NEUROMUSCULAR PHARMACOLOGY[1,2,3]

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Neuromuscular Transmission

The skeletal neuromuscular junction continues to be a subject of intense study because this chemically activated, cholinergic synapse affords unusual opportunities for the study of electrophysiological, biochemical, and ionic factors controlling the release and action of the transmitter substance. Both post- and presynaptic action of the transmitter and of drugs is possible, and the relative importance of these actions is of basic significance. Moreover, the pertinent studies gained in depth with the development of microelectrode techniques and electrophoretic methods of application of drugs at localized post- and presynaptic regions.

Neuromyal transmission is not only of interest in its own right but serves as a model for investigation of autonomic and central synapses. It becomes increasingly clear that all synapses may operate as miniature brains, with as many as ten or more (cf. the concluding section) loci of drug action and mechanisms for regulating and controlling the transmission process. It is not surprising therefore that some 1500 investigations (cf. 9) were published in the 18 months since the last review on the subject appeared in this periodical (273). This necessitates selection; the present review will not deal with comparative aspects of the problem, with intrafusal transmission (cf. 259), or transmission in the homologous electric organs. Even within the restricted area of vertebrate, primarily mammalian and amphibian material, this review cannot describe all pertinent investigations.

Morphological and physiological aspects will be described first; pharmacological studies second. Since drugs active at the neuromyal junction affect

[1] The survey of the literature pertaining to this review was concluded in July 1966.

[2] The following abbreviations will be used: ACh (acetylcholine); AChE (acetylcholinesterase); ChE (cholinesterase); BuChE (butyryl cholinesterase); SCh (succinylcholine); DFP (diisopropyl phosphorofluoridate); e.p.p. (end plate potential); m.e.p.p. (miniature end plate potential); TEA (tetraethylammonium); Amb (ambenonium); MeAmb (methoxyambenonium); TEC (triethylcholine).

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more than one site or exhibit more than one mechanism of action, the pharmacological studies will be organized under major drug types rather than in terms of specific sites of drug action.

Morphology and Related Findings

The main features of the prejunctional and subneural apparatus of the skeletal neuromyal junction, presented in classical studies by Couteaux (73) and expanded by De Robertis & Bennett (85) and Palay & Palade (239) to include the synaptic vesicles, remain virtually unchallenged on the basis of both light and electron microscopy [for references to earlier literature cf. (4, 23, 83, 84, 190, 205, 299, 300)]. The terminal motor axon loses its sheath close to its branching, the branches making a series of contacts with the motor end plate. The terminal is surrounded by thin projections of Schwann cell cytoplasm that extends over the synaptic cleft. The muscle surface membrane is infolded to accommodate secondary synaptic clefts. An amorphous, moderately electron dense basement membrane material (252), presumably derived from surface membranes of the Schwann cell and of the muscle fibers, fills the primary and secondary synaptic clefts (84, 299). The synaptic vesicles are grouped in clusters near, or almost directly upon the synaptic portion of the sarcolemma.

However, Birks (22, 23) employing acrolein rather than osmium fixation, found two types of tubules among the population of synaptic vesicles in the frog neuromyal junction. Short tubules were interspersed among the vesicles abutting on the axolemma, while thinner and much longer tubules were noticed further away from synapsing regions, meandering throughout the axoplasm. He suggested (22), on the basis of his studies of penetration of thorium dioxide particles into both tubules and synaptic vesicles, that the short, large diameter tubules open into the synaptic cleft, while the long tubules open only into the axon Schwann cell space. While Birks did not suggest that synaptic vesicles are artifacts of fixation, he considered them to be delicate and likely to break down "during fixation and perhaps even during life" (22). He finally proposed that tubules form and store acetylcholine (ACh) and named them "synaptic tubules." It should be pointed out that, regardless of the fixative used, "synaptic tubules" were not observed at sites other than the frog neuromyal junction (240, 253).

It is well known that by ultracentrifugation of brain tissues, subcellular fractions have been obtained which contained both ACh and choline acetylase (83, 84, 86, 87, 137); it is not known to this reviewer whether similar work has been carried out in the case of the neuromyal junction.

Acetylcholinesterase (AChE) concentrates in the end plate, as known since 1938 (111, 220) and as confirmed by studies using the Cartesian microdiver (132) and histochemistry (light microscopy) (187, 205). Subsequent adaptations of the histochemical methods for electron microscopy confirmed the presence of AChE in subneural as well as in axonal terminations [cf. for instance (192, 209)]. Particular concentrations of the enzyme were noticed at

the plasma membrane of the axon terminal and at the junctional folds of the muscle (13). Sarcoplasmic mitochondria contained AChE (278, 300); and possibly axonal mitochondria also (cf. 13, 300). The presence of the enzyme at both pre- and postsynaptic sites is important; certainly, the earlier view reiterated in a recent review (273) that AChE "is . . . localized almost exclusively in the postsynaptic membrane", is no longer tenable. However, because of infolding, the postjunctional surface area is very large compared to the presynaptic area; even if the concentrations of the pre- and postjunctional AChE are equal, the postsynaptic enzyme seems functionally more important.

Recent electron microscopy findings indicate the presence of AChE at two strategic sites: in the primary and secondary synaptic clefts (12, 13, 209, 300) and, possibly, in the synaptic vesicles (13, 300). While Zacks & Blumberg (300) occasionally, and Koelle et al. (192) practically never, found the enzyme in the vesicles, Barrnett (13) and Miledi (227) consistently found it associated with the vesicular material. Barrnett (13) also described differential sensitivity of the enzyme within various structures to diisopropyl phosphorofluoridate (DFP) and to eserine, that of synaptic vesicles being some ten times more resistant.

The presence of the enzyme in the vesicles which also contain ACh is not teleological (13); while the substrate employed in electron microscopy application of the histochemical method may not always have been specific for AChE [as in the case of thiolacetate employed by Zacks & Blumberg (300)], acetylthiocholine was employed as substrate by Miledi (225). However, fixation procedures led to frequent rupture of the vesicles (13), and the origin of the stain may have been extravesicular.

Species differences in the pharmacology of the neuromyal junction make comparison of end plates of various species of interest. Unfortunately, little comparative electron microscopy work has been done but generally, unbranched terminals ["terminaison en ligne" (70)] characterized lower vertebrates such as toads and turtles (cf. also 299).

Physiology of Neuromyal Transmission

Cholinergic nature of neuromyal transmission.—Chemical and morphological interrelationships imply operation of the cholinergic system in the neuromuscular junction. Release of ACh upon motor axon stimulation and cholinergic transmission across this junction has been accepted since the classical work of Dale, Feldberg & Vogt (78) and Kuffler's (203) review of the evidence. Near-panic arose therefore among physiologists and pharmacologists when Hayes & Riker (145) found no quantitative difference between liberation of ACh upon the stimulation of the normal or denervated rat diaphragm. However, Mitchell & Silver (228) and Krnjević & Straughan (202) showed that mean release of ACh in resting denervated rat diaphragm was one half of that in normal muscle. Moreover, Krnjević & Straughan (202) and Bowman & Hemsworth (42) reported that direct stimulation of the

normal right hemidiaphragm increased the release fivefold; only a small increase was recorded in the case of denervated hemidiaphragm. The leech muscle and the much less sensitive frog rectus bioassay were utilized by Krnjević & Straughan (202) and by the Cornell group, respectively, in evaluating the release of ACh. Thus, the release of ACh upon prejunctional stimulation again appears to be incontrovertible [for earlier literature on this subject cf. (167, 202)]. Krnjević & Straughan (202) ascribed the release of ACh in the resting denervated muscle to Schwann cells and other cell remnants (25). Of course, some ACh is presumably present in denervated muscle (cf. 167).

Altogether, ample evidence exists for cholinergicity of the neuromyal junction; it is sufficient here to quote Eccles' (94) statement "that the vertebrate neuromuscular junction provides the site par excellence at which chemical . . . cholinergic . . . transmission has been most rigorously established," and to stress one additional piece of evidence. Demonstration of synaptic delay, i.e., "interval between pre- and post-synaptic membrane currents" (180) is an important criterion for accepting chemical transmission; it was, however, difficult to establish synaptic delay for the neuromyal junction. In fact, only in a few preparations was it possible to insert intracellular electrodes into both pre- and postsynaptic sites to demonstrate absence of electrical coupling as well as synaptic delay (61, 270). However, Katz & Miledi (179, 180) applied the method of recording "focal" potential changes in the frog nerve sartorius preparation to the measurement of the synaptic delay. In a calcium-free medium, the motor nerve impulse could release ACh and produce postsynaptic potential only in a localized region immediately under the recording electrode whenever calcium was allowed to diffuse from the latter. At 20° C, the synaptic delay had a minimal duration of 0.4 to 0.5 msec and a modal value of 0.75 msec. Furthermore, when ACh was applied electrophoretically to highly sensitive end plate sites and nearby depolarization recorded intracellularly, the reaction time of ACh with the receptor was 150 μsec (180). As cross-synaptic diffusion time must be of the order of 1 μsec, the synaptic delay should be mainly due to the release process.

This process, rather than diffusion or receptor action, was found to be temperature sensitive ($Q_{10}=3$); the release of the transmitter was dispersed in time at low temperature (182). Altogether, contrary to the postulate of Liley (211), release of ACh does not seem synchronous with the nerve terminal action potential.

Presynaptic events.—Acetylcholine release from the motor nerve terminal may be related to active propagation; alternatively, the nerve terminal, unable to carry the action potential, may be passively depolarized by electrotonic spread (94). An external electrode a few microns from the outer surface of the terminal membrane should record a biphasic potential if a current flows through the membrane; however, a biphasic potential, although of slightly different pattern, may be recorded also in the case of electrotonic spread (cf. 94).

In the rat diaphragm preparation (158) and in the frog nerve sartorius preparation (52, 179), extracellular recording of miniature end plate potentials (m.e.p.p.'s) in the case of the rat and the frog or of focal end plate potentials (e.p.p.'s) in the case of the frog was employed to establish that the microelectrode tip was in very close proximity to a presynaptic terminal. A biphasic or triphasic potential was recorded with both preparations. The conduction velocity of the potential was 30 to 40 cm/sec (52, 179); it was minimal near the transition from the myelinated to the nonmyelinated portions of the motor nerve and it increased toward the terminal. In the frog preparations, however, spike polarity was reversed when the recording electrode was relatively close to the "parent" myelin sheath. Katz & Miledi (179) suggested that this was the result of special local circuit conditions at the closed end of the terminal (55, 94), rather than to the invasion failure of the action potential.

In the frog preparations in which nerve terminals run for 100 to 200 μm along the muscle fiber, external two-point recordings showed that the focal e.p.p.'s, i.e., the release of the transmitter, occurred along the whole length of the terminal. However, the polarization of the preterminal part of the motor axon increased the frequency of the m.e.p.p.'s. Some attenuation of the response occurred at the distal compared to the proximal nerve terminal point. These results suggest transmitter release by a decremental, depolarizing electrotonic spread. On the other hand, antidromic response could be initiated, both in the rat and frog preparation (52, 158, 175), indicating that the impulse can be propagated in the nerve terminal.

Release of acetylcholine.—Depolarization along the nerve terminal caused by the arrival of the propagated potential, or perhaps by electrotonic spread, leads to release of ACh. Several factors might control the release: (*a*) storage and synthesis of ACh; (*b*) polarity of the membrane; (*c*) ions, particularly sodium and calcium; (*d*) mobilization of the transmitter, particularly following repetitive stimulation of the terminal; (*e*) possible positive feedback action of ACh upon the nerve terminal (190, 191).

(*a*) The problems related to ACh synthesis and storage in the nerve terminals were recently reviewed (146, 273, 292). Storage of ACh in synaptic vesicles is generally agreed upon. However, whether choline acetylase is located in synaptic vesicles or in separate cytoplasmic components is controversial (146, 292). Vesicles may concentrate ACh synthesized elsewhere in the cytoplasm, and a relationship may exist between the concentrations of ACh at these two sites (106, 292). It seems also that not all the ACh is equally available in response to the impulse reaching the nerve terminal, and Birks & MacIntosh (26) favored the concept of "reserve," "available," and "most readily releasable" ACh (cf. also 89). While the latter must be related to the population of synaptic vesicles or tubules immediately adjacent to the presynaptic membrane, the two former fractions may consist of ACh from synaptic vesicles located at various distances from the membrane; strength of binding of ACh in these vesicles may vary.

There is no doubt that ACh synthesis depends upon activity (26). Furthermore, activity may push the vesicles toward the presynaptic membrane (94). Cytoplasmic ACh may also act as negative feedback in control of ACh synthesis, which should be particularly effective when activity is decreased. Finally, Na may be needed in ACh synthesis, since decrease of ACh output was noticed in low Na media (21); this finding is, however, controversial (64, 128, 184).

The advent of hemicholiniums, which inhibit ACh synthesis, aided the development of many of the concepts presented above (cf. 273). Recent results obtained with these agents will be described below.

(*b*) It has been generally accepted that the amount of ACh released depends upon the magnitude of depolarization produced by the impulse reaching the nerve terminal; this in turn depends upon the resting membrane potential. Hyperpolarization leads to an increased presynaptic spike as well as to an increased end plate potential (160, 161), the latter being the measure of the amount of the transmitter liberated (cf. 94). However, the increase in the magnitude of the e.p.p. predominates in these experiments. Lack of relationship between the size of the action potential and ACh release, as indicated by the size of the e.p.p., was noticed particularly after conditioning of neuromyal transmission in the rat and frog (52, 158).

Mobilization of the transmitter may also play a role in the buildup of the postsynaptic event by hyperpolarization (see below). Additionally, hyperpolarization may lead to a local "dielectric breakdown" of the membrane (82) and to high frequency bursts of miniature end plate potentials. Recent experiments of Katz & Miledi (179) indicated that this phenomenon resides in the nerve terminal itself. Hyperpolarization of the terminal may be regulated by an inside-outside sodium concentration gradient; block of the sodium pump by drugs or poisons such as ouabain, 2,4-dinitrophenol, and sodium azide may result in Na accumulation in the cell and lowering of the potential. This may prevent post-tetanic facilitation (127) and subsequently depolarization and ACh release (126). The final effect may depend on the concentrations of Mg and Ca (74), and the extent of sodium pump inhibition (103).

(*c*) A previous review (273) described the dependence of ACh release upon Mg and Ca. In summary, either high Mg or low Ca blocks ACh release; the quantal ACh content of the end plate potentials is affected in parallel. Calcium may be the essential cofactor for either coupling between synaptic vesicles and "acting zones" of nerve terminals, or for the discharge of the contents of the former (176; cf. 94), thus increasing "the probability of quantal transmitter release . . . by . . . depolarization of nerve endings" (181). Since a fraction of m.e.p.p.'s seemed to persist in the absence of Ca (154, 155), it was suggested that the release of some of the ACh may be independent of Ca. More recently, however, all m.e.p.p.'s were found to disappear after six hours EDTA treatment of rat nerve-diaphragm preparation (103). Ca may be strongly bound in the terminals, and a low intraterminal

concentration of Ca (10^{-10} M) may suffice to maintain activity under usual conditions (cf. also 24). Results predictable on this basis were obtained using agents that promote Ca efflux or mobility such as caffeine and ouabain (19, 103, 217); caffeine with ouabain further decreased m.e.p.p. frequency in EDTA-treated preparations (103).

Katz & Miledi (181) applied Ca electrophoretically to the frog nerve terminal bathed in high Mg, low Ca medium and demonstrated release by Ca of ACh from the terminals as evidenced by focal postsynaptic potentials. Similar results were obtained by Elmqvist & Feldman (103) using the rat nerve-diaphragm preparation treated with EDTA. Additionally, lack of Ca had no effect upon propagation of nerve impulse, thus disposing of the argument that the failure of the release was due to failure of the potential in the Ca-free medium (117). These results as well as those of Birks et al. (24) suggest that ACh release is determined by intraterminal ionized Ca and that the latter is derived from influx, accelerated by depolarization and dependent on extracellular Ca, as well as from the variable membrane store of bound Ca controlled by membrane potential. Changes in action potential of the nerve terminal may not always be related to changes in ACh release (181), indicating that Ca and nerve terminal depolarization may constitute two independent controls of ACh release from the nerve terminal. However, Ca release maybe influenced by the state of depolarization and by an interplay between the latter and such drugs as physostigmine (234).

Recently, Birks (21) and Gage & Quastel (cf. 128) suggested that intraterminal Na is also involved in the release of ACh. Blockade of the sodium pump by cardiac glycosides (258) caused fasciculation and increased frequency of the m.e.p.p.'s (21, 104). Birks (21) proposed that Na entering the nerve terminal with each action potential facilitates ACh release as it concentrates briefly at the terminal membrane; during continuous activity this effect becomes more enduring as intraterminal Na concentration increases, both actions being potentiated by block of Na extrusion. Reversal of action of glycosides by low Na medium appears to favor Birks' (21) suggestion; however, ouabain-induced accumulation of Na may also cause nerve terminal depolarization and thus ACh release (135). Alternately, ouabain may act by increasing the mobility of calcium, as suggested by Elmqvist & Feldman (104). Indeed, many substances which block the Na extrusion pump caused depolarization as well as increased ACh release (cf. 104). A suggestion was made (126) that glycosides produced only an increase of amplitude of the m.e.p.p.'s in absence of the change of the quantal content of the e.p.p. (sensitization). The increase of the frequency of the m.e.p.p.'s was ascribed to the alcoholic content of the glycoside preparations. However, Elmqvist & Feldman (104) employed nonalcoholic media.

Further investigation is required in this area. If Na indeed serves as facilitator of ACh release, some coupling between Na and Ca mechanisms may exist. For instance, calcium influx may be accelerated by high intracellular sodium (24; cf. also 21, 184, 236, 277). Certain apparent discrepancies

between the role of Ca at axonal and muscle membranes and at the nerve terminal also have to be elucidated. At the former membranes, Ca is related to the resting state; its removal by various means—including depolarization by K or by electric stimulation—leads to an active membrane [(118, 277); for review of recent results cf. (195)]. It is of interest that Mg can substitute for Ca as a controller of membrane permeability, but at the nerve terminal Ca caused ACh release (103, 181) and Mg and Ca were mutually antagonistic in the control of ACh liberation. A reconciliation of these differences is suggested by the work of Nishi, Soeda & Koketsu (233) who demonstrated, on spinal ganglia of the frog, that bivalent ions, including Ca, exerted dual actions. Besides binding the membrane and controlling and limiting its permeability, they could also act as charge carriers by penetrating the membrane. This second effect which is Na-like, may be involved in ACh release.

(*d*) In recent reviews (94, 230, 273) the case for presynaptic mobilization of ACh as the source of facilitation following conditioning stimulus and tetanus was well presented. In brief, increased frequency of m.e.p.p.'s, as well as increased amplitude of e.p.p., was recorded by many investigators following conditioning, provided the experiment did not lead to transmitter depletion (155) or to postsynaptic desensitization [for references cf. (94)]. These phenomena may be related to increased nerve terminal action potential, which in turn may be related to after-hyperpolarization. An alternative explanation is that the transmitter is mobilized either via increased synthesis or because synaptic vesicles may move to the presynaptic membrane (93, 94). Possibly, the mechanism which operates depends on the species. In the frog, for instance, the nerve terminal potential may decrease rather than increase, and hyperpolarization may be small or absent during the period of post-tetanic facilitation and of increased frequency of m.e.p.p.'s (52, 53). It should be remembered that following a stimulus, a negative and positive after-potential can be demonstrated in the nerve terminal (91, 158), the evidence for the negative phase being mostly indirect (159); cf. also below under (*e*). This may obscure the relationship between nerve terminal membrane phenomena and facilitation, particularly in the case of experiments involving short conditioning (53).

(*e*) Released from the nerve terminal, ACh classically activates transmission by its exclusive postsynaptic action. An action of ACh on the nerve terminal was suggested by the discovery that ACh and anticholinesterases (anti-ChE's) produce antidromically propagated action potentials (221; cf. 94, 167, 250). Furthermore, Werner (290) and Barstad (15) proved that ephaptic transmission is not involved and that the potentials originated in the nerve terminal, and indirect evidence (159) suggested that nerve terminal activation was not due to anti-ChE, ACh-induced K release, or both as suggested by Diamond [(90) for further references cf. (159)]. Possibility of ACh action at the nerve terminal is of particular interest in view of Koelle's (190, 191) "percussion" hypothesis of two-step release of ACh, which pos-

tulates that the initial quanta have a positive feedback action upon the nerve terminal, resulting in a synchronized, mass release of the transmitter.

However, Hubbard et al. (159) found that ACh does not increase the frequency of m.e.p.p.'s or the quantal content of the e.p.p.'s in the rat phrenic nerve-diaphragm preparation in which the quantal component of the e.p.p. was reduced by high Mg. In the presence of prostigmine, ACh decreased the quantal content of m.e.p.p.'s and still had no effect on the frequency of ACh release. ACh-produced reduction of the number of quanta released by each nerve impulse was also noticed in the frog neuromyal junction by Ciani & Edwards (69) who employed relatively large (6×10^{-6} to 8×10^{-5} g/ml) concentrations of ACh which blocked transmission. Yet ACh lowered the threshold of the nerve terminal response to cathodal pulse. Thus, paradoxically, the first set of experiments indicated that ACh does not, while the second set suggested that it may depolarize the terminal (284). Hubbard (156, 159; cf. also 262, 263) proposed therefore that ACh depolarized at a site above the terminal, specifically at the node or nodes of Ranvier. The depolarization at this site should then be insufficient to increase ACh release, but sufficient to lower the threshold. Endogenous ACh, released by nerve impulses, should also be capable of action at this site, particularly in the presence of anti-ChE's, leading to fasciculation and to antidromic firing. Hubbard (156) concluded that contrary to Koelle's (190, 191) hypothesis, "ACh does not appear to have any essential role in transmitter release at the neuromuscular junction."

Riker and his associates (250) also stressed the limitations of action of ACh on the nerve terminal. They emphasized that antidromic response to ACh as well as ACh-induced repetitive antidromic response to motor nerve stimulation are much less extensive than similar actions produced by anti-ChE's and related facilitatory drugs. They denied therefore the possibility that ACh may act "as an intermediary in facilitatory drug actions at the nerve terminal," that the facilitatory compounds may have nerve terminal action by causing ACh accumulation, and that massive release of ACh may be due to a priming concentration of ACh, as suggested by Koelle. It should be pointed out that Hubbard's (cf. 159) experiments may have no bearing on momentary facilitation of ACh release upon priming ACh liberation and that generally his data and particularly those of Ciani & Edwards (69) dealt with effects of long duration. It is also not quite clear why Riker (250) should expect the effects of ACh to be quantitatively comparable to those of combinations of ACh and anti-ChE agents. ACh injected into the blood stream usually produces responses which are qualitatively similar and quantitatively inferior to those elicited by ACh in anti-ChE-treated animals (199). The additional question of the possibility of the direct, cholinomimetic action of facilitatory agents on the nerve terminal remains unanswered.

Postsynaptic events.—The membrane effects arising upon synchronous release of ACh at the nerve terminal, amounting to an increase of the permeability of the membrane to all ions (81), were described in earlier reviews

(94, 230, 273, 291). The "short circuit" hypothesis of Fatt & Katz (108) seems today to be proven.

The ability of ACh to produce the "short circuit" is basically restricted to the end plate region [for review cf. (273)] and, at a somewhat lower level, to the muscle-tendon region (177) which was found to be one-thousandth as sensitive as the end plate, and yet much more sensitive than nerve-free zones.

ACh depolarization of the end plate is a relatively short-lived process; in the frog neuromyal preparation kept continually in an effectively depolarizing solution of ACh, repolarization occurred within seconds or slower, depending on the concentration of ACh employed [(173, 186, 271); for additional references cf. (134, 230, 273)]. With, or without repolarization, the end plate becomes desensitized to ACh and does not respond by twitch or by depolarization; *d*-tubocurarine increases the block at this time. The extent of desensitization may be increased by Ca and antagonized by Na (230). Following conditioning with low concentrations of ACh, high concentrations depolarized less than in unconditioned preparations. In parallel with this adaptation of membrane, concentrations of ACh capable of depressing neuromyal transmission became ineffective following conditioning (186). Thus, desensitization may underlie such phenomena as decline of e.p.p. amplitude after a high frequency stimulation (cf. 273) as well as the so-called "phase II" block arising under similar circumstances. Karczmar speculated that desensitization and adaptation may play a minor part, postsynaptically, in regulating response to ACh (186). It should be remembered that the above data were gathered in frog neuromyal junction, even though cholinomimetic, depolarizing agents also produce desensitization in red (slow) mammalian muscles of some species (302).

Cholinergic Receptor

Structure-activity relationship (SAR) studies.—Cholinergic transmission seems essentially similar at the neuromyal junction, at autonomic ganglia, and at certain central synapses, such as that between motor axon collateral and the Renshaw cell. All these synapses can be classified as nicotinic and they react in a predictable manner to cholinomimetics and anticholinesterases. Until recently, it was generally believed that nicotinic sites were not activated by muscarine and muscarinic agents. At present, muscarinic sites have been described at autonomic ganglia (cf. 283) and the Renshaw cell exhibits a weak delayed response to muscarinic agents (75). The neuromyal junction is now the only nicotinic site presumably devoid of muscarinic receptors (cf. 167).

Agonists of the nicotinic neuromyal, ganglionic, and central sites differ from those of the muscarinic sites [for references cf. (11, 167)]. The nicotinic site is activated by small doses of nicotine (and blocked by large doses) and by simple onium salts, such as triethyl ammoniums, which are either inactive or act only as antagonists at parasympathetic (muscarinic) sites. Mus-

carine is ineffective, while muscarones exhibit nicotinic activities (285, 286). It is surprising that such a typical nicotinic agent as acetyl-β-methylcholine (283) apparently has not been studied at the neuromyal junction.

That blockers of the nicotinic and muscarinic sites also differ is well known; *d*-tubocurarine and dihydro-β-erythroidine on the one hand and atropine on the other are effective at these two sites, respectively. On the basis of SAR studies of agonists and antagonists effective at the nicotinic sites, models for the nicotinic cholinergic receptor were proposed (cf. 11, 167, 286). Ariëns' concept (7), that anticholinergic and cholinergic agents do not act via a common receptor, was mainly based on the finding that compounds without amino or onium groups can exhibit anticholinergic action.

Isolation attempts.—Another approach to the problem of cholinergic receptor has been to isolate receptor substances from muscle or its homologue, the electric eel electroplax. A technique based on precipitation of electric organ extract of *Electrophorus electricus* with *d*-tubocurarine (96–98) led to isolation of a drug-binding protein. However, in solution, the protein combined weakly or not at all with decamethonium (C_{10}), ACh, carbamylcholine, and prostigmine. A somewhat different approach was employed by Chagas' group (cf. 143, 144). Dialysis of electric organ homogenates against H_2O yielded macromolecules in the aqueous phase which bound *in vitro* certain curare-like agents and other quaternary ammonium bases. Acidic mucopolysaccharide of the hyaluronic type was thought to be the active macromolecule.

Finally, Grob & Namba (139, 229) isolated from human muscle a ribonucleoprotein with differential affinity for ACh and *d*-tubocurarine which exhibited AChE activity. Data were presented to indicate that none of the three types of macromolecules isolated can be accepted as the neuromyal cholinergic receptor (cf. 98, 99).

Autoradiography of the cholinergic receptor.—Waser (286, 287) employed radioactive *d*-tubocurarine, C_{10}, toxiferine, muscarone, and ^{14}C and ^{32}P in the mouse nerve-diaphragm preparation. The drug concentrations used were those which produced clear-cut pharmacological end points. End plate receptors which could bind well-defined numbers of molecules of toxiferine and *d*-tubocurarine were demonstrated, as well as receptors, embracing larger areas around the end plate, which bound specific amounts of curaremimetic and depolarizing agents. Presumably, separate centers of AChE activity could also be established. Denervation produced spreading of sites binding depolarizers and disappearance of those binding *d*-tubocurarine (287). A drastic change of the type of action of *d*-tubocurarine, following denervation (cf. below) is of interest in this context.

Cholinergic receptor and cholinesterases.—Several attempts identify AChE (98, 99) or its anionic center (17, 18, 304–306) with the cholinergic receptor, while Wurzel (297, 298) synonymized the "muscarinic receptor" with AChE and "nicotinic receptor" with butyryl cholinesterase (BuChE). The data of Waser (287): weakness of direct neuromyal junction

action of many ChE inhibitors including oxamides which react both with cationic and anionic (esteratic) centers of ChE molecule; conversely, weak anticholinesterase action of *d*-tubocurarine and atropine, all militate against identification of ChE's or their centers with the cholinergic receptor (cf. also 167, 168).

Pharmacology of the Neuromyal Junction

Depolarizing agents.—Subsequent to demonstration of skeletal neuromyal blockade by succinylcholine [SCh (38)] and of the depolarizing action of SCh and C_{10} on the end plate (241, 242), it was generally assumed that these slender, elongated molecules ("leptocurares," to employ Bovet's term) produced muscular relaxation by prolonged postsynaptic depolarization. The cationic head and the structure of the leptocurares are constituted so that they can closely approach the receptor molecule, contrary to curaremimetic, competitive blockers ("pachycurares") (273). The antagonism between these two classes of compounds is well known [for recent results cf. (38a, 231)]. Besides SCh and C_{10}, compounds such as choline carbachol and choline esters of ω-amino substituted fatty acids and their secondary, tertiary, and quaternary methylamino derivatives (56, 114, 115), tetramethylammonium, phenyltrimethylammonium, as well as several hydroxyaniliniums such as 3-hydroxyphenylmethylammonium (247) may belong to this category of agents. Many of these have a highly charged, penetrating onium head characterized by little steric hindrance thus resembling SCh and C_{10}, even though they lack the elongated shape of the latter compounds.

Ester compounds among leptocurares, such as SCh and fatty acid esters of choline, are hydrolyzed by BuChE but not by AChE (113, 115). Not all species possess BuChE's capable of hydrolyzing fatty acid esters of choline (115), and genetic differences in man with regard to the hydrolyzing capacity of plasma cholinesterase (BuChE) are well known (164). C_{10} and SCh are weak inhibitors of AChE [for references cf. (36, 212)], as are the newer fatty acid esters of choline, some of which also inhibited BuChE (115).

All leptocurares exhibit biphasic actions upon skeletal neuromyal junction and they produce contracture of smooth, including extraocular (92), muscle as well as of the frog rectus. At the skeletal neuromyal junction, leptocurares first produce facilitatory effects including fasciculations and subsequent blockade; only facilitatory effects may be noticed with lower doses. Repetitive stimulation enhanced the blockade produced by SCh, as well as increased the twitch produced by depolarizers [(282); cf., however (162)]. Volle interpreted these results as dependent on accumulation of ACh at the junction following repetitive stimulation (282).

The postsynaptic actions of these compounds were described (273) as dependent on two processes; first, increase of the membrane permeability and depolarization resembling the effect of ACh (269) but exaggerated so as to inactivate the sodium-carrying mechanism responsible for the generation of the action potential; second, desensitization of the end plate which may

occur after repolarization took place (173, 271; cf. 230, 273). This desensitization differs from the competitive blockade by curaremimetics, since it was found to be generally not competitive and responded poorly to high concentrations of ACh or to anti-ChE's (186, 273).

This picture of action of depolarizers may apply especially to the amphibian neuromyal transmission. In the mammal, the second phase of action may be missing or be competitive and antagonizable by anti-ChE's (302). In certain mammalian species the blocking effect of depolarizers on the so-called slow (red) muscle may be competitive in its entire course (302).

Recently, attention has been directed to the action of depolarizers on the nerve terminal and on structures other than the postsynaptic membrane. Basically, this phenomenon is similar to that following the administration of anti-ChE's and related agents (cf. next sections). As known since the first description of the phenomenon by Feng & Li (110), tetanic stimulation also produces nerve terminal activity (261, 289, 290).

C_{10} and SCh were shown to produce antidromic responses (1, 95, 257, 261, 293) whether following orthodromic "conditioning" or in the absence of motor nerve stimulation (35, 174, 264). The repetitive potential in the ventral root which followed either the tetanic stimulation of the axon, the administration of the depolarizer, or both occurred some 1 msec before the muscle potential, thus indicating that the neuronal activity originated in the terminal (174, 261, 264). This, however, is not equivalent to the demonstration of the nonephaptic character of antidromic response to anti-ChE's (15, 290); in fact, part of the antidromic response to large doses of SCh may have been due to the ephaptic response (174).

The nerve terminal action of SCh and C_{10} was related by the Cornell group to the increase in twitch response to indirect stimulation, either observed during the early phase of the action of blocking doses, with small doses, and with the blockade. As shown by Standaert & Adams (264), the smallest effective intra-arterial dose of SCh (0.01 μg/kg) reduced post-tetanic repetitive firing; doses two to three times higher evoked a repetitive antidromic response following single ventral root stimulation; while still higher doses elicited repetitive nerve terminal response in absence of stimulation. Finally, from five to ten times the initial dose was necessary to depress the evoked twitch. The Cornell group suggested that the repetitive antidromic response to single stimulation observed with SCh "presumably caused" the increase in muscle twitch response "coincident with" the repetitive response. Standaert & Adams (264) further concluded that the block of transmission was due to impairment of the nerve terminal by SCh which follows its earlier facilitatory action. Blaber (29) did not obtain with SCh increase in frequency of the m.e.p.p., although in the same preparation SCh was about one hundred times more potent a depolarizer of the end plate than ACh. Similar negative data were obtained with ACh by Hubbard et al. (159). Thus, it is unlikely that SCh depolarizes—and hence blocks—the nerve terminal. Standaert & Adams (264) further suggested that the end

plate depolarization by SCh or C_{10} "may merely reflect the . . . induced depolarization of the motor nerve terminal." Thus, the Cornell group distinctly preferred the nerve terminal to the postsynaptic membrane as the site of blocking and facilitatory actions of depolarizers as they did with regard to facilitatory drugs (cf. 265).

However, the attenuation by SCh of the repetitive antidromic responses to ventral root tetanus occurred at doses some ten times less than those leading to depression of transmission; in fact, the latter seemed to coincide with excitatory nerve terminal actions of SCh. Secondly, repetitive muscle responses were frequently observed by Standaert & Adams (264) in absence of nerve activity; while this may suggest the tendency of SCh to act post- rather than presynaptically, Standaert & Adams (264) ascribed this effect to the "SCh-induced failure of propagation from the terminal nerve branches to the parent axon." This, however, seems improbable in view of the dependable nerve terminal effect of SCh obtained in the course of "reversal" [(35); cf. next sections]. Finally, the argument that nerve terminal depolarization may be the cause of the end plate depolarization seems to suggest an effective electrotonic spread across the synaptic cleft. Yet, Hubbard & Schmidt (158) found an extreme attenuation of the presynaptic potential when its electrotonic spread was recorded postsynaptically in the rat nerve-diaphragm preparation. Moreover, nerve terminal depolarization by anodal current produced disappearance of the end plate depolarization [or of the e.p.p. (281)], contrary to the suggestion of Standaert & Adams (264).

Altogether, it seems difficult at present to associate undeniable nerve terminal actions of SCh with its blockade of neuromyal transmission, particularly since SCh did not seem capable of effective depolarization of the terminal (29); postsynaptic mechanism of SCh blockade on the whole explains the available data satisfactorily (cf. also 282).

Two other actions of depolarizers were demonstrated by many investigators: fasciculations and, upon intra-arterial administration of these compounds, muscle twitch (35). Small doses given intra-arterially may produce only fasciculations (35). Fasciculations were also observed in human beings in whom muscle relaxants were employed, as noted first probably by Churchhill-Davidson [(66); for recent clinical observation cf. (293)]. Since Hubbard et al. (159) proposed that ACh may increase nerve terminal excitability by depolarizing action at the first node, it was suggested by Blaber & Karczmar (35) that depolarization of the first node by cholinomimetic agents such as SCh may cause propagation to several terminals of the same motor unit and thus, depolarization. The proposed nodal action of ACh was antagonized by *d*-tubocurarine (159); analogously, fasciculations due to depolarizers are readily blocked by curaremimetics. A different interpretation was given by Kato & Fujimori (174) on the basis of their elegant experiments in which afferent discharges from the motor spindle, monosynaptic reflex of extensor motoneurons, random motoneuron discharges and antidromic firing, as well as electromyograms were simultaneously recorded prior to and following

SCh, administered intra-arterially. An increase in the afferent discharge was associated with a decrease in the amplitude of the monosynaptic reflex and with an increase in random motoneuron discharge (124, 125, 150, 174, 175). The Hokkaido group suggested that fasciculation may result from an interaction between increased random motoneuron discharge caused by activation of the muscle spindles by the depolarizers (3, 124) and the partial blockade of the end plates. Indeed, fasciculations disappeared when motoneuron contribution was prevented by the section of ipsilateral ventral roots. Yet, the latter phenomenon was not uniformly observed (223).

Muscle twitch caused by intra-arterial administration of ACh, SCh, carbachol (178), and other cholinomimetics was related by the Cornell investigators (264) to an activation of the "numerous subdivisions of the terminal arborization"; cf. above for the reason given by these investigators for the absence of the antidromic response at this time. Since, however, muscle twitch can also be obtained in the chronically denervated muscle [cf. for instance (28, 178)], the classical explanation of the twitch induced by depolarizers as a result of rapid postjunctional depolarization seems more plausible.

Thus, the depolarizers appear capable of acting at numerous sites within the neuromyal junction. Their inhibitory actions against AChE and BuChE have already been described; these actions may be more important than suspected since the "reversible" inhibition caused by these compounds cannot be measured reliably, and since sometimes they were applied in large concentrations to a small area, as when administered intra-arterially.

Anticholinesterase agents.—As known since the experiments of Brown, Dale & Feldberg (57), anti-ChE's affect the neuromyal junction, whether activated by neural stimulation or by short, intra-arterial injection of ACh. For classical neuromyal actions of anti-ChE's such as increase of the twitch response to indirect stimulation and anti-*d*-tubocurarine actions which include also prolongation and augmentation of the e.p.p. obtained when the postjunctional spike is blocked by fatigue or by *d*-tubocurarine, cf. recent reviews (166, 167, 273, 291). Effects described more recently include actions on m.e.p.p.'s and on the presynaptic membrane. The prejunctional effects were first discovered in the early 40's (111, 221), and subsequently many reversible and organophosphorus anti-ChE's were shown to activate the terminal. These actions were analyzed pharmacologically and their importance stressed within the last ten years by the Cornell group (cf. 250).

The extent to which these compounds exert their pharmacological actions by actually inhibiting AChE is the subject of many investigations beginning with that of Bacq & Brown (10; cf. 167, 291). Particular controversy exists with regard to hydroxyanilinium compounds, especially edrophonium (Tensilon). That the inhibition of ChE constitutes the mechanism of their action has been denied by Riker and his associates [(247–249); cf. also below]. The crux of this matter is that in view of a particularly rapid dissociation of the edrophonium-AChE complex (260, 294), reliable measurement of the in-

hibition which may occur *in vivo* following edrophonium is well nigh impossible, particularly when manometric techniques which involve homogenization and dilution are employed. A maneuver, originally developed by Koelle (188), was employed recently (136) to answer this question. After a "reversible" inhibitor is used to bind enzymatic sites preventing their occupation by the organophosphorus compound, histochemical procedures demonstrated the protected sites after washing away from the slide the reversible anti-ChE. Edrophonium did not protect the enzyme when given to cats in a dose sufficient to increase twitch responses, prior to a 100 per cent inhibitory dose of DFP; or when used *in vitro* [in a concentration calculated (28) to correspond to the dose effective *in vivo*] prior to the application of an inhibitory concentration of DFP. Blaber & Bowman (27, 28, 30) employed an additional approach; they evaluated enzymatic inhibition *in vivo* by ascertaining whether or not the pertinent compounds could potentiate twitch responses to close intra-arterially injected ACh; in the case of neostigmine, a bisquaternary oxamide ambenonium (see below) and edrophonium, their anticholinesterase action, *d*-tubocurarine antagonism, and twitch increase could not be correlated. A similar conclusion based on a related technique was reached by Lands et al. (207).

This reviewer cannot conclude, on the basis of these data, that many potent anti-ChE's, such as ambenonium, do not act via enzymatic inhibition. Potentiation of response to injected ACh may not be equivalent to facilitation of transmission activated by endogenous transmitter, since the topographical relations may be different in these two instances; the doubts expressed with regard to experiments involving edrophonium are pertinent with regard to rapidly reversible inhibition.

The site of action of anti-ChE's remains an open question. Inhibition of either the pre- or postsynaptic enzyme may lead to accumulation of ACh which in turn may act presynaptically via Koelle's "percussion" mechanisms, or by classic prolongation and intensification of postsynaptic action of the transmitter. Moreover, whether or not enzymatic inhibition takes place, the compounds may exert a direct, either pre- or postjunctional action. Finally, this direct effect may be cholinomimetic in nature leading either to release of ACh from the presynaptic terminal or to the activation of the postjunctional membrane, or it may be independent of cholinomimetic activity, as preferred by Riker. These questions will be pursued further below.

Oxamides.—Oxamides are bisquaternary compounds, analogues of methoxyambenonium (MeAmb) and ambenonium (Amb), investigated originally by Karczmar, Lands, and their associates (165, 208), and recently better known than almost any other group of neuromyally active drugs. These compounds vary greatly in their *in vitro* anti-AChE action, ranging from several times more potent (Amb) to several times less potent (MeAmb) than neostigmine (8, 206–208; cf. also 212). They are poor inhibitors of BuChE. Even in the case of Amb, the relationship of its pharmacological action to its anti-AChE action *in vivo* remains controversial (28, 166, 189, 208).

Almost without exception, oxamides are potent antagonists of *d*-tubocurarine; on the other hand, not all of these compounds increased the muscle twitch response to indirect stimulation. Amb increased the twitch at intravenous doses as small as $\frac{1}{2}$ μg and antagonized *d*-tubocurarine at 3 μg/kg. MeAmb antagonized *d*-tubocurarine at 6 μg/kg but exhibited no effect on the twitch. Fractions of these doses were effective via the intra-arterial route (27).

In the frog, Amb augmented and prolonged the end plate potential and ACh depolarization (172, 173); MeAmb augmented but did not prolong these phenomena. Conventional anti-ChE's augment and conspicuously prolong the end plate potential and ACh depolarization (cf. 94, 291).

Some preliminary information suggests that these compounds may have biphasic activities on m.e.p.p.'s, first increasing their frequency, then augmenting their amplitude and prolonging their duration (29, 169).

At the nerve terminal, Amb produced repetitive firing with or without electric orthodromic stimulation (32, 33). While incapable of this action, MeAmb seemed to increase the duration and frequency of nerve terminal action due to SCh (34, 35).

Finally, oxamides block at the neuromyal junction [(27); cf. also next section]. This effect was obtained at intravenous doses of 500 μg/kg or higher (165). While this block could be antagonized by the tetanus and by depolarizing agents (27, 34, 35), it was not affected by anti-ChE's, and thus it resembled the block produced by benzoquinonium rather than the one produced by *d*-tubocurarine (cf. *infra*).

Hydroxyaniliniums.—Hydroxyaniliniums are generally twitch-potentiating, anti-*d*-tubocurarine agents, exhibiting to various degree anti-ChE potencies; edrophonium, 3-OH phenylethyldimethylammonium, and its trimethyl and diethylmethyl analogues inhibited eel AChE at ID_{50} values ranging from 5×10^{-6} to 1.3×10^{-5} M (cf. 212). These compounds exhibited also, to a varying degree, a cholinomimetic, depolarizing action at the end plate (247) and finally, a blocking action. Immediate blockade may be due to initial depolarization by these compounds (250); a more sustained blockade may be caused by a curaremimetic action, which can be antagonized by tetanus and by depolarizing agents, but only partially by anti-ChE's (35).

At the frog neuromyal junction, some hydroxyaniliniums augmented but did not prolong the e.p.p. and ACh depolarization (173, 193); thus, these compounds resembled oxamides. Furthermore, while it was not possible to demonstrate AChE inhibition by edrophonium by the method described above (136), edrophonium caused augmentation of the response to ACh when both drugs were applied electrophoretically to the end plate region of the amphibian or tortoise muscle (183, 210, 226).

Presynaptic actions of hydroxyaniliniums, while not unique to these agents, were emphasized repeatedly by the Cornell group (247–250). As with other compounds studied similarly, hydroxyaniliniums produced, depending on the dose, repetitive antidromic firing either following conditioning orthodromic stimulation or in absence of nerve stimulation.

Several characteristics of these responses should be stressed. First, hydroxyaniliniums, while weak as twitch potentiators, effective intravenously in mg/kg dose ranges, and at 25-100 μg/kg when given intra-arterially, were found somewhat more potent in producing nerve terminal effects. Some of the oxamides proved some hundred times more potent as twitch potentiators, *d*-tubocurarine antagonists, or activators of the nerve terminal. Second, in the frog, in which hydroxyaniliniums were also capable of twitch-potentiating and anticurare action (173, 193, 204a), the nerve terminal phenomena were not readily evoked; in fact, only one of them could induce antidromic firing (173, 204). These two points may suggest that, first, the compounds in question may have acted via inhibiting AChE and affecting the nerve terminal indirectly by accumulating ACh. Anti-ChE mechanism of their neuromyal facilitation was suggested on various grounds by earlier investigators (cf. 167, 291); as already stated, many potent AChE inhibitors exhibited nerve terminal action. Second, the nerve terminal effect may not be related to facilitatory phenomena; facilitation is certainly not a required phenomenon as obvious from frog data. Finally, many of the results, such as the hydroxyaniliniums increasing the sensitivity of the end plate to ACh, are consistent with a postsynaptic mechanism of action, either dependent on inhibition of postsynaptic AChE or on a direct, sensitizing postsynaptic action (173). On the contrary, Riker [cf. for instance (246, 250)] associated facilitation by hydroxyaniliniums with their nerve terminal action. He and his associates pointed out the differences—mostly quantitative rather than qualitative—between nerve terminal actions of ACh and of hydroxyaniliniums, and they emphasized the sensitivity of the terminal to these drugs. Altogether, they concluded "that these drugs act on the motor nerve terminal directly and in a distinct way, not through ACh intermediation" and that "the motor nerve terminal is primarily the site of facilitatory drug action" (250). They postulated that this "distinct," presumably noncholinergic event was due to prolongation of a nerve terminal negative after potential (157); possibly, the proposed effect may reside at the node (159) rather than at the terminal. However, this effect was explained by the Canberra group as cholinomimetic in nature, a concept which is not accepted by the Cornell investigators.

Interaction between depolarizers and facilitators.—Classically, anti-ChE agents were shown to antagonize *d*-tubocurarine and to have no action on or increase the blocking effect of the depolarizers [for references cf. (166, 273, 291)], at least in the case of the fast (white) muscle. However, in 1951, DeBeer et al. (80) described certain amides as capable of antagonizing both *d*-tubocurarine and SCh or C_{10} blockade. Karczmar (165, 166) described similar action of the bisquaternary oxamides and demonstrated a novel phenomenon, "reversal," in the course of which the action of blocking doses of depolarizers upon twitch response to indirect stimulation was converted by methoxyambenonium into a pure excitatory, twitch-potentiating action.

It was pointed out (165, 166) that "reversal" could not be explained in

terms of anti-ChE action of MeAmb, and of depolarizing action of SCh or C_{10}, if only these mechanisms were involved.

In subsequent investigations (27, 34-36), "reversal" was produced by several other oxamides. It was particularly surprising that hydroxyaniliniums were also capable of "reversal" of SCh and C_{10} when given intravenously but not intra-arterially. In the frog, oxamides and some of the hydroxyaniliniums could also antagonize blockade by both *d*-tubocurarine and depolarizers and "reverse" the effect of the latter (172, 173). Moreover, these compounds could augment the e.p.p. obtained by blocking neuromyal transmission either by C_{10} or by *d*-tubocurarine; anti-ChE's, on the other hand, decreased rather than increased the e.p.p. produced by depolarizers.

When the facilitatory—anti-*d*-tubocurarine and twitch-potentiating—effects as well as curaremimetic and "reversal"-producing actions of oxamides and hydroxyaniliniums were investigated simultaneously, many of the compounds in question exhibited "reversal" within or near the range of the curaremimetic effect (34–36). While prostigmine per se did not "reverse" depolarizer blockade, it could do so when, prior to the administration of SCh or C_{10}, its own twitch potentiation was blocked by *d*-tubocurarine (35). Finally, MeAmb, while incapable of activating the nerve terminal when given alone, increased SCh-induced antidromic firing. Thus, the mechanism of "reversal" may depend on a balance of curaremimetic and facilitatory properties characterizing some oxamides and hydroxyaniliniums, and the excitatory component of "reversal" may be due to activation of the nerve terminal by the oxamide- or hydroxyanilinium-depolarizer combinations; in fact, two distinct sites could be involved as suggested by the differential effect of MeAmb at these sites (36). There are some exceptions to this generalization (35), and the "reversal" could also be obtained in the frog where the nerve terminal is relatively inactive. As suggested by Karczmar, the peculiar, sensitizing, postsynaptic action of oxamides and hydroxyaniliniums may be involved in their antagonism of depolarizers (169, 186).

Organophosphorus agents, oximes, and NaF.—Recent results with organophosphorus agents including those obtained with several insecticidal components such as EPN and malathion (170) confirmed the earlier impression that their effects at the neuromyal junction are similar to those of carbamate and oxamide drugs. The actions of organophosphorus agents included nerve terminal effects leading to antidromic repetitive firing (14, 15, 224, 245, 279). At doses many times those necessary to facilitate, such drugs as soman and sarin blocked neuromyal transmission. The tetanic response was particularly vulnerable (213, 214). For more complete review of neuromyal actions of organophosphrus agents see (100, 101, 152, 167, 291). Some question remains, however, whether or not their blocking actions were related to inhibition of AChE (214).

The earlier impression that neuromyal actions of organophosphorus agents can be readily reversed by quaternary oxime reactivators, i.e., agents capable of disengaging the phosphoryl moiety from the phosphorylated

enzyme, was confirmed. Moreover, phosphoryl-grouping may undergo a transformation leading to "aging" and inactivation of the enzyme [for review cf. (100, 101, 151, 167)]. Some organophosphorus agents may immediately phosphorylate the enzyme so that it cannot be reactivated at all, or with difficulties. For instance, soman blockade was only partially reversed by oximes (213).

A reactivator of a novel chemical type, NaF, was shown recently to reactivate the phosphorylated enzyme (147, 148) and to reverse completely the block of neuromyal transmission due to several organophosphorus agents in the frog and in the cat (2, 196, 198). It is of interest that NaF exhibits in the frog actions resembling those of oxamides (196, 197) and that its antagonism of organophosphorus block may in part be a result of its "sensitizing" action on the neuromyal junction. NaF also exhibited some curaremimetic effects.

Blocking agents: d-*tubocurarine, benzoquinonium, miscellaneous drugs.*—d-*Tubocurarine.*—*d*-Tubocurarine was reviewed recently (39, 273) as a nondepolarizing blocking agent, competitively antagonizing ACh at the neuromyal junction which first converts the spike into the e.p.p. and then reduces the latter. As a competitive blocker, *d*-tubocurarine is antagonized by agents which release ACh, such as TEA [(194); for further references cf. (273)], by depolarizers, and by anticholinesterases. Its blocking action on transmission is particularly pronounced during the tetanus; it is cumulative and frequency-dependent, although this latter effect is not as pronounced as in the case of drugs inhibiting the synthesis of ACh.

All these actions of *d*-tubocurarine are consistent with its competitive blockade of the postsynaptic membrane. However, Riker and his associates (cf. 246,250) believe that the nerve terminal is the major site involved; the antidromic firing in the motor axon due to hydroxyaniliniums and related compounds was readily antagonized by small doses of *d*-tubocurarine (246–249, 290). Moreover, the lowering of the nerve terminal threshold by conditioning volleys, by ACh, or by ACh-prostigmine combinations was readily antagonized by *d*-tubocurarine (157, 159). More recently, Standaert (261, 263) abolished with small doses of *d*-tubocurarine the repetitive firing originating in the nerve terminal following tetanic stimulation of the motor nerve. He suggested that *d*-tubocurarine suppressed "the after-potentials of the nonmyelinated terminal, thereby preventing the development of the generator potential," and he explained neuromyal transmission blockade by this agent as a result of the drug-induced decay of the terminal action potential. It should be emphasized that, as in the case of facilitators, the action of the drug upon the nerve terminal seemed, dose-wise, dissociated from its effect on neuromyal transmission; *d*-tubocurarine was "25 times as potent in abolishing post-tetanic firing as in blocking transmission" (263). Standaert (263), in fact, suggested that additive pre- and postjunctional actions may be involved in *d*-tubocurarine-induced failure, but he preferred the prejunctional site as the major locus of neuromyal blocking action of *d*-tubocurarine.

Generally, Riker & Standaert (250, 265) feel that any action of a drug that seems to inculpate the postsynaptic membrane may be referrable to the prejunctional site, since any drug bathing the junctional region cannot avoid acting on both structures, and that the sensitivity of the terminal is indicative of its primary role even if this sensitivity is demonstrable only at doses a small fraction of those that affect transmission. Yet, primary postsynaptic sites of action of both *d*-tubocurarine and of facilitators cannot be denied when these agents decrease and increase, respectively, the amplitude of the miniature end plate potentials, as known since the work of Fatt & Katz (109). Recently, Katz & Miledi (179), by developing a technique which enabled them to stimulate locally the nerve terminal and to initiate antidromic responses, recorded simultaneously with the latter the e.p.p.'s and demonstrated differential blockade of the postsynaptic response by *d*-tubocurarine; the antidromic response could still follow even a 200/sec stimulus. Using the related technique of focal recording of the terminal spike (cf. above), Katz & Miledi (179) concluded that *d*-tubocurarine has "powerful postsynaptic blocking action" . . . and . . . "comparatively small or absent presynaptic effect."

Difficulties of a less basic nature resulted from the recent work of Hubbard et al. (159). As already stated, *d*-tubocurarine blocked ACh-induced threshold decrease of the nerve terminal when applied electrophoretically during the period of threshold decrement; yet, used in the absence of ACh, *d*-tubocurarine decreased the threshold. In fact, this effect of *d*-tubocurarine amounted to "profound depolarizing action." Moreover, in curarized preparations, ACh produced very readily the decrease in threshold. Finally, following a conditioning stimulus, *d*-tubocurarine shortened the duration of the period of increased excitability of the terminal but it also shortened the refractory period (158, 159). In view of these paradoxical findings, nerve terminal antagonism between *d*-tubocurarine and ACh cannot be considered as proven, and the action of *d*-tubocurarine itself appears to be composed of facilitatory and blocking components.

Benzoquinonium.—This interesting agent is a nondepolarizing blocker used at one time clinically as a muscle relaxant; it differs from both depolarizing and competitive blockers in being a much more potent anti-ChE, *in vitro* one third as potent as prostigmine (167). It was shown by Hoppe (153) that its block was poorly antagonized by anti-ChE agents; another difference between benzoquinonium and competitive, nondepolarizing curaremimetics. It was thought that this lack of antagonism between benzoquinonium and anti-ChE may be caused by its own anti-ChE action (153) and by accumulation of ACh. That this was not so was demonstrated by Karczmar (166, 171). Subsequently, Blaber & Bowman (31, 32) stressed that benzoquinonium exhibits potent blocking actions at the nerve terminal which cannot be readily antagonized by anti-ChE's. In fact, TEA, a compound which increases the release of ACh, differed from all other facilitators in antagonizing the blocking effects of benzoquinonium (32, 204). On the other hand, Karczmar (166)

stressed potent postsynaptic anticholinergic actions of this compound (216, 288) and considered this action as the reason for the poor antagonism between benzoquinonium and the anticholinesterases.

Miscellaneous drugs.—Aryl tropine esters (119–121, 276) are acetates in which acetate hydrogens were substituted by one or two phenyls or by halogen-, methyl-, or methoxy-substituted phenyls. On the whole, these compounds showed weak anti-ChE actions, blocking effect at the node, and biphasic muscle effect. Most compounds inhibited both directly and indirectly elicited muscle twitch in the rat nerve-diaphragm preparation at comparable concentrations. However, tropine *p*-chlorophenylacetate blocked differentially (at 9×10^{-4} *M*) the indirect twitch response and did not produce initial facilitation (276). Certain suggestions were made on the basis of these data with regard to stereospecificity of "neuromuscular receptors" (120, 121). However, multiplicity of actions of the compounds in question, high concentrations necessary, and the fact that the block was not analyzed as to its site and mechanism preclude any conclusions.

Recently, the ganglionic blockers mecamylamine, dimecamine, pempidine, and their quaternary metho salts were shown to exhibit typical curaremimetic blocking action, antagonizable by prostigmine, calcium, and by tetanus (37). They showed also weak anti-AChE actions (72). In the case of the tertiary amines, the active form was probably an extracellularly acting cation. Quaternary compounds, but not the tertiaries, exhibited weak depolarizing activity (72, 133).

Among newer curaremimetics were bisquaternaries of piperidyl-propanol esters of α-truxillic acid (185). Several conventional antihistamines were shown recently to exhibit weak neuromyal blocking actions resistant to prostigmine and synergistic with those of *d*-tubocurarine (65).

Holothurin A, a mixture of steroid glycosides and related Quillaja saponin, produced, in relatively high concentrations, contracture and blockade of both directly and indirectly induced twitch. Physostigmine protected differentially the response to indirect stimulation (275). Inasmuch as these substances produced pathological changes in the axoplasm in and around Ranvier's node, jointly with excitability and conduction changes, as well as other cell-damaging actions (122, 274), the sites of the neuromyal junction actions of these drugs are obscure.

Catecholamines.—The effects of catecholamines upon the neuromyal junction and the muscle tissue have been investigated since the observation that epinephrine increases ACh contracture of the isolated kitten diaphragm and that it exerts a biphasic effect on the potentiation by prostigmine of the twitch response to indirect stimulation (58, 77; cf. 41, 62, 291). The studies of the last thirty years disclosed several actions of catecholamines, including hyperpolarizing and recruitment action on muscle fibers; sensitizing effect on the denervated muscle rendered refractory by ACh; biphasic actions on *d*-tubocurarine blockade, as well as presynaptic actions including acceleration of discharge rate of m.e.p.p.'s (200; cf. 291).

Neuromyal or muscular effects of the catecholamines in intact animals

were frequently thought to be due to the vascular effects of these drugs; in fact, sometimes no effects could be demonstrated in isolated preparations. In a recent investigation, however, Bowman & Raper (50) demonstrated that a number of actions of three catecholamines upon the neuromyal effect of *d*-tubocurarine, C_{10}, and anti-ChE's were independent of blood pressure and blood flow.

It can be accepted today that norepinephrine and epinephrine increase anti-ChE-produced twitch potentiation, and repetitive firing (32, 49). They exert biphasic actions on *d*-tubocurarine block, the synergistic effect being generally delayed. Isoproterenol, almost exclusively a β-receptor stimulant, exerted only the inhibitory actions, exhibiting no anti-*d*-tubocurarine effect. Norepinephrine and epinephrine, but not isoproterenol, augmented potentiation of the indirectly evoked muscle twitch due to prostigmine and C_{10}. Again biphasic actions could be observed as all three catecholamines given prior to depolarizers reduced their blocking potency. The partial antagonism of depolarizers by the catecholamines may be analogous to a curaremimetic action since the amines also depressed the twitch response to intra-arterially administered acetylcholine. It may also be related to the hyperpolarizing effect of catecholamines on the muscle fiber, exhibited by all the three compounds (49, 50), particularly as they reduced depolarization of the end plate due to SCh or C_{10}. The α- and β-receptor blockers inhibited, respectively, the facilitatory effects of the catecholamines.

Bowman & Raper (50) concluded that facilitatory actions of epinephrine and norepinephrine are due to nerve terminal effects of these substances. The facilitatory effects were obtained in spite of blocking actions on ACh twitch whether obtained by intra-arterial or by electrophoretic application (200), while epinephrine increased the e.p.p. produced by motor nerve stimulation and relieved presynaptic failure of transmission which occurred following repeated nerve stimulation (163, 200). This effect was similar to that produced by hyperpolarization of the nerve ending (201) which augmented release of the transmitter [for additional references cf. (211, 272)]. Indeed, epinephrine increased the discharge rate of m.e.p.p.'s without affecting their amplitude (200). Finally, catecholamines augmented fasciculations and repetitive firing produced by depolarizers [(40, 50) cf. above)]. The nerve terminal effects were thus associated by Bowman & Raper (50) with α-receptor activity of catecholamines but inhibitory actions of catecholamines on muscle membrane, which may explain their inhibitory effects on transmission, were related to β-activity. Several monoamine oxidase inhibitors also produced neuromyal blockade and antagonized response to ACh (5); however, Condouris (71) reported conduction blockade by these agents, and their nerve terminal blocking action may also be involved (cf. also 5). It should be added that SCh-evoked contracture of the (slow) extraocular muscle, which was blocked by *d*-tubocurarine but not by atropine, was antagonized by a β-receptor blocking agent as well as by epinephrine (92).

It is felt by this reviewer that facilitatory, α-receptor-dependent action of catecholamines upon nerve terminals cannot as yet be fully accepted. Indeed,

while blocking actions of catecholamines upon end plate responses to ACh were stressed by Bowman & Raper (50), facilitatory effects were also reported [for references cf. (291)]. Moreover, facilitatory effects of epinephrine were demonstrated in the denervated diaphragm (222), although the latter may not be comparable to the fast (white) muscles employed by Bowman & Raper (50). Finally, while postsynaptic blocking actions of catecholamines, possibly analogous to those described above for the neuromyal junction, were also shown for other synapses such as those in ganglia, facilitatory postjunctional effects were also described (283).

Hemicholiniums and choline analogues.— The hemicholiniums synthesized by Schueler (255) have been extensively studied since his suggestion that they may depress the neuromyal junction by depressing ACh synthesis (cf. 256). Their delayed and frequency-dependent (107) blocking action, antagonizable by choline and potentiated by a choline-deficient diet (6), was reviewed recently (167, 274). The most potent member of the group is 2,2′-dimethylaminoethanol-4,4′-biacetophenone (HC-3).

More recently, other compounds were shown to exhibit actions similar to those of HC-3. While only general pharmacological characteristics were described in the case of 3,6-bis(3-diethylaminopropoxy)pyridazine bismethiodide [WIN 4981; (130, 131)] a more extensive investigation was carried out by Bowman and his associates with the triethyl-analogue of choline, N-triethylaminoethanol [triethyl 2-hydroxyethylammonium, triethylcholine (TEC)], and bisquaternary choline analogues related to decamethonium.

In mammals and chicks, TEC caused progressive muscular weakening of conscious animals and frequency-dependent transmission block, antagonizable by choline but not by anti-ChE's. It had only weak action on end plate sensitivity, and no effect upon nerve conduction or muscle contractility (44, 46, 47). Roberts (251) blocked, with triethylcholine, the response of isolated frog muscle to electrophoretically applied ACh but did not demonstrate a block of response to indirect stimulation. Indeed, in the frog, triethylcholine may be predominantly curaremimetic and may exhibit its presynaptic action only in mammals and chicks in which it resembled HC-3 in all its actions (44).

Several other analogues of choline besides triethylcholine showed similar presynaptic action (48). These were dimethyl-N-propyl, dimethyl-1-propyl choline, diethylmethyl choline, and triethyl analogues in which the two carbon chain of choline was slightly modified. Compounds with smaller substitutions on the quaternary nitrogen depolarized, while those with larger substituents exhibited curaremimetic, postsynaptic action.

The direct proof that triethylcholine acted presynaptically by changing ACh output was also provided by the Brunswick Square group (42, 60). In fact, decrease in ACh liberation paralleled blockade of transmission, and choline restored both ACh output and transmission. Moreover, triethylcholine depressed ACh synthesis by "mitochondrial" homogenate fraction and by fresh frozen section of rabbit brain and this effect was also antagonized by choline (59, 60).

However, the inhibition of synthesis could be achieved only with relatively

high concentrations of TEC; in fact, direct rather than competitive inhibition with choline may be possible under these circumstances (149). Another possibility for presynaptic action of TEC raised by Bowman (47, 48) was that TEC may be acetylated by choline acetylase in place of choline and act as a "false transmitter," less effective than acetylcholine. Indeed, choline acetylase is capable of acetylating a number of choline analogues (79, 149). A mechanism involving competition between choline and TEC for access to the enzyme, resembling therefore that suggested for hemicholiniums (129, 219), explains most readily the action of triethylcholine.

Neuromyally active agents usually exhibit more than one type of action, and hemicholiniums (167) and particularly choline analogues are not exceptions. Weak anti-ChE and weak postsynaptic curaremimetic actions of TEC as well as depolarizing actions of some TEC analogues have already been mentioned. An interesting effect of TEC is its slight initial potentiation of indirect muscle twitch response (49), accompanied by repetitive firing occurring in a limited number of muscle motor units (44). This action may be coupled with the weak anticurare effect which can be demonstrated for TEC under certain conditions (44, 251). By carefully comparing TEC with tetraethylammonium (TEA) Roberts (251) and the Brunswick Square group suggested that, like TEA (cf. 173, 194, 266), TEC may cause a release of preformed ACh from the nerve terminal; indeed, initial increase of ACh output with TEC could be demonstrated (45).

A series of dicholine analogues of decamethonium, studied by Bowman and his associates (43, 45), resembled the series of monocholine analogues. Among both series of compounds were drugs capable of depolarizing curaremimetic and presynaptic (inhibiting or increasing ACh output) actions. Many compounds exhibited a dual block which resembled HC-3 blockade, being frequency-dependent and antagonized by choline. The decamethylene derivative iuhibited the output and, *in vitro*, the synthesis of ACh; both inhibitions were reversed by choline.

Bowman (45) reminds us that many, if not all quaternaries may, whatever their other actions, either decrease or exhibit biphasic actions on ACh output. Scattered reports in the literature indicate that C_6 (45), *d*-tubocurarine (16, 218), and a quaternary agent of an entirely different type, bretylium, may block ACh output, while certain ganglionic blocking agents may initially enhance release of ACh from the nerve terminal (37). Bowman further suggests that the well-known change in type of block occurring after prolonged administration of depolarizers to animals or human beings may not be due, as hitherto supposed, to conversion from depolarization to competitive blockade, but to delayed effect on ACh output and synthesis.

Myasthenia Gravis

This chronic disease, characterized by weakness and abnormal fatiguability of the skeletal muscle, with puzzling temporary remissions, is of great pharmacological interest. The fatigued myasthenic, neuromyal junction can

be activated by anti-ChE's and is very sensitive to *d*-tubocurarine, but resistant to depolarizers (cf. 167). Excessive AChE activity cannot account for the weakness (20); in fact, it may be subnormal in the final stages of the disease, when the end plates appear pathologic (cf. below).

Desmedt (cf. 88) and more recently the Scandinavians (76) proposed as the underlying mechanism a deficiency in the release and synthesis of ACh. On the other hand, Churchill-Davidson & Richardson (67) and Grob and his associates (141) suggested that myasthenia is caused by changes in the postsynaptic sensitivity or in the quality of postsynaptic response. The first hypothesis was reviewed recently by Thesleff & Quastel (273). In brief, postsynaptic sensitivity of the isolated intercostal muscle of a myasthenic was unchanged while the amplitude of m.e.p.p.'s and the calculated quantal size were diminished (102, 272). As a result of the reduced amount of ACh released by each nerve impulse at the myasthenic junction, the e.p.p.'s which decrease in tetanic train in the myasthenic as well as in the normal muscle, become readily subthreshold in the myasthenic. Thesleff (272) stressed that the number of ACh quanta per m.e.p.p., but not ACh content of each quantum, is decreased by lack of calcium, high magnesium concentration, neomycin, botulinum toxin, HC-3, or TEC. He concluded that ACh release rather than synthesis is affected in myasthenia, and that a fixed smaller "myasthenic quantum" arises, presumably due to changes in the structure of attachment sites in synaptic vesicles or in canaliculi (104).

On the other hand, Desmedt (89) stressed the similarities between HC-3 action and the behavior of myasthenic muscle. He compared post-tetanic potentiation of transmission in myasthenics and in curarized as well as HC-3-treated animals. In both myasthenics and in HC-3-treated animals, the post-tetanic facilitation was short lived; a block more intense than that observed prior to tetanus followed. On the other hand, prolonged post-tetanic potentiation was observed in curarized muscles, and the subsequent block was identical with that observed prior to tetanus. Desmedt (89) explained these results as due to the fact that in both myasthenic and HC-3-treated muscle the postactivation facilitation, caused by an increase of the presynaptic spike and by improved ACh release, is limited because of a subnormal store of available ACh, and leads rapidly to "postactivation" exhaustion. He argued further that on the basis of Thesleff's hypothesis of reduced, fixed-size "myasthenic quantum," the postactivation cycle should resemble that which is obtained in curarized rather than in HC-3-treated animals.

A particular problem arises in view of C_{10} resistance of myasthenics. Desmedt (89) pointed out that this resistance was manifest in uninvolved muscles (cf., however, 138, 140), and that it was not specific for myasthenia. Thesleff (272, 273) felt that in conditions of maximal (normal) transmission, depolarizers may show primarily their "desensitizing" action, while their depolarizing action, which produces the decrease of the threshold and facilitation, predominates in the myasthenic muscle. To this reviewer, change of action of depolarizers, including ACh, in the myasthenic, remains baffling.

Besides stressing changed responsivity in myasthenia to SCh and C_{10}, Grob (140) also emphasized a change in response to ACh. Intra-arterially administered ACh caused short-lasting depression, somewhat less pronounced in myasthenic than in normal muscle, followed by a secondary depression which was particularly pronounced in myasthenia. ACh and anti-ChE's antagonized the secondary depression in myasthenics and increased it in normal muscle. Patients not responding clinically to anti-ChE's exhibited no initial depression (140); additional types of responses to ACh and anti-ChE's led Grob to postulate several types of block, all depending on changed postsynaptic receptor reactivity.

It should be pointed out that cholinergic crises, manifested by either refractoriness to anti-ChE treatment or muscle weakness following an overdose of the anti-ChE agent, may be explained by desensitization and tolerance processes which presumably can occur postsynaptically.

Since the early work of Coërs (68), Russell (254), and Coërs & Desmedt (68a), pathology of the myasthenic neuromyal junction has frequently been described. Many changes have been reported, such as elongated or shrunken end plates (296) and myopathy-resembling changes induced by inflammation and denervation (54, 112, 254). Recently, the myasthenic syndrome was reported for the first time in dogs (142, 237); one case was thoroughly examined pharmacologically, neurophysiologically, and anatomically (301). Again, changes in nerve endings, in the end plate, and, particularly, increase in the size of the synaptic cleft were described. Occasionally, changes of this type could be described only for the clinically affected but not for the normal muscle of the myasthenic patient. Also, specific pathology could be found in muscles not responding to anti-ChE therapy. Some changes were ascribed to compensatory processes leading to elongation of terminal aborizations (197). The changes described appear consistent with either a presynaptic or postsynaptic myasthenic mechanism. The picture presented is that of denervation and until end plates began to degenerate, the synaptic vesicles were normal in appearance and number; subsequently, their number appeared reduced (197).

These inflammatory and degenerative changes are consistent with the recently stressed autoimmune aspects of myasthenia (cf. 232, 268, 295). In brief, since the demonstration of Strauss et al. (267) of the presence of a factor in myasthenic sera reacting specifically with the skeletal muscle proteins, it seems generally accepted that this factor has the characteristics of an antibody, muscle-specific and γ-globulin in character, present perhaps in a minority of myasthenic sera. The type of antigen, either autologous or heterologous, has been established. Finally, thymus, believed to be the source of lymphoid cells participating in autoimmune processes, is occasionally hyperactive in myasthenia (cf. 63), and some myasthenics benefit from thymectomy or thymic irradiation (280). However, particularly because of an appreciable frequency of myasthenic sera in which no muscle-specific antibodies were found, and for other reasons (232, 296), circulating

autoantibody probably does not play a primary part in the disease, although it may appear in the serum as the result of a basic lesion of the junction.

Therapy of myasthenia.—Long-acting anti-ChE's continue to constitute the major therapy of myasthenia. While quaternary and bisquaternary carbamates are most often used, quaternary organophosphorus derivatives, as for instance echothiophate [phospholine, cf. (138)], have been employed on an experimental basis. Short-acting AChE inhibitors, such as edrophonium (Tensilon), are useful in diagnosis as well as in the evaluation of whether the treatment is optimal, suboptimal, or excessive. Its use was recently reviewed by Osserman & Jenkins (238).

Several recent reports attest to the success of anti-ChE therapy in myasthenia (138); moreover, a good agreement was established between the relative therapeutic efficacy of prostigmine and other clinically useful drugs, and their anti-AChE (but not anti-BuChE) potency, as well as, generally, between the clinical improvement with effective doses of these agents and the inhibition of red blood cell ChE which they produced (116). Ambenonium proved to be an exception, possibly because of its preferential localization at the junction. In view of these data, it is somewhat difficult to understand the statement in a recent review (273) that while "superficially, it might appear that drugs" such as anticholinesterases "should be effective . . . their efficacy is far less than would be expected . . . Anticholinesterase drugs depress postsynaptic sensitivity . . . , and this hardly desirable." It seems to this reviewer that Thesleff & Quastel (273) have an exaggerated opinion of the depression of postsynaptic activity by anti-ChE's (cf. 167) and unduly deprecate their ability to antagonize *d*-tubocurarine.

Denervated Muscle and Its Drug Sensitivity

Expansion of sensitivity of denervated muscle to ACh was reviewed recently (273). The sensitization was explained as a result of the increase, following denervation, of the area which can be depolarized by ACh, and of increased membrane resistance.

When frog muscle was divided *in situ*, the nerve-free segments showed increased sensitivity; sensitivity of the neural segment also increased (178). Thus, development of denervation sensitivity does not require the presence of the "active end plate center," and myotomy may oppose neural influence which normally restricts (225) sensitivity to ACh to the junctional area. It is of great interest that in the denervated muscle of young rats new foci of high AChE activity were demostrated (215); this effect occurred besides the well-known decrease of AChE activity at the end plates (cf. 191), characteristic for the denervated muscle of both adult and young animals (303).

Effects of many agents, besides ACh and ions, are increased by denervation, and *d*-tubocurarine depolarized the denervated muscle [for review cf. (167, 273)]. Catecholamines raised the resting potential of denervated muscle, but also increased the frequency of spontaneous electric activity and muscle tension (49, 51). The capacity of organophosphorus anti-ChE's to produce

twitch in denervated muscle is controversial; large doses of DFP, sarin, and soman seemed capable of such an effect (214).

It is well known that botulinus toxin produces sensitization similar to that induced by denervation (cf. 273). Similar sensitization as well as electromyographic changes, including fibrillation, were produced in rabbits by the tetanus toxin and by means of vitamin E deprivation (123, 235, 243, 244).

Conclusions

The complexity of physiological and drug control of skeletal neuromyal transmission should be evident from this review. Presumably, the structuring of this transmission is aimed at preserving efficiency, whether in terms of servo and negative feedback systems, or multiplying and positive feedback arrangements. Thus, tetanus leads to hyperpolarization of the terminal and, consequently, to increased potential and secretion of ACh. Additional factors with regard to post-tetanic phenomena are the mobilization of the transmitter and equilibria between various transmitter compartments within the terminal. The release of ACh may constitute its own positive feedback system reacting upon the terminal; this hypothesis is one of the many controversies in this area (cf. 159, 191). On the contrary, postsynaptic action of the transmitter may lead to desensitization and to adaptation processes (186, 230); negative feedback arrangement. Moreover, ionic factors are effective at all these levels.

The same characteristics of multifactorial design apply to drug action, and it would be a major oversimplification to speak of any drug or group of drugs in terms of a single site or mechanism of action. Among major sites and mechanisms available, the following can be listed jointly with their appropriate subdivisions: (*a*) The nerve terminal, within which possibly two sites and mechanisms may be distinguished (36); release of the transmitter as well as agonist and antagonist actions are possible at this site. (*b*) The first node of Ranvier in the motor axon. (*c*) The mechanisms and loci involved in ACh synthesis. (*d*) Anti-ChE actions which theoretically can involve both the pre- and postsynaptic AChE, and possibly BuChE of the glia. (*e*) Agonist and antagonist actions at the postsynaptic membrane. The types of action at these sites include cholinomimetic depolarization and facilitation, sensitization (172, 186), desensitization, and anticholinergic or curaremimetic action. A good example of drugs with multiple actions are the cholinomimetic agents such as succinylcholine, which acts on nerve terminals, has a postsynaptic effect including both depolarization and desensitization, exhibits anti-ChE action, and, finally, a possible hemicholinium-like effect (45). Similar types of action are exhibited by certain hydroxylpolymethylene bisquarternaries (43). Still another example is that of oxamides which have potent anti-ChE as well as presynaptic and postsynaptic actions, the latter consisting, depending on dose, of sensitizing, anti-ChE and curaremimetic action.

To this reviewer it appears that the common denominator among these drugs is their cholinomimetic nature. An anticholinesterase must be stereo-

chemically related in some degree to cholinomimetic drugs and to the cholinergic receptor. This predictable fact led to the hypothesis relating the ChE molecule to the cholinergic receptor, which, as stated above, appears untenable. Similarly, pharmacological analysis suggests that the site concerned with ACh synthesis and with hemicholinium-like action is also cholinoceptive (36, 43). Moreover, actions at the nerve terminal, which involve antagonisms between *d*-tubocurarine on the one hand and anti-ChE and cholinomimetic agents on the other (36, 159), seem to deal with a cholinoceptive site.

It appears, however, that, for any particular drug, not all the actions may be equally important. Location, concentration, or activity of AChE may earmark the site significance, as for instance in the case of its presynaptic localization at the autonomic ganglia (191, 283). While at the neuromyal junction, AChE is present at both the pre- and postsynaptic site, the infolding at the latter site seems to render it functionally more important. Furthermore, species-wise, the presynaptic site appears only facultative rather than obligatory (172).

It should also be remembered that many agents seem unable to affect transmission at doses exhibiting presynaptic actions, although there are exceptions to this rule. Altogether, it seems that with many curaremimetic, cholinomimetic, and anticholinesterase agents, the postsynaptic site—itself a complex entity—continues to be of major functional importance, subject possibly to modulatory influences arising presynaptically.

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CONTENTS

PHARMACOLOGY IN OLD AND MODERN MEDICINE, *C. Heymans* . . . 1
BIOCHEMICAL MECHANISMS OF DRUG ACTION, *Curt C. Porter and Clement A. Stone* . . . 15
MECHANISMS OF DRUG ABSORPTION AND EXCRETION, *I. M. Weiner* . 39
METABOLIC FATE OF DRUGS: BARBITURATES AND CLOSELY RELATED COMPOUNDS, *Milton T. Bush and Elaine Sanders* . . . 57
PARASITE CHEMOTHERAPY, *Paul E. Thompson* . . . 77
CANCER CHEMOTHERAPY WITH PURINE AND PYRIMIDINE ANALOGUES, *Charles Heidelberger* . . . 101
ELECTROLYTES AND EXCITABLE TISSUES, *Juan A. Izquierdo and Iván Izquierdo* . . . 125
CARDIOVASCULAR PHARMACOLOGY, *Theodore C. West and Noboru Toda* 145
RENAL PHARMACOLOGY, *Gilbert H. Mudge* . . . 163
THE AUTONOMIC NERVOUS SYSTEM, *C. B. Ferry* . . . 185
HISTOCHEMISTRY OF NERVOUS TISSUES: CATECHOLAMINES AND CHOLINESTERASES, *Olavi Eränkö* . . . 203
PHARMACOLOGY OF THE CENTRAL CHOLINERGIC SYNAPSES, *Z. Votava* 223
NEUROMUSCULAR PHARMACOLOGY, *Alexander G. Karczmar* . . . 241
NARCOTIC AND NARCOTIC ANTAGONIST ANALGESICS, *H. F. Fraser and L. S. Harris* . . . 277
PSYCHOTOMIMETIC AGENTS, *Sidney Cohen* . . . 301
PESTICIDES, *Alastair C. Frazer* . . . 319
AFLATOXINS, *Regina Schoental* . . . 343
TOXICOLOGICAL SAFETY OF IRRADIATED FOODS, *H. F. Kraybill and L. A. Whitehair* . . . 357
ANTIFERTILITY AGENTS, *Edward T. Tyler* . . . 381
WHY DO THIAZIDE DIURETICS LOWER BLOOD PRESSURE IN ESSENTIAL HYPERTENSION?, *Louis Tobian* . . . 399
REVIEW OF REVIEWS, *Chauncey D. Leake* . . . 409
INDEXES
AUTHOR INDEX . . . 419
SUBJECT INDEX . . . 444
CUMULATIVE INDEX OF CONTRIBUTING AUTHORS, VOLUMES 3 TO 7 461
CUMULATIVE INDEX OF CHAPTER TITLES, VOLUMES 3 TO 7 . . . 462