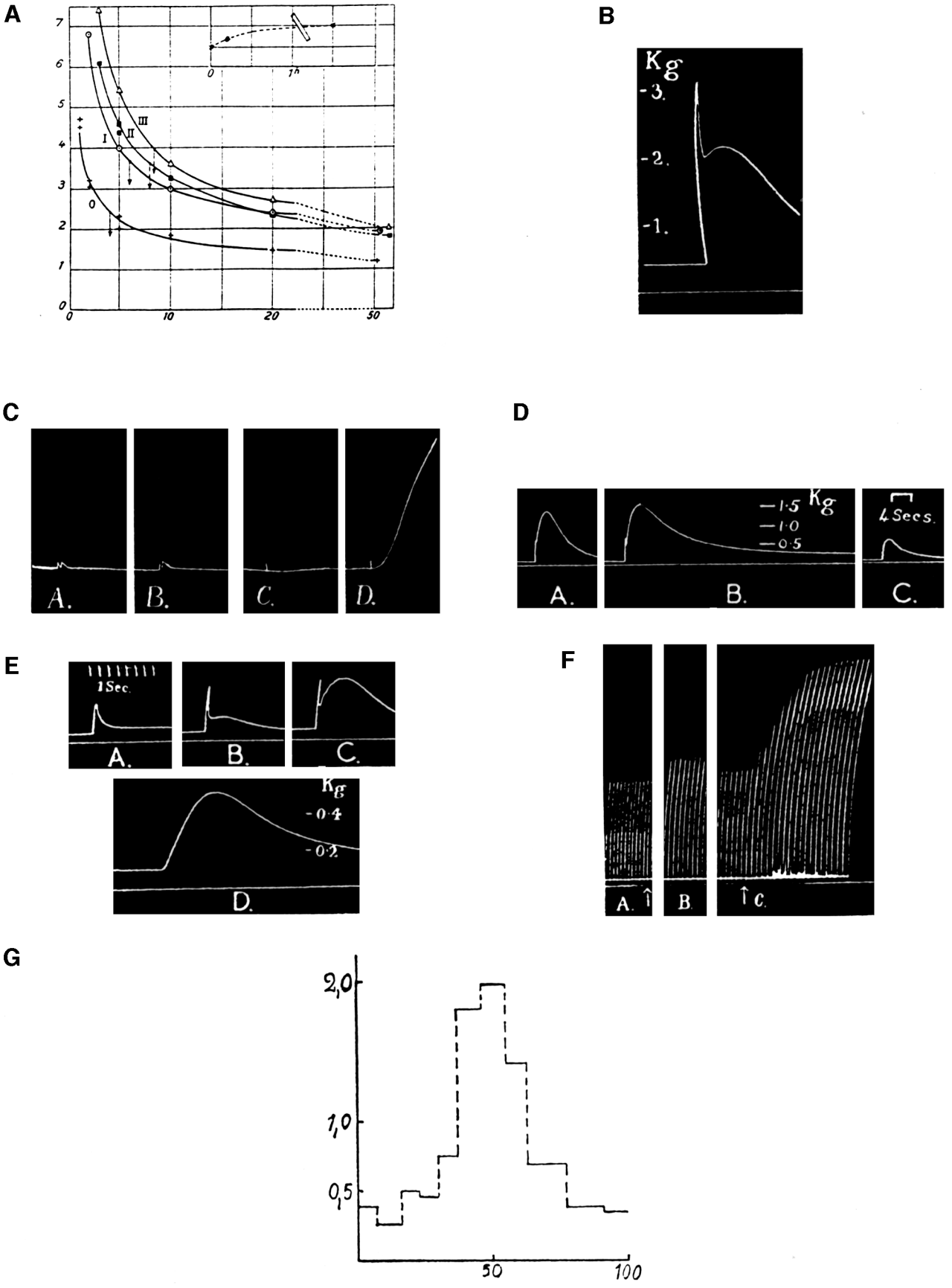
> (1) that acetylcholine, identified as such, is liberated by impulses at motor nerve endings; (2) that acetylcholine, when suitably injected into the muscle, produces the kind of contraction which the transmitter should produce; and (3) that a suitable dose of eserine causes the muscle to give a short, waning, tetanic response to a single, synchronous volley of nerve impulses.

Many of these actions of applied acetylcholine on muscle were subsequently examined by measuring the rate of impulse firing in the muscle by Brown (1937). He found that close arterial injection of acetylcholine into a denervated muscle gave rise to a quick initial contraction (Fig. 7E) that was accompanied by a burst of action potentials in the muscle. He comments that:

> The facts presented give incidental support to the suggestion of a concentration of cholinesterase at the mammalian motor nerve endings.

That this is the case was shown by Couteaux and Nachmansohn (1940) who determined the concentration of cholinesterase in the middle portion of the guinea pig's gastrocnemius. This work showed that cholinesterase was indeed found at relatively high levels in the central region of the muscle where the nerve endings are located (Fig. 7G).

By 1940, acetylcholine was believed to be the agent of transmission between motor nerve and muscle. However, it should be noted that Eccles, who together with O'Connor (1938) had just recorded for the first time the electrical signs of transmission at the endplate (the so called 'endplate potential'; see also Gopfert and Schaefer, 1937), did not come around to this opinion until he worked on motor nerve transmission to muscle with Katz and Kuffler a few years later (Eccles et al., 1941). Indeed Loewi at the time of being awarded the Nobel Prize with Dale in 1936 was not persuaded of chemical transmission at the motor endplate. Nevertheless, most students of transmission accepted by 1940 that the 'receptive substance' of Langley could be identified as a receptor for acetylcholine, although the full flavour of the opposition to the concept of chemical transmission from the time of Loewi's experiments after the Great


A
B
Kg
-3.
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C
A.
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D
1·5 Kg
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4 Secs.
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E
1 Sec.
A.
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Kg
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F
A.
B.
C.
G
2,0
1,0
0,5
50
100

Fig. 7.

Fig. 7. The identification of acetylcholine as the transmitter substance at the somatic neuromuscular junction. (A) Strength–capacity curves on a toad's gastrocnemius; O, before curare; I, II, III, after a small dose of curare. In the upper right-hand corner, variation of the chronaxie with the time in hours as abscissa (from Fig. 2 in Lapicque, 1934). (B) Spinal cat. Record from denervated gastrocnemius, 14 days after nerve section. Perfusion with Locke's solution. Effect of 1 μg of acetylcholine by close arterial injection. No time calibration is given (from Fig. 5 in Brown et al., 1936). (C) Effect of venous fluids from gastrocnemius muscles of cat, in 75% dilution, on contraction of eserinized leech muscle: A and B from denervated muscle, A resting, B during stimulation; C and D from normal muscle, C resting, D during stimulation. No time or contraction calibration is given (from Fig. 5 in Dale et al., 1936). (D) Contractions of decerebrated cat gastrocnemius 20 days after nerve section, in response to close arterial injections of: A, 2–5 μg acetylcholine; B, 50 μg carabaminoylcholine; and C, 21 min later, 10 μg acetylcholine (from Fig. 5 in Brown, 1937). (E) A–C, Decerebrate cat, contractions of denervated (12 days) gastrocnemius in response to 0.25 μg, 1.0 μg and 2.5 μg acetylcholine by close arterial injection respectively; D, spinal cat, contraction of denervated gastrocnemius in response to 20 μg acetylcholine by 'distant' injection into inferior mesenteric artery (from Fig. 1 in Brown, 1937). (F) Spinal cat, 9 days after lumbosacral sympathectomy. Contractions of gastrocnemius in response to maximal shocks to nerve at 10-s intervals: A, arterial injection of 5 μg eserine; B, 10 min later; C, arterial injection of 20 μg eserine. No time or contraction calibration is given (from Fig. 4 in Bacq and Brown, 1937). (G) Concentration of choline esterase in the middle portion of the interior section of a guinea pig's gastrocnemius cut in 11 slices of similar thickness and weight. Each horizontal line corresponds to one slice and indicates its weight in % of the total weight. Abscissae: region from which the tissue was obtained in terms of order of consecutive slices. Point 50 corresponds to the center region where the nerve endings are situated. Ordinate: choline esterase in mg acetylcholine hydrolyzed per hour by 100 mg of fresh tissue (from Fig. 1 in Couteaux and Nachmansohn, 1940).

War up to the 1950s can only be gauged by reading a first hand account of the controversies (for example that of Bacq, 1983).

10. The discovery of the physiological action of single acetylcholine receptors

The study of the physiological action of single acetylcholine receptors began in 1970 with the discovery by Katz and Miledi of membrane noise at the endplate in response to the steady action of acetylcholine from a micropipette. They hypothesised that during such a steady application:

> the statistical effects of molecular bombardment might be discernible as an increase in membrane noise, superimposed on the maintained average depolarisation.

This is what in fact they observed with an intracellular electrode as can be readily ascertained by inspection of Fig. 8A. A simple relationship was then used that connects the size of the elementary voltage (a) due to the opening of a single channel as a consequence of acetylcholine binding to a receptor to the average depolarisation (V) and the root mean square value of its fluctuation (E), namely $a=2E^2/V$. Applied to the results of Fig. 8A this gave a value for the elementary event of 0.29 μV. In 1971, the same authors determined the approximate time course of the elementary conductance change underlying the elementary event by recording the extracellular voltage fluctuations due to the bombardment of receptors with acetylcholine. In this case they ascertained the power spectrum of the acetylcholine induced noise, that is the relationship between ($E^2/\Delta f$) and the frequency (Fig. 8B). This gave an average time constant of the elementary event of about 1 ms when the event is treated as decaying exponentially and a net charge transfer across the open channel of about 5×10^4 univalent ions, with a channel conductance of about 10^{-10} Siemens.

Direct recording of the electrical signs of individual acetylcholine receptor channels was made by Neher and Sakmann (1976). They introduced the technique of recording from a small membrane area of the muscle, so as to decrease background noise (Fig. 8C). The tip of a glass pipette of 3–5 μm diameter, with fire polished edges, was connected up in the circuit shown in Fig. 8C after being filled with Ringer's solution and an acetylcholine receptor agonist, in this case suberyldicholine (SubCh). This pipette was then applied to the surface of a muscle fibre, denervated so as to ensure an abundance of receptors all over the surface of the muscle and subjected to enzyme treatment for the digestion of connective tissue and the basement membrane. Discrete conductance changes, like those shown in Fig. 8D, could only be resolved if the conductance between the pipette interior and the bath decreased by a factor of at least four after the pipette came into contact with the muscle membrane. Inspection of Fig. 8D shows that the amplitude of the single channel conductance is about 3.4 pA, giving a channel conductance of 28×10^{-12} Siemens. This conductance is about the same as that determined by Katz and Miledi (1973) for the agonist SubCh using the noise method of Fig. 8A, namely 28.6×10^{-12} Siemens.

11. Conclusion

The saga of the concept of the receptor has been followed from its beginnings in the hands of Langley and Ehrlich to the triumph of recording the electrical signs of the opening of a single acetylcholine receptor channel. This work took almost exactly a century to accomplish, from the experiments of Langley in 1874 on pilocarpine and atropine to those of Neher and Sakmann (1976). The structure of the receptor molecule was also opened up

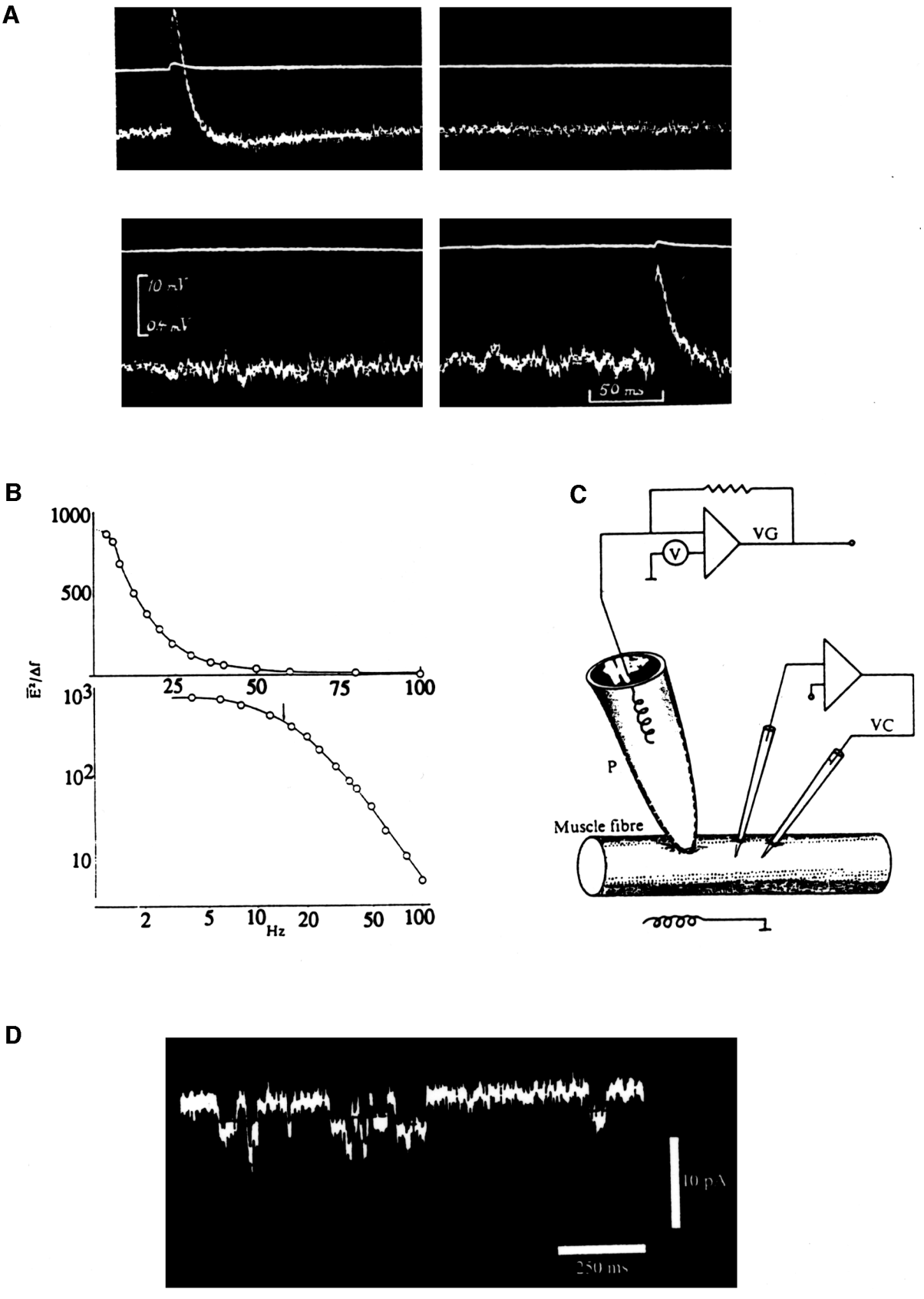
A
10 mV
0.5 mV
50 ms
B
1000
500
$\bar{E}^2/\Delta f$
25
50
75
100
10^3
10^2
10
2
5
10
Hz
20
50
100
C
VG
V
VC
P
Muscle fibre
D
10 pA
250 ms

Fig. 8.

Fig. 8. Discovery of the physiological action of single acetylcholine receptors at the somatic neuromuscular junction. (A) Intracellular recordings of membrane potential from end-plate region of frog muscle fibre. In each block, the upper trace was recorded on a low gain d.c. channel (10 mV scale); the lower was simultaneously recorded on a high gain a.c. coupled channel (0.4 mV scale). The records in the upper row are controls (no acetylcholine); the lower row shows membrane noise during acetylcholine application, by diffusion from a micropipette. In the lower records, the increased distance between a.c. and d.c. traces shows upward displacement of the d.c. trace because of acetylcholine-induced depolarization. Two spontaneous m.e.p.p.s are also seen (from Fig. 1 in Katz and Miledi, 1970). (B) Power spectrum of intracellularly recorded acetylcholine noise. Temperature 5.5°C. Linear plot of $\bar{E}^2/\Delta f$, (see text), in relative units against frequency in Hz in the upper part; double-log plot in lower part. (from Fig. 1 in Katz and Miledi, 1971). (C) Schematic circuit diagram for current recording from a patch of membrane with an extracellular pipette. VC, Standard two-microelectrode voltage clamp circuit to set locally the membrane potential of the fibre to a fixed value. P, Pipette, fire polished, with 3–5 μm diameter opening, containing Ringer's solution and agonist at concentrations between 2×10^{-7} and 6×10^{-5} M d.c. resistance of the pipette: 2–5 MΩ. The pipette tip applied closely on to the muscle fibre within 200 μm of the intracellular clamp electrodes. VG, Virtual ground circuit, using a Function Modules Model 380K operational amplifier and a 500-MΩ feedback resistor to measure membrane current. The amplifier is mounted together with a shielded pipette holder on a motor-driven micromanipulator. V, Bucking potential and test signal for balancing of pipette leakage and measuring pipette resistance (from Fig. 1 in Neher and Sakmann, 1976). (D) Oscilloscope recording of current through a patch of membrane of approximately 10 μm^2. Downward deflection of the trace represents inward current. The pipette contained 2×10^{-7} M SubCh in Ringer's solution. The experiment was carried out with a denervated hypersensitive frog cutaneous pectoris (*Rana pipiens*) muscle in normal frog Ringer's solution. The record was filtered at a bandwidth of 200 Hz. Membrane potential: −120 mV. Temperature: 8°C (from Fig. 2 in Neher and Sakmann, 1976).

by the discovery in the late 1960s by Lee and his colleagues of toxins that could irreversibly bind to the receptor (Lee and Chang, 1966; Lee, 1972), and so allow for its isolation. But that story takes us too far from the main theme of this essay, which has been the establishment of the reality of the transmitter receptor.

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