NEUROMUSCULAR PHARMACOLOGY[1,2,3]

By S. Thesleff and D. M. J. Quastel[4]
Department of Pharmacology, University of Lund, Sweden

Neuromuscular Transmission

In recent years, the physiological processes underlying synaptic transmission have been extensively investigated, such studies showing a remarkable similarity between different kinds of synapses (36). This review will be concerned primarily with the pharmacology of transmission at the neuromuscular junction, as studied by electrophysiological microtechniques. The advantage of these methods is that they permit the exploration, in considerable detail, of the individual steps involved in the transmission process and the modification of these steps by drugs. The information obtained from such study of the effects of a drug at individual junctions, usually *in vitro*, may not necessarily enable one to predict its net effect on neuromuscular transmission in a whole animal, but an understanding of its mode or modes of action at the end plate usually provides a reasonable explanation for what is observed grossly.

A complete bibliography covering all recent work on neuromuscular pharmacology has not been attempted and some important papers may be referred to only indirectly, or perhaps not at all. The views expressed regarding the site and mode of drug action are those of the authors and may not always reflect generally accepted beliefs.

Physiology of Neuromuscular Transmission

There are a number of excellent and comprehensive monographs and reviews covering this subject (27, 36, 61, 67, 86). The following will be a very brief outline of the transmission mechanism with the emphasis on those processes which drugs modify.

Intracellular recording at the end plate region of a muscle fibre reveals spontaneous depolarizations known as miniature end plate potentials (m.e.p.p.'s). These have been shown to be due to the effects on the postsynaptic membrane of presynaptically released multimolecular "quanta" of

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[3] The following abbreviations used: ACh (acetylcholine) e.p.p. (end plate potential); DFP (diisopropyl flavophosphate) m.e.p.p. (miniature end plate potential); and TEA (tetraethylammonium).

[4] Post-graduate Fellow, Muscular Dystrophy Association of Canada. Present address: The Department of Physiology, The Australian National University, Canberra, Australia.

acetylcholine (ACh). Under normal conditions, a nerve impulse causes the simultaneous release of a large number of these units of transmitter, which causes a postsynaptic depolarization, the end plate potential (e.p.p.) which triggers a propagated action potential in the muscle fibre.

The effect of an action potential at the nerve terminal is to increase enormously the probability of quantal liberation, the number of quanta released per impulse being apparently a Poisson variable. Although quantal release by a nerve impulse is evidently a function of the extent and duration of presynaptic depolarization (53, 75), this cannot be the only factor involved. The release of ACh is delayed up to a millisecond following the crest of the nerve action potential (63), and calcium and magnesium ions have a large influence on the quantum content of e.p.p.'s without modifying to any extent the presynaptic spike (62). One must postulate the existence of a mechanism, sensitive to these ions, which couples depolarization to release.

Evidence from sympathetic ganglia and neuromuscular junctions shows that the total store of transmitter is large, sufficient for several thousand impulses (9, 40); but the amount accessible for immediate release constitutes only a small part of the total, sufficient for only a few impulses (76), and is a variable upon which depends the amount liberated by a nerve impulse. For example, the decline in size of sequential e.p.p.'s at the beginning of a tetanus (early tetanic rundown) can plausibly be attributed to partial depletion of that part of the transmitter store which is available for immediate release (76, 96).

Following a high frequency tetanus, there may be a period during which the amount of ACh released by a single impulse is augmented. During this time of "posttetanic" potentiation, there is also an increased rate of early tetanic rundown, indicating an increased fractional release of transmitter (76). Marked facilitation of ACh release evidently takes place also during the tetanus, though this is normally obscured by the reduction of the amount of store immediate available for release; i.e., during repetitive nerve activity there are two opposing processes, increased fractional release but concomitant depletion of the accessible store. The level at which the store is maintained is determined by the fraction discharged from it per-unit-time and the rate at which transmitter is "mobilized" to become available for liberation. Thus, during high frequency stimulation, the rate of release of ACh quickly becomes limited by the rate of its mobilization from a relatively more distant or less accessible presynaptic store, and increase of either the rate of stimulation or fractional release can have little effect (9). The mechanism by which mobilization is controlled remains obscure. Recently evidence has been presented that hyperpolarizing current at the nerve terminals can accelerate transmitter mobilization (54).

When a quantum of ACh comes into contact with the postsynaptic membrane by diffusion, the result is a very localized increase of the membrane permeability to sodium and potassium ions. Formally, this is equivalent to shunting the electrical resistance of the membrane; the resulting depolariza-

tion is proportional to the conductance increase relative to the "input resistance" of the cell and to the electrochemical driving force of the ions involved (42, 87, 117). When a number of quanta act simultaneously at a number of spots, as during an e.p.p., the net conductance increase is simply proportional to the number of quanta. For the same reasons the depolarization produced by a given concentration of ACh is a function not only of the chemosensitivity of the membrane but also of the area of the membrane which is sensitive.

Since under physiological conditions only the end plate region and its immediate surroundings are sensitive to ACh, it can be said that the ACh receptors are restricted to that part of the muscle fibre membrane. The action of many drugs is attributable to interaction with the ACh receptors and it is probable also that drugs may interfere with the permeability change normally consequent upon the ACh-receptor combination. The enzyme, acetylcholinesterase, which at the neuromuscular junction is situated postsynaptically (68), functions to terminate rapidly the action of transmitter, thus permitting the conduction of impulses at high frequencies.

When an e.p.p. is large enough, it triggers a self-regenerating action potential in the muscle fibre. Just how large it need be, i.e., the threshold for initiation of a spike, is determined by a variety of factors such as ions, the membrane potential, the rapidity of depolarization, and various drugs.

Finally, it should be emphasized that the end result of the neuromuscular transmission process, the muscle contraction, depends not only upon the steps outlined above but also upon the complex and little understood processes by which membrane phenomena lead to a contractile response.

The diagram presented (Figure 1) shows, schematically, the main steps involved in neuromuscular transmission.

Drugs Affecting Neuromuscular Transmission

For the purposes of this review, we have chosen to classify drugs on the basis of their sites of action on the transmission process. It will become apparent that many have more than one effect. There is nothing inherently surprising in this, since evidently the drugs largely owe their activity to a chemical resemblance to the transmitter and may therefore compete with it to varying extents, at a number of locations.

Drugs Acting Presynaptically

In this section will be considered those agents whose main site of action is at one or more of the presynaptic steps indicated in the scheme.

Acetylcholine synthesis and storage.—Acetylcholine is enzymatically synthetized from choline and acetyl-CoA in motor nerve terminals and is subsequently stored there. Certain drugs have been shown to interfere at this level of the transmission mechanism.

In 1955, Schueler described a series of quaternary ammonium compounds with two choline moieties in the molecule (hemicholiniums) and suggested

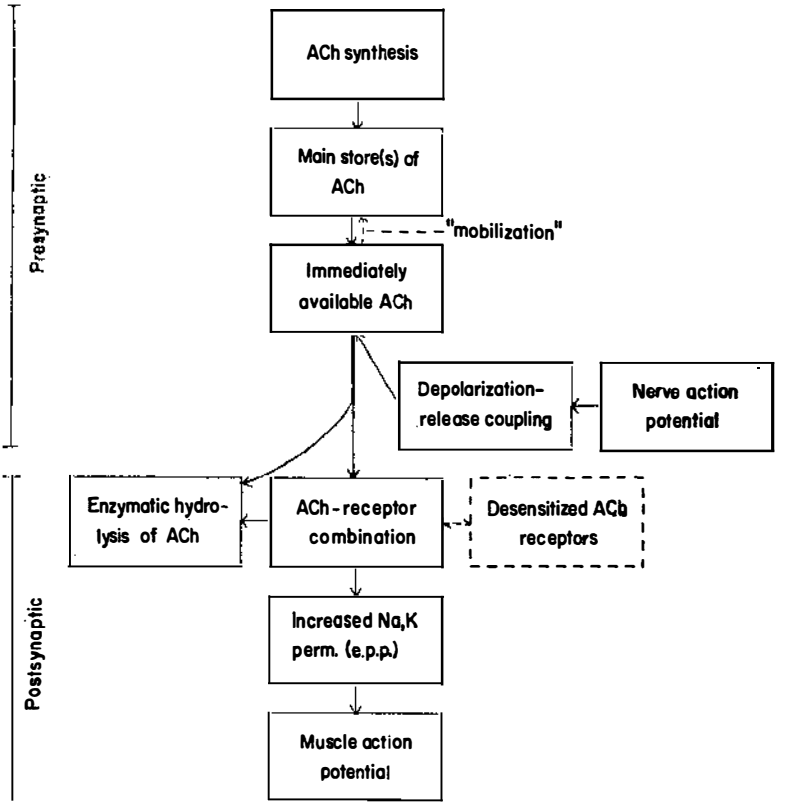

FIG. 1. Steps Involved in Neuromuscular Transmission.

that their depressant action on neuromuscular transmission might be due to an interaction with ACh metabolism (106). It was subsequently demonstrated that the compound hemicholinium No. 3 (HC-3) inhibits the synthesis of ACh in intact nervous tissue (85) and that this action might be due to blockade of active transport of choline to the site of ACh synthesis. Support for this theory is provided by the observation that choline is an excellent antidote to HC-3 poisoning (106) and acts as a competitive antagonist to HC-3 *in vitro* (107).

Neuromuscular blockade by HC-3 is characteristically slow in onset and occurs first at junctions which are activated most. The block evidently develops when the presynaptic store of ACh has become depleted because of release, the main action of the drug being to prevent maintenance of the store by synthesis (126). It has recently been shown that the presynaptic action of HC-3 is manifest at the neuromuscular junction in a progressive reduction of the amount of ACh contained in the quanta released. When

HC-3 is used in a concentration which blocks ACh synthesis virtually completely (4×10^{-6} to 2×10^{-5} M), a progressive diminution of e.p.p.'s due to change in quantal size, eventually to extinction, takes place during prolonged indirect stimulation (41). The change in quantum size is presumably related to emptying of the presynaptic ACh store rather than to a direct action of the drug. By following the change in quantum size as transmitter is released, it can be shown that the total releasable amount of ACh at a single junction corresponds to several hundred thousand full quanta (38, 40).

Triethylcholine is another drug which shows many resemblances in its action to HC-3 (15). Like HC-3 it blocks synthesis of ACh in intact tissues (22), its action being weakened by treatments which disrupt tissue organization. Choline antagonizes its effect. The onset of neuromuscular blockade is delayed and dependent upon the rate of transmitter release, and it can be observed that the quanta of ACh become reduced in size (40).

Like most quaternary ammonium compounds, HC-3 and triethylcholine depress the postsynaptic end plate sensitivity to ACh (15, 127). However, for both drugs it takes a higher concentration to block neuromuscular transmission by this action.

It is quite possible that a number of drugs have an action on ACh synthesis similar to that of HC-3 and triethylcholine but that this action is obscured by more powerful effects on other sites. Ganglion perfusion experiments have shown that ACh synthesis may be inhibited by high concentrations of eserine, tetraethylammonium (TEA), or digitalis, and by sodium ion deficiency (8, 9, 10, 84). Whether these do the same at the neuromuscular junction has not yet been ascertained.

Since the effect of synthesis inhibition becomes apparent only when nerve terminal stores become depleted of transmitter, it follows that drugs with this action should block first the most activated cholinergic junctions. On this basis, Bowman & Rand suggested that triethylcholine might be effective in selectively reducing the spasms of tetanus intoxication (16), and animal experiments have borne this out (73). The idea of selectively blocking hyperactive synapses by partial inhibition of transmitter synthesis is an intriguing one, and may well have therapeutic applications.

Acetylcholine release.—For neuromuscular transmission to take place, the nerve impulse must cause the release of an amount of transmitter sufficient to elicit a propagated action potential in the muscle fibre. Normally the quantity liberated is more than adequate, there being a large safety margin for transmission. Consequently, it is only when neuromuscular transmission has been depressed to such an extent that the e.p.p.'s are only a little above or below fibre threshold that transmission is particularly sensitive to changes in the amount of ACh released. Under normal circumstances facilitation of the release process is quite devoid of effect and a relatively large inhibition of release is necessary before transmission becomes blocked.

Many agents have been shown to influence the release of transmitter by a nerve impulse. The effects of calcium and magnesium ions have been studied

in particular detail and may be considered to exemplify the effects of drugs which respectively facilitate or inhibit the fractional release of transmitter from the nerve terminals. Presynaptically these two ions oppose each other; either a high concentration of magnesium or lowering of the calcium concentration can block transmission, and raising the calcium concentration antagonizes a block caused by magnesium. That this block is due to diminished transmitter release can easily be observed, the quantum content of e.p.p.'s being reduced (18, 25, 26, 74).

Conversely, the effect of increased calcium is to raise the quantum content. When e.p.p.'s have a low quantum content because of excess magnesium, the antagonistic effect of calcium can be observed directly, and when transmission is blocked instead by a postsynaptically depressant drug such as tubocurarine, raised calcium also increases e.p.p. size. Under the latter conditions, the second and subsequent e.p.p.'s in a train fall more steeply (82, 96). As previously pointed out, the early tetanic rundown of transmitter output can be considered to be caused by partial depletion of the store of transmitter immediately available for release (76, 96). The increase of its rate by calcium is therefore a good indication that the effect of the ion is to augment fractional release rather than the amount of transmitter available for release.

By external recording from motor nerve terminals it has been shown that the effect of calcium on release by an impulse is not associated with any observable change in the amplitude or duration of the presynaptic action potential (62). Moreover, the acceleration of quantal release by high concentrations of potassium ions is dependent upon the presence of calcium, and inhibited by magnesium (50, 75). Since these ions do not change the extent of membrane depolarization produced by potassium ions, this too would indicate that the effects of calcium and magnesium are on the mechanism which links transmitter release with presynaptic depolarization.

There is no evidence that posttetanic potentiation is affected by calcium or magnesium ions, but, in the presence of a high concentration of magnesium, fractional release is much diminished and significant depletion of available transmitter does not occur. Under these conditions the effect of posttetanic potentiation to increase release is not obscured by depletion, and augmentation of e.p.p. size is easily observed (81, 82).

The antibiotics neomycin, streptomycin, and kanamycin, which are structurally related, can cause a neuromuscular blockade, which is antagonized by calcium (32, 33, 56). The block caused by neomycin has been studied using intracellular recording from end plates and is found to be very similar to that produced by calcium lack or high concentrations of magnesium, the quantum content of e.p.p.'s being reduced and the acceleration of m.e.p.p. frequency by potassium inhibited. These effects were reversed by an increase of calcium concentration (39).

The toxin produced by *Clostridium botulinum* is a potent blocker of cholinergic transmission (23, 72). The action of the toxin is to inhibit trans-

mitter release drastically, the e.p.p.'s resembling those found in the presence of excess magnesium, until the inhibition becomes complete. Unlike magnesium, the toxin also has a great effect on the spontaneous release of quanta (19). The effect of the toxin can only partially be reversed by raising the calcium concentration (19, 124). Since there is no evidence that it affects the propagation of nerve impulses or the ACh stores, the action of the toxin is presumably on the depolarization-release coupling system. From observations made in electronmicroscopic studies with ferritin-tagged toxin, it has been suggested that toxin molecules may mechanically obstruct the sites of quantal efflux in the presynaptic nerve terminals (130). The action of the toxin at the neuromuscular junction is protracted, complete restoration of function taking place only after several months.

Tetraethylammonium (TEA) is one of a group of agents which, like calcium ions, have been shown to increase e.p.p. size without increasing postsynaptic sensitivity to ACh (70, 110, 111). It also causes an increase in the rate of early tetanic rundown, showing that it augments fractional release of transmitter (70). Thus, TEA, like an excess of calcium ions, increases the amount of transmitter released by a nerve impulse (31), but apparently it differs from calcium in its site of action. It has been shown to prolong markedly the duration of the presynaptic spike potential (70), and this can be expected to increase fractional release and thus the number of quanta per impulse. There is no need to suppose that the compound affects the mechanism by which depolarization elicits transmitter liberation.

A number of phenols, including catecholamines, have effects similar to those of TEA, and so does guanidine (95, 97). These all increase the quantity of transmitter released per impulse and accelerate early tetanic rundown. Whether they influence the time course of the action potential is not known. Guanidine also produces spontaneous twitching in muscle (43), and intracellular recording has shown this to be caused by the appearance of spontaneous multiquantal end plate potentials some of which reach fibre threshold and therefore trigger the conducted muscle action potentials responsible for the twitching (95). This finding suggests that guanidine may owe its action on transmitter release to a modification of the electrical excitability of the nerve terminals rather than to an effect on the release mechanism. If these agents act by facilitating the depolarization-release coupling, then, like calcium ions, they could be expected to antagonize the depression by magnesium of the m.e.p.p. acceleration caused by potassium ions. Unfortunately, this has not as yet been investigated.

A moderate elevation of the external potassium ion concentration (5 mM to 10 mM) results in some increase of the quantum content of e.p.p.'s (37, 118) and also in an acceleration of early tetanic rundown (76). Since it is difficult to imagine that this could be attributable to an increase in amplitude or duration of the presynaptic spike, it may be that potassium has some effect on the ability of the spike to release ACh, i.e. on depolarization-release coupling.

It has also been found that pyridine-2-aldoxime (2-PAM) increases the number of quanta in e.p.p.'s of low quantum content because of excess magnesium. An interesting observation is that succinylcholine alone apparently reduces quantum content, but seems to have the opposite effect in the presence of 2-PAM (37).

In low concentrations, local and general anaesthetics reduce and, in high concentrations, block the release of transmitter from nerve terminals (90, 114). This is presumably attributable to an action of the agents on the selective sodium conductance change responsible for the initial part of the action potential (113, 120). Since the rate of rise and the peak amplitude of the action potential are directly proportional to the rate of entrance of sodium ions, i.e. to the selective sodium conductance (47), an inhibition of this mechanism by anaesthetics can be expected to reduce and eventually to abolish the presynaptic action potential. Tarichatoxin (or tetrodotoxin) has presumably a similar mode of action (21, 59, 91).

Cardiac glycosides, known to be inhibitors of the sodium pump, have been shown to have significant effects on ACh release. According to Birks (7, 8) they have five effects, namely: acceleration of m.e.p.p. frequency, increased quantum content of isolated e.p.p.'s, failure of impulse propagation in the nerve terminals, spontaneous multiquantal release of ACh, and structural changes in nerve terminals. From the time course of these events and their dependence upon the concentration of sodium in the external medium, it has been deduced that the effects are secondary to the accumulation of sodium ions in the nerve terminals (7, 8).

2-4-Dinitrophenol, a potent metabolic inhibitor, is also known to block the sodium pump. As such, it might be expected to have actions on the neuromuscular junction similar to those of digoxin. It has been reported to cause a progressive increase in m.e.p.p. frequency and failure of nerve conduction. Whether these effects are related to the sodium concentration was not determined. In contrast to what has been observed with digoxin, quantum content of e.p.p.'s seemed to be progressively decreased (71).

Recently, an interesting study has been made in which metabolic inhibitors have been shown reversibly to abolish posttetanic potentiation (51). Antimycin A, sodium azide, and digoxin, all inhibitors of sodium transport and of the posttetanic hyperpolarization of peripheral nerve fibres (45, 103), act in this way (51). This finding adds considerable support to the postulated connection between posttetanic presynaptic hyperpolarization and posttetanic potentiation of transmitter release (53, 79)

It seems possible that the spontaneous multiquantal release of ACh which is observed when nerve terminals have been in prolonged contact with digoxin (8) is associated with the same process as is guanidine and is perhaps an instability of the presynaptic membrane potential resulting from differential depolarization of the terminal nerve filaments.

The suggestion has been made that tubocurarine reduces the amount of ACh liberated by nerve impulses during high frequency stimulation and that

this action is responsible for the decline in amplitude of successive e.p.p.'s in a train (77). However, quantitative studies of e.p.p.'s, while the concentration of tubocurarine was increased, showed no apparent change in early tetanic rundown of e.p.p.'s (96); it is therefore unlikely that the phenomenon should be the result of an action of tubocurarine. Under suitable conditions, early tetanic rundown of e.p.p.'s may be observed in the absence of any drug (100).

Drugs Acting Postsynaptically

Many tertiary and quaternary ammonium compounds have been shown to depress postsynaptic sensitivity to transmitter released presynaptically, and to artificially applied ACh. With the majority of the drugs, it is believed that the blocking actions are attributable to association of the drug with the receptor molecule to ACh, thus preventing ACh from reacting with its receptor.

The combination between blocking drug and receptor may or may not lead to ionic permeability changes like those which are the normal sequelae to the combination of ACh with receptor. On the basis of whether or not a drug causes postsynaptic depolarization one can classify it as either a nondepolarizing or a depolarizing receptor-blocking drug.

To a large extent, such a classification must be considered artificial, there probably being a considerable overlap of mode of action by any one drug. The molecules intermediate in structure between TEA and tetramethylammonium apparently have both nondepolarizing and depolarizing actions, the latter increasing with substitution of methyl for ethyl groups. Tubocurarine is a classical example of a nondepolarizing receptor blocker but in mammalian muscle with a large receptor surface resulting from chronic denervation, it may depolarize (17).

Since the ACh receptor molecule has neither been isolated nor chemically characterized, it is generally difficult to determine whether a nondepolarizing blocking agent works by (*a*) interference with ACh-receptor combinations, (*b*) changes of receptor properties or (*c*) interference with permeability changes in the membrane. The situation is further complicated by the fact that depolarizing blocking agents cause neuromuscular block not only by their depolarizing action but by a subsequent "desensitization" of ACh receptors which is unaccompanied by any changes of membrane permeability or potential. In this block, the receptors evidently are rendered inactive rather than merely protected from combination with the transmitter, as is the case when a competitive blocking agent such as tubocurarine is used. With tubocurarine there is a parallel shift of the dose-response curve to ACh without a reduction in the maximum response, indicating simple competition (55). Quantitative dose-response curves to ACh, in the presence of desensitizing agents, have not been made, but the evidence available indicates that the peak response to ACh is considerably diminished, and that therefore the nature of the block is noncompetitive (119).

Nondepolarizing receptor-blocking agents.—With a few exceptions, receptor-blocking agents are completely ionized tertiary or quaternary ammonium salts; bis-quaternaries are, in general, more active than monoquaternaries. Sterically, the molecular structure is bulky and rigid or has hindering groups attached to the onium N atom. It is believed that the agents owe their blocking activity to electrostatic bonding with anionic sites in the postsynaptic ACh-receptor molecule. Since the bonding force varies inversely with the square of the distance between charged centers, it follows that molecules with steric hindrance at their cationic head, preventing a close approach to the receptor, cannot be firmly bound to them. It is also probable that such molecules can be displaced from the receptor by quaternaries having a smaller steric hindrance.

The best known example of a nondepolarizing receptor-blocking drug is *d*-tubocurarine. This agent does not affect the electrical properties of the end plate or those of the muscle membrane. Its sole effect appears to be to reduce reversibly the depolarizing effect of the transmitter or applied ACh at the end plate.

When tubocurarine is added in increasing concentrations to a skeletal muscle, it progressively reduces the amplitude of the e.p.p. until eventual extinction. When the e.p.p. becomes less than the threshold of the muscle membrane for generation of an action potential, a neuromuscular block results.

The antagonism between ACh and tubocurarine has been examined by the method of determining the effect of known concentrations on the end plate depolarization evoked by bath-applied ACh (55). The results obtained from this study are consistent with the hypothesis that tubocurarine competes with ACh on a 1:1 basis for receptors at the end plate.

Failure of neuromuscular transmission during a tetanus is very pronounced in the presence of curare. This is a simple consequence of the progressive decline in the amount of ACh released by successive nerve impulses so that, because of the depressed postsynaptic sensitivity, e.p.p.'s soon fail to depolarize the end plate beyond the fibre threshold. The acceleration, by a drug, of failure of a muscle to maintain mechanical tension in response to indirect stimulation in the presence of tubocurarine, has therefore been used as an index of augmented early tetanic rundown, i.e. increased fractional release of transmitter (14); but this is obviously not specific.

Drugs which increase the amount of ACh released per impulse are effective curare antagonists. Calcium and potassium ions, TEA, phenols, and guanidine have all been shown to relieve a neuromuscular block produced by tubocurarine. Similarly, posttetanic facilitation of transmitter release also temporarily relieves the block. Anticholinesterase agents are effective antagonists insofar as they prevent the enzymatic destruction of ACh and thereby increase its concentration and area of action at the postsynaptic membrane.

Conversely, a reduction in the amount of transmitter released, either as

the result of drug action or in a pathological condition, will "sensitize" neuromuscular transmission to the blocking effect of tubocurarine. Neuromuscular transmission is highly vulnerable to the blocking effect of tubocurarine in the presence of botulinum toxin, or low calcium, and/or high magnesium ion concentration. The same applies to transmission in myasthenia gravis and after sufficient stimulation in the presence of HC-3, which are also conditions in which the presynaptic transmitter release is deficient.

A great many tertiary and quaternary ammonium compounds, e.g. gallamine, β-erythroidine, TEA, and probably the hemicholiniums (88), have a mode of action similar to that of tubocurarine; but quantitative studies of their interaction with ACh at end plate receptors are still lacking.

Depolarizing receptor blocking agents.—The combination between these agents and end plate receptors produces an initial depolarization of the end plate region which is followed by a reduction in the sensitivity of the end plate to the transmitter, i.e. by receptor desensitization.

Drugs with this mode of action are, like many nondepolarizing receptor blockers, mono- or bis-quaternary compounds; but the molecule is slender and flexible and the cationic head has a minimum of steric hindrance, presumably allowing a close approach of the onium atom to the receptor. Typical depolarizing receptor blockers are ACh itself, choline, carbachol, succinylcholine, decamethonium, and tetramethylammonium. It is tempting to speculate that the aforementioned structural properties are responsible for the difference in mode of action between depolarizing and nondepolarizing receptor blocking agents. A less hindered quaternary group might be capable of greater penetration in depth of the postsynaptic membrane and thereby producing changes in its structure which would lead to higher ionic permeability. Moreover, quaternaries capable of close attachment to the receptor could be expected to be less readily displaced by the transmitter, and this could account for the noncompetitive nature of the "desensitization" block.

The mechanism responsible for the initial depolarizing effect of these agents is presumably similar to that of the transmitter and involves a local conductance increase which is independent of the level of membrane potential. As shown for ACh by Fatt & Katz (42) and Takeuchi & Takeuchi (117), the permeability of the end plate membrane to both sodium and potassium ions is greatly enhanced, resulting in depolarization. Persistent depolarization of the muscle fibre results in inactivation of the sodium-carrying mechanism responsible for the generation of the action potential. Therefore, when the end plate is depolarized excessively, e.p.p.'s become unable to elicit propagated responses, and transmission block results. A small depolarization, on the other hand, may increase the safety margin for transmission by making smaller the further depolarization necessary to trigger an action potential (44). This may well account for the observations that, with nonblocking concentrations of depolarizing agents, the mechanical tension of the muscle in response to indirect tetanic stimulation is well maintained (14), and that neuromuscular transmission in conditions with a reduced amount of trans-

mitter output per impulse is not particularly sensitive to the initial actions of these agents.

The localized end plate depolarization produced by these agents necessarily causes current to flow through the adjoining fibre membrane, depolarizing it, and the result is sometimes the generation of action potentials—isolated or in trains. Whether action potentials occur is determined by the extent of the depolarization, the rapidity with which it is brought about, and the electrical properties of the fibre membrane.

The neuromuscular block produced by the administration of a depolarizing agent seems to be generally caused at first by the persistent and excessive depolarization of the end plate, but subsequently an important role is played by receptor desensitization which may or may not be accompanied by some residual depolarization (92). Once this stage is reached, the effect of tubocurarine is simply to intensify the block (121).

The time course of the desensitization process has been studied at single end plate regions by the use of ionophoretic micro-application of drugs and intracellular recording of resulting membrane depolarizations (2, 29, 64). This technique permits high-speed drug application to a localized area of the postsynaptic membrane and the recorded effects are those following immediately upon the drug-receptor interaction. The results obtained in different species were qualitatively similar, and show that the degree and speed of desensitization increase with the concentration of the depolarizer, while recovery time apparently is independent of concentration. Desensitization of receptors to half their original sensitivity was observed to occur within a few seconds or less after drug application: the recovery half-time upon withdrawal of the drug was 3 to 5 sec. If the drug is not withdrawn, the membrane slowly becomes repolarized to its normal resting potential, despite the continued presence of the drug, as receptors become desensitized. The release of transmitter or additional application of a depolarizer, then, has little or no effect on membrane potential. After prolonged bath application, recovery of receptor sensitivity is a slow process taking 15 min or more (119). Evidently, when receptor molecules are exposed to a depolarizing agent, they are slowly changed into a form that is no longer able to produce or maintain a high ionic permeability of the postsynaptic membrane. Because of the noncompetitive nature of the drug-receptor combination, local increases in transmitter concentration are not particularly effective in antagonizing the block. Some antagonism may be observed, however, when neuromuscular transmission is studied under circumstances when e.p.p.'s are almost threshold size, and changes in e.p.p. amplitude by only a few per cent decide whether or not neuromuscular transmission occurs in a majority of fibres.

The rapidity at which receptor desensitization develops under the influence of the depolarizing action of ACh has led to the suggestion that the progressive decline in e.p.p. amplitude early in a high frequency train of impulses might in part be attributable to postsynaptic desensitization by the transmitter (2). Support for this hypothesis was obtained when it was observed that the postsynaptic sensitivity to a pulse of ionophoretically applied

ACh was sometimes reduced during a few tenths of a second following a train of nerve impulses at frequencies above 20 per sec (122). However, other investigators have been unable to confirm this observation (96) and it is therefore doubtful, at the moment, whether in fact desensitization plays a significant role for the development of neuromuscular depression or "Wedensky inhibition" during high frequency stimulation. Under all circumstances, presynaptic transmitter depletion is a far more important cause for the early tetanic rundown of e.p.p.'s.

Anticholinesterase agents.—Besides diffusion, the enzyme acetylcholinesterase is most responsible for the rapid removal of ACh from end plate receptors. This enzyme, capable of hydrolyzing ACh into acetic acid and choline, is, at the neuromuscular junction, localized almost exclusively in the postsynaptic membrane and there only in apposition to the nerve terminals (68). It is generally accepted that two groups at the active site of the enzyme are essential for bringing about the hydrolysis of substrate: the esteratic site where the ester bond is activated; and the anionic site, consisting of one or more negative groups which interact by ionic bonding with the cationic head of the ACh molecule. There is reason to believe that the ACh receptor and the enzyme are localized close to each other in the membrane and that the chemical forces binding ACh to the enzyme are very similar to those between ACh and its receptor.

Numerous agents from all classes of pharmacologically active drugs have been reported to inhibit acetylcholinesterase. A description of all these drugs is beyond the scope of this presentation. For detailed and extensive information about this subject the reader is referred to (67).

It is important to note that the pharmacological activity of a drug which inhibits acetylcholinesterase is not always exclusively, or even predominantly the result of inhibition of the enzyme. Generally speaking, it can be said that drugs which inhibit acetylcholinesterase, including the organophosphorus compounds, to some extent also react with the ACh receptor. Many anticholinesterase agents evidently also produce repetitive activity in motor nerve terminals. This action will be treated under a separate heading on page 276.

Prevention of enzymatic hydrolysis of the transmitter allows it to react longer, at a higher concentration and thereby upon a larger area of the postsynaptic membrane. At the resting myoneural junction, the net result is an increase in amplitude and duration of the m.e.p.p.'s. Occasionally, when a few m.e.p.p.'s are summated, they may trigger action potentials and so produce "spontaneous" twitches in single muscle fibres. Similar effects on e.p.p.'s may be observed. The amplitude and the duration of e.p.p.'s are found to be markedly increased and prolonged, and e.p.p.'s in close succession, to some extent, add on to each other. The increase in amplitude of individual e.p.p.'s may be sufficient to relieve a transmission block which is the result of either a deficient release of transmitter or a reduced postsynaptic receptor sensitivity. As previously discussed, transmission failure caused by a depolarizing and noncompetitive receptor blocking agent is less likely to be

relieved than that caused by a competitive agent, and, obviously, the blocking action of choline esters which are hydrolysed by cholinesterases is intensified by cholinesterase inhibitors. The prolonged end plate potential caused by the inhibition of acetylcholinesterase may act as a local "sink" for current flow, and thereby result in the generation of trains of action potentials—essentially the same situation as was described in the discussion of the action of depolarizing receptor blocking agents. In this way, a single nerve impulse may, because of the inhibition of acetylcholinesterase, produce in a muscle fibre a short tetanic response which may be easily mistaken for an augmented twitch.

All acetylcholinesterase inhibitors, in sufficient concentration, have a "curarizing" action and block neuromuscular transmission. Some indication as to whether the block is attributable to an excess of ACh or to depression of receptors has been obtained by studies of the effect of tubocurarine. The antagonism between the cholinesterase inhibitors and tubocurarine is rather limited. Only under certain conditions, when the dose of the inhibitor is relatively low and its application has been of short duration, can antagonism be shown. More generally, the blocking effect of cholinesterase inhibitors adds significantly to that of tubocurarine (1). Moreover, it has been shown that TEA, to a considerable extent, restores transmission to both single and tetanic stimuli, when transmission has been partially blocked by di-isopropyl fluorophosphate (DFP) (112). Since the action of TEA is to increase transmitter release per impulse, it would appear very unlikely that the block by DFP was mediated in these experiments by excess ACh. The accumulation and persistence of ACh at the end plate during repetitive stimulation might be involved in the neuromuscular blocking activity of anticholinesterase drugs, but it is difficult to account on this basis for the observed depression of responses to isolated stimuli. The type of receptor blockade, which anticholinesterase drugs produce, evidently varies with their structure and can be either predominantly depolarizing or nondepolarizing.

The possibility that anticholinesterase agents might interfere with presynaptic ACh synthesis by preventing the hydrolysis of released ACh, and thereby the formation and subsequent uptake of choline by nerve terminals, is interesting but has as yet not been investigated (99).

To some extent, the effects of cholinesterase inhibition would be mimicked by an increase in receptor sensitivity or in the response of the postsynaptic membrane to receptor activation. To show that a drug had this effect it would be necessary to demonstrate an increase in the depolarization produced by stable ACh analogues, such as carbachol or decamethonium. Such studies have been carried out for only a few compounds, without showing evidence of this kind of action. On the contrary, the depolarization produced by carbachol or decamethonium is depressed by those cholinesterase inhibitors which have been tested (60, 65).

Drugs which influence the ionic permeability of the muscle membrane.—The combination of ACh and its receptor causes a permeability change in the postsynaptic membrane tantamount to the insertion of a shunting conduc-

tance across it. This results in electric charge being transferred inwards at the end plate, causing current to flow through the adjoining muscle membrane. When depolarization reaches a critical amplitude—the threshold of the membrane—there occurs a high, selective, and brief increase in the permeability of the membrane, at first to sodium and then to potassium ions. These two selective and consecutive conductance changes are responsible for the action potential.

Chemical agents can interfere with the aforementioned events by affecting (*a*) the permeability changes and the electrochemical potential of the ions involved, (*b*) the electrical resistance of the resting membrane, or (*c*) the selective permeability changes responsible for the action potential. Let us now consider these three possibilities.

(*a*) The specific change caused by ACh at the end plate membrane is to increase, simultaneously, permeability to sodium, potassium, and perhaps calcium ions but not to chloride ions (117). It has been shown that increasing the external calcium concentration reduces the sodium conductance change produced by ACh with little or no effect on potassium conductance (116), and that the external potassium concentration also has an influence on the permeability changes (115).

Local and general anaesthetics (28, 104, 105, 120) depress the postsynaptic depolarizing action of the transmitter and of applied ACh. In view of the known action of these agents generally to inhibit membrane permeability changes (108, 109), it appears reasonable to suppose that they depress the conductance increase in the end plate membrane by an effect on the permeability change rather than by interaction with the receptor.

Changes in intra- or extracellular sodium or potassium concentrations, or in the resting membrane potential, alter the electrochemical gradient of these ions and, of course, thereby modify the depolarization caused by ACh.

(*b*) The electric resistance of the resting membrane is a function mainly of its permeability to potassium and chloride ions. Substitution of chloride by anions which less readily permeate the membrane increases membrane resistance and hence the depolarization caused by a shunting conductance. Evidently, nitrate, sulphate, and iodide increase the e.p.p. and m.e.p.p. (48) by this mode of action (94).

(*c*) Ions and drugs which affect the permeability change in the end plate membrane caused by ACh are likely to alter similarly the more selective conductance increases leading to the action potential. Thus, sodium lack, high calcium or magnesium, high potassium, local and general anaesthetics, and certain neurotoxins all block the generation of action potentials (108, 109). Calcium lack and the anions, nitrate, sulphate, and iodide, lower the threshold for excitation (49).

Antidromic Activity in Motor Nerves

This subject is a large and confusing one, recently well discussed by a number of authors (12, 13, 36, 129). Following a single maximal shock applied to a motor nerve, a double discharge can be recorded in some muscle

fibres and concomitantly a short-lived antidromic activity is observed in the motor nerve (20). In the presence of anticholinesterases and a number of other agents, both the back response to a single nerve stimulus and the repetitive muscle action potentials are markedly prolonged and intensified (12, 13, 89, 101). In the absence of drugs, the summed action potentials of the muscle fibres can ephaptically re-excite the motor nerve terminals and thus re-excite muscle fibres by an axonal reflex (78). To some extent, the action of the anticholinesterase is evidently to make the nerve terminals more readily re-excited by this mechanism (52). Whether this action may be referred to the preservation of released ACh in the synaptic cleft, or to a direct action of the drugs on the nerve terminals, or even secondary to the prolonged action of ACh postsynaptically, remains obscure. The hypothesis of a direct action of ACh on the presynaptic nerve membrane gains some support from the finding of a direct action of ACh on mammalian nonmyelinated nerve fibres (35, 102), and from the fact that curare reduces the enhanced excitability of motor nerve terminals following a conditioning stimulus (52). However, application of ACh to end plates has always failed to reveal any effect on the frequency of miniature end plate potentials (27), which is an index of presynaptic depolarization (75), or to cause twitches as a result of axon reflexes in motor units. Moreover, there is apparently no correlation between anticholinesterase potency and potentiation of maximal twitch response to indirect stimulation (11).

Tetraethylammonium (TEA), veratrine, and guanidine also cause repetitive muscle activity and backfiring in motor nerves. The action of these drugs is not blocked by curare (89). TEA and veratrine both markedly prolong the duration of the action potential in nerve (34), and it is reasonable to assume that the repetitive nerve activity is caused by these drugs delaying repolarization in the thin unmyelinated nerve terminals to a greater extent than in nerve axons. The situation would be analogous to that observed on local application of veratrine to skeletal muscle (24). It is of course possible that with these drugs, too, ephaptic excitation is involved. Anticholinesterase drugs may well cause, directly or indirectly, a similar but less marked effect on the presynaptic after-potential (128), so that not all the backfiring observed in their presence is necessarily secondary to muscle fibre activity. Barstad (4) has recently shown that the antidromic activity in the motor nerve following a stimulus can, in the presence of prostigmine or DFP, occur in the complete absence of muscle action potentials or twitches, the muscle fibres in his preparation being cut to make them become depolarized.

It should be emphasized that even if presynaptic nerve terminals are responsive to ACh this does not necessarily mean that a feedback system is involved in the ACh release mechanism (69). The evidence seems to be that quantum content is not depressed by curare (18, 87, 96) nor is a rise in quantum content a necessary consequence of anticholinesterase action (97).

The action of low doses of curare in preventing repetitive backfiring caused by anticholinesterases, may explain the reduction by tubocurarine of ACh output by an indirectly stimulated phrenic-diaphragm preparation,

since in these experiments DFP was of course present in order to permit the collection and assay of ACh (5, 6).

Myasthenia Gravis

From the pharmacological point of view one of the most important and interesting neuromuscular diseases is myasthenia gravis, which occurs only in man, and is characterized by rapid fatigue of neuromuscular transmission. It has recently been shown by electrophysiological techniques that in this disease the quanta of ACh acting at the postsynaptic membrane are diminished in size, being about one-fifth the normal. Postsynaptic sensitivity is apparently normal and it would therefore seem that the quanta are deficient in ACh content. Their size is not affected by prolonged stimulation or rest, and it is therefore unlikely that they are small as a result of synthesis blockade like that caused by HC-3 or triethylcholine. The small quantal size accounts entirely for the small e.p.p.'s which are found at myasthenic end plates—the e.p.p.'s containing a normal allotment of quantal components. The weakness and rapid failure of neuromuscular transmission in the disease are readily explained by the early decline of e.p.p.'s in tetanic trains. This occurs in the normal way but entails rapid neuromuscular blockade simply because the quanta are small and even the first e.p.p.'s are close to threshold (38).

In view of the fact that the reduced amount of ACh, released by each nerve impulse at a myasthenic junction, often produces subthreshold e.p.p.'s, it follows that inhibition of acetylcholinesterase will improve transmission to a certain extent. However, as has already been pointed out, anticholinesterase drugs depress postsynaptic sensitivity, to various degrees, and this is hardly desirable. Superficially, it might appear that drugs which facilitate transmitter release should be effective, but inasmuch as such drugs also increase early tetanic rundown, mobilization not being augmented as much as fractional release, their efficacy is far less than would be expected from the results of testing with isolated stimuli. The ideal treatment for myasthenia gravis would be to correct the defect in quantal size but, failing this, a drug which facilitated mobilization of transmitter to become available for release, or one which increased the depolarization of the postsynaptic membrane by ACh, or one which lowered fibre threshold, would be valuable in treatment. It is probable that the action of decamethonium to reduce fibre threshold is responsible for myasthenic patients not being particularly sensitive to the neuromuscular blocking action of this drug (30, 98). An anticholinesterase activity of decamethonium (29, 119) probably also plays a part. Neuromuscular transmission in myasthenia gravis shows the expected high sensitivity to tubocurarine (98), suggesting that the reduction of postsynaptic sensitivity by this drug is unaccompanied by any significant action to facilitate transmission.

Chronic Denervation

Following motor denervation, a skeletal muscle fibre becomes spontaneously active, electromyography showing fibrillation potentials. At the same time, the entire membrane becomes uniformly sensitive to applied ACh, the

increase in size of the receptor area starting at the end plate and spreading towards the tendons. By one to two weeks after denervation, the whole surface of a mammalian muscle fibre is as sensitive as the former end plate region, which has maintained its original responsiveness to the drug. The new ACh receptors which are formed in the muscle membrane have, as far as is known, pharmacological properties similar or identical to those at an innervated end plate. However, drugs with anticholinesterase activity do not significantly potentiate the effects of locally applied ACh, indicating that the denervated membrane, despite its high ACh sensitivity, is effectively devoid of acetylcholinesterase, which is in agreement with the results of histochemical studies (46, 123).

The increase of receptor surface which occurs following denervation is responsible for the chemical supersensitivity of a chronically denervated muscle. As previously mentioned, the action of ACh on a skeletal muscle fibre is to change the ionic permeability of that part of the membrane which is covered by receptors, the resulting depolarization being determined by the relative reduction in the effective resistance across the membrane produced by this shunt. With a small membrane area, even a large increase in its permeability has little effect on the total "input resistance" of the fibre and hence the resulting depolarization is small. With a large receptor surface, as in a chronically denervated fibre, the permeability increase affects the whole cell membrane and therefore a proportionally larger depolarization is produced (3). As an example of how the depolarization by a given concentration of ACh varies with the size of the receptor area, it may be useful to recall the difference in amplitude between m.e.p.p.'s and e.p.p.'s. In this case, the concentrations of ACh at the membrane are presumably the same but when, following nerve stimulation, there is action of a number of quanta at many spots, i.e. on a larger area of the postsynaptic membrane, a much greater depolarization is produced.

Another factor which contributes to denervation supersensitivity is reduction in the ionic permeability of the resting membrane (66, 93) The membrane resistance increases about twofold and therefore the depolarization produced by a given electric current is enhanced. The increase in membrane resistance is also of interest, in that it, together with a reduction in the resting membrane potential, probably accounts for the spontaneous electrical activity, "denervation fibrillation," observed in chronically denervated muscle (83, 125).

From the foregoing, it can be seen why the observed degree of chemical supersensitivity varies in denervated muscle according to the mode of ACh application. Close-arterial injection, for instance, brings the drug in contact with only a part of the cell and thereby gives a different estimate of supersensitivity from that obtained by adding the drug to the external solution *in vitro*. The conversion of the whole muscle membrane into an ACh-sensitive surface allows drugs to depolarize simultaneously the entire length of a muscle fibre and provides an explanation for the electrically "silent" contrac-

tures produced by depolarizing drugs in chronically denervated skeletal muscles.

Unlike innervated mammalian muscles, chronically denervated ones respond by mechanical contraction to tubocurarine, epinephrine, and norepinephrine (17, 80). These actions probably indicate that the drugs interact with receptors and depolarize, too weakly to cause a measurable depolarization in the small end plate region, but strongly enough to cause threshold depolarization when the chemoreceptors cover a large membrane area.

Of considerable interest is the observation that despite the morphological integrity of the neuromuscular junction in botulinum poisoning, the muscle fibres change in a way similar to that observed following denervation (124). The fibres atrophy and become spontaneously active, showing typical fibrillation potentials on the electromyogram (57, 58). As following denervation, the whole surface of the muscle fibre becomes uniformly sensitive to ACh. Since degenerative changes are not observed in the motor nerve or its terminals, it may be reasonable to suppose that lack of the transmitter, or of some agent released with the transmitter, is responsible for the changes produced in a muscle cell following denervation or poisoning with botulinum toxin.

LITERATURE CITED

1. Axelsson, J., Gjone, E., and Naess, K., *Acta Pharmacol. Toxicol.*, **13**, 319–36 (1957)
2. Axelsson, J., and Thesleff, S., *Acta Physiol. Scand.*, **43**, 15–26 (1958)
3. Axelsson, J., and Thesleff, S., *J. Physiol. (London)*, **149**, 178–93 (1959)
4. Barstad, J. A. B., *Experientia*, **18**, 579 (1962)
5. Beani, L., and Bianchi, C. *Boll. Soc. Ital. Biol. Sper.*, **37**, 504–7 (1961)
6. Beani, L., and Bianchi, C., *Ibid.*, **37**, 1150–54 (1961)
7. Birks, R. I., *Can. J. Biochem. Physiol.*, **40**, 303–15 (1962)
8. Birks, R. I., *ibid.*, **41**, 2573–97 (1963)
9. Birks, R. I., and MacIntosh, F. C., *ibid.*, **39**, 787–827 (1961)
10. Birks, R. I., and Quastel, D. M. J. (In preparation)
11. Blaber, L. C., *Brit. J. Pharmacol.*, **20**, 63–73 (1963)
12. Blaber, L. C., and Bowman, W. C., *Intern. J. Neuropharmacol.*, **2**, 1–16 (1963)
13. Blaber, L. C., and Bowman, W. C., *Brit. J. Pharmacol.*, **20**, 326–44 (1963)
14. Blackman, J. G., *ibid.*, **20**, 5–16 (1963)
15. Bowman, W. C., Hemsworth, B. A., and Rand, M. J., *Brit. J. Pharmacol.*, **19**, 198–218 (1962)
16. Bowman, W. C., and Rand, M. J., *Lancet*, **I**, 480–81 (1961)
17. Bowman, W. C., and Raper, C., *Nature*, **201**, 160–62 (1964)
18. Boyd, I. A., and Martin, A. R., *J. Physiol. (London)*, **132**, 74–91 (1956)
19. Brooks, V. B., *ibid.*, **134**, 264–77 (1956)
20. Brown, M. C., and Matthews, P. B. C., *ibid.*, **150**, 332–46 (1960)
21. Buchwald, H. D., Durham, L., Fischer, H. G., Hasada, R., Mosher, H. S., Kao, C. Y., and Fuhrman, F. A., *Science*, **143**, 474–75 (1964)
22. Bull, G., and Hemsworth, B. A., *Nature*, **199**, 487–88 (1963)
23. Burgen, A. S. V., Dickers, F., and Zatman, L. J., *J. Physiol. (London)*, **109**, 10–24 (1949)
24. Burns, B. D., Frank, G. B., and Salmoiraghi, G., *Brit. J. Pharmacol.*, **10**, 363–70 (1955)
25. del Castillo, J., and Engbaek, L., *J. Physiol. (London)*, **124**, 370–84 (1954)
26. del Castillo, J., and Katz, B., *ibid.*, **124**, 560–73 (1954)
27. del Castillo, J., and Katz, B., *Progr. Biophys. Biophys. Chem.*, **6**, 121–70 (1956)
28. del Castillo, J., and Katz, B., *Proc. Roy. Soc. (London), B*, **146**, 339–56 (1956)
29. del Castillo, J., and Katz, B., *ibid.*, **146**, 369–81 (1956)
30. Churchill-Davidson, H. C., and Richardson, A. T., *J. Neurol. Psychiat.*, **15**, 129–33 (1952)
31. Collier, B., and Exley, K. A., *Nature*, **199**, 702–3 (1963)
32. Corrado, A. P., and Ramos, A. O., *Rev. Brasil. Biol.*, **20**, 43–50 (1960)
33. Corrado, A. P., Ramos, A. O., and De Escobar, C. T., *Arch. Intern. Pharmacodyn.*, **121**, 380–94 (1959)
34. Cowan, S. L., and Walter, W. G., *J. Physiol. (London)*, **91**, 101–26 (1937)
35. Douglas, W. W., and Ritchie, J. M., *ibid.*, **150**, 501–14 (1960)
36. Eccles, J. C. in *The Physiology of Synapses* (Springer Verlag, Berlin-Göttingen-Heidelberg, 316 pp., 1964)
37. Edwards, C., and Ikeda, K., *J. Pharmacol. Exptl. Therap.*, **138**, 322–28 (1962)
38. Elmqvist, D., Hofmann, W. W., Kugelberg, J., and Quastel, D. M. J., *J. Physiol. (London)*, (In Press)
39. Elmqvist, D., and Josefsson, J. O., *Acta Physiol. Scand.*, **54**, 105–10 (1962)
40. Elmqvist, D., and Quastel, D. M. J., *J. Physiol. (London)* (In press)
41. Elmqvist, D., Quastel, D. M. J., and Thesleff, S., *J. Physiol. (London)*, **167**, 47–48P (1963)
42. Fatt, P., and Katz, B., *ibid.*, **115**, 320–70 (1951)
43. Feng, T. P., *Chinese J. Physiol.*, **13**, 119–40 (1938)
44. Gissen, A. J., and Nastuk, W. L. *Federation Proc.*, **23**, 365 (1964)
45. Greengard, P., and Straub, R. W., *J. Physiol. (London)*, **161**, 414–23 (1962)
46. Gutmann, E. in *The Denervated Muscle* (Czechoslovak Acad. of Sci. Prague, 486 pp., 1962)
47. Hodgkin, A. L., *Biol. Rev.*, **26**, 339–409 (1951)
48. Hofmann, W. W., Feigen, G. A., and Genther, G. H., *Nature*, **193**, 175–76 (1962)

49. Horowicz, P., *Pharmacol. Rev.*, **16**, 193–221 (1964)
50. Hubbard, J. I., *J. Physiol. (London)*, **159**, 507–17 (1961)
51. Hubbard, J. I., and Gage, P. W., *Nature*, **202**, 299–300 (1964)
52. Hubbard, J. I., and Schmidt, R. F., *Nature*, **191**, 1103–4 (1961)
53. Hubbard, J. I., and Schmidt, R. F., *J. Physiol. (London)*, **166**, 145–67 (1963)
54. Hubbard, J. I., and Willis, W. D., *ibid.*, **163**, 115–37 (1962)
55. Jenkinson, D. H., *ibid.*, **152**, 309–24 (1960)
56. Jindal, M. N., and Deshpande, V. R., *Brit. J. Pharmacol.*, **15**, 506–9 (1960)
57. Jirmanova, I., Sobotkova, M., Thesleff, S., and Zelena, J., *Physiol. Bohemoslov.* (In press)
58. Josefsson, J. O., and Thesleff, S., *Acta Physiol. Scand.*, **51**, 163–68 (1961)
59. Kao, C. Y., and Fuhrman, F. A., *J. Pharmacol. Exptl. Therap.*, **140**, 31–40 (1963)
60. Karczmar, A. G., Kim, K. C., and Koketsu, K., *ibid.*, **134**, 199–205 (1961)
61. Katz, B., *Proc. Roy. Soc. (London)*, *B*, **155**, 455–79 (1962)
62. Katz, B., and Miledi, R., *J. Physiol. (London)*, **171**, 10P (1964)
63. Katz, B., and Miledi, R., *ibid.*, **172**, 26P (1964)
64. Katz, B., and Thesleff, S., *ibid.*, **138**, 63–80 (1957)
65. Katz, B., and Thesleff, S., *Brit. J. Pharmacol.*, **12**, 260–64 (1957)
66. Klaus, W., Lüllmann, H., and Muscholl, E., *Arch. Ges. Physiol.*, **271**, 761–75 (1960)
67. Koelle, G. B. in *Cholinesterases and Anticholinesterase Agents* (Springer Verlag Berlin-Gottingen-Heidelberg, 1220 pp., 1963)
68. Koelle, G. B. in *Cholinesterases and Anticholinesterase Agents*, 187–298 (See ref. 67)
69. Koelle, G. B., *J. Pharm. Pharmacol.*, **14**, 65–90 (1962)
70. Koketsu, K., *Am. J. Physiol.*, **193**, 213–18 (1958)
71. Kraatz, H. G., and Trautwein, W., *Arch. Pathol. Pharmacol.*, **231**, 419–39 (1957)
72. Lamanna, C., *Science*, **130**, 763–72 (1959)
73. Laurence, D. R., and Webster, R. A., *Lancet*, **I**, 481–82 (1961)
74. Liley, A. W., *J. Physiol. (London)*, **133**, 571–87 (1956)
75. Liley, A. W., *ibid.*, **134**, 427–43 (1956)
76. Liley, A. W., and North, K. A. K., *J. Neurophysiol.*, **16**, 509–27 (1953)
77. Lilleheil, G., and Naess, K., *Experientia*, **16**, 550 (1960)
78. Lloyd, D. P. C., *J. Neurophysiol.*, **5**, 153–64 (1942)
79. Lloyd, D. P. C., *J. Gen. Physiol.*, **33**, 147–70 (1949)
80. Luco, J. V., and Sanchez, P. in *Curare and Curare-like Agents*, 405–8 (Elsevier Publ. Co., New York, 1959)
81. Lundberg, A., and Quilisch, H., *Acta Physiol. Scand.*, **30**, Suppl. 111, 111–20 (1953)
82. Lundberg, A., and Quilisch, H., *ibid.*, **30**, Suppl. 111, 121–29 (1953)
83. Lüllmann, H., *Klin. Wochschr.*, **38**, 1169–71 (1960)
84. MacIntosh, F. C., *Can. J. Biochem. Physiol.*, **41**, 2555–71 (1963)
85. MacIntosh, F. C., Birks, R. I., and Sastry, P. B., *Nature*, **178**, 1181 (1956)
86. McLennan, H. in *Synaptic Transmission* (W. B. Saunders Company, Philadelphia-London, 134 pp., 1963)
87. Martin, A. R., *J. Physiol. (London)*, **130**, 114–22 (1955)
88. Martin, A. R., and Orkand, R. K., *Can. J. Biochem. Physiol.*, **39**, 343–49 (1961)
89. Masland, R. L., and Wigton, R. S., *J. Neurophysiol.*, **3**, 269–75 (1940)
90. Matthews, E. K., and Quilliam, J. P., *Brit. J. Pharmacol.*, **22**, 415–40 (1964)
91. Narahashi, T., Deguchi, T. Urakawa, N., and Ohkubo, Y., *Am. J. Physiol.*, **198**, 934–38 (1960)
92. Nastuk, W. L., and Gissen, A. J. (Personal communication)
93. Nicholls, J. G., *J. Physiol. (London)*, **131**, 1–12 (1956)
94. Oomura, Y., and Tomita, T., *Tohoku J. Exptl. Med.*, **73**, 398–415 (1961)
95. Otsuka, M., and Endo, M., *J. Pharmacol. Exptl. Therap.*, **128**, 273–82 (1960)
96. Otsuka, M., Endo, M., and Nonomura, Y., *Japan. J. Physiol.*, **12**, 573–84 (1962)
97. Otsuka, M., and Nonomura, Y., *J. Pharmacol. Exptl. Therap.*, **140**, 41–45 (1963)
98. Pelikan, E. W., Tether, J. E., and Unna, K. R., *Neurology*, **3**, 284–96 (1953)
99. Perry, W. L. M., *J. Physiol. (London)*, **119**, 439–54 (1953)

100. Quastel, D. M. J., and Thesleff, S. (Unpublished observation)
101. Riker, W. F., *Arch. Neurol.*, **3**, 488–99 (1960)
102. Ritchie, J. M., and Armett, C. J., *J. Pharmacol. Exptl. Therap.*, **139**, 201–7 (1963)
103. Ritchie, J. M., and Straub, R. W., *J. Physiol. (London)*, **134**, 698–711 (1956)
104. Sabawala, P. B., and Dillon, J. F., *Anesthesiology*, **19**, 473–77 (1958)
105. Sabawala, P. B., and Dillon, J. F., *ibid.*, **22**, 564–68 (1961)
106. Schueler, F. W., *J. Pharmacol. Exptl. Therap.*, **115**, 127–43 (1955)
107. Schueler, F. W., *Intern. Rev. Neurobiol.*, **2**, 77–97 (1960)
108. Shanes, A. M., *Pharmacol. Rev.*, **10**, 59–164 (1958)
109. Shanes, A. M., *ibid.*, **10**, 165–273 (1958)
110. Stovner, J., *Acta Physiol. Scand.*, **41**, 370–83 (1957)
111. Stovner, J., *Acta Pharmacol. Toxicol.*, **14**, 317–32 (1958)
112. Stovner, J., *ibid.*, **15**, 55–69 (1958)
113. Straub, R., *Arch. Intern. Pharmacodyn.* **107**, 414–30 (1956)
114. Straughan, D. W., *J. Pharm. Pharmacol.*, **13**, 49–52 (1961)
115. Takeuchi, N., *J. Physiol. (London)*, **167**, 128–40 (1963)
116. Takeuchi, N., *ibid.*, **167**, 141–55 (1963)
117. Takeuchi, A., and Takeuchi, N., *ibid.*, **154**, 52–67 (1960)
118. Takeuchi, A., and Takeuchi, N., *ibid.*, **155**, 46–58 (1961)
119. Thesleff, S., *Acta Physiol. Scand.*, **34**, 218–31 (1955)
120. Thesleff, S., *ibid.*, **37**, 335–49 (1956)
121. Thesleff, S., *Acta Anaesthesiol. Scand.*, **2**, 69–79 (1958)
122. Thesleff, S., *J. Physiol. (London)*, **148**, 659–64 (1959)
123. Thesleff, S., *Physiol. Rev.*, **40**, 734–52 (1960)
124. Thesleff, J., *J. Physiol. (London)*, **151**, 598–607 (1960)
125. Thesleff, S. in *The Effects of Use and Disuse on Neuromuscular Functions*, 41–52 (Czechoslovak Acad. of Sci., 1963)
126. Thies, R. E., *Pharmacologist*, **5**, 220 (1962)
127. Thies, R. E., and Brooks, V. B., *Federation Proc.*, **20**, 569–78 (1961)
128. Werner, G., *J. Neurophysiol.*, **23**, 453–61 (1960)
129. Werner, G., *ibid.*, **24**, 401–13 (1961)
130. Zacks, S. I., Metzger, J. F., Smith, C. W., and Blumberg, J. M., *J. Neuropathol. Exptl. Neurol.*, **21**, 610–33 (1962)

CONTENTS