PHARMACOLOGY OF MOTOR NERVE TERMINALS[1,2]

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INTRODUCTION

The importance of motor nerve terminals as sites of drug action has become increasingly apparent in the last decade. This has involved drugs classically associated with neuromuscular pharmacology as well as drugs acting on a broad range of excitable cells. Therefore, this review will emphasize the principal approaches that have led to this, as well as the pharmacologic facts which have emerged.

Since pharmacologic dissection has played so prominent a part in the anlysis of neuromuscular function and is presently no less implicated, it is essential to stress the need for thorough pharmacologic evaluation of drugs that are to be used for physiologic studies. This seemingly evident point is too often overlooked in concern with mechanism of drug action. The latter consideration, though undoubtedly a most appealing aspect, does not by itself constitute an adequate pharmacologic definition, since a mechanism inconsequential to a major drug effect may be misconstrued. Ideally, one should know the sites at which the drug acts, the quantitative definition of each responding system as a function of dose, the way or ways in which the drug reaches and leaves the sites, and the fate of the drug. With selectivity clearly defined, relevant mechanisms of drug action and biological function are more likely to be realized.

Even circumscribing drug action to the neuromuscular junction, there are, grossly speaking, two possible sites of action: nerve and muscle. Subdividing motor nerve into unmyelinated terminal and its contiguous myeli-

[1] The survey of literature pertaining to this review was concluded in July 1968.

[2] The following abbreviations are used in this review: ACh (acetylcholine), ChE (cholinesterase), C6 (hexamethonium), C10 (decamethonium), EPP (end-plate potential), HC-3 (hemicholinium), MEPP (miniature end-plate potential) 3-OH DEMA (3-hydroxydiethylmethylammonium), 3-OH PTEA (3-hydroxyphenyltriethylammonium), 3-OH PTMA (3-hydroxyphenyltrimethylammonium), PDR (post-drug repetition), PTEA (phenyltriethylammonium), PTMA (phenyltrimethylammonium), PTP (post-tetanic potentiation), PTR (post-tetanic repetition), SCh (succinylcholine), TEA (tetraethylammonium), TMA (tetramethylammonium).

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nated axon with Ranvier nodes further complicates this; the muscle and the specialized post-junctional area add to the difficulty. Nevertheless the relative involvement of these components in drug action must first be identified. Assuming that site of action can be distinguished, dose-response analysis should indicate any differential susceptibility. In turn, drugs recognized as selective should reveal the functional significance of a component site, since drugs serve only to suppress or enhance function. Therefore, this review emphasizes for discussion those facts that either explicitly describe the pharmacology of motor nerve terminals or which are, in the reviewers' opinion, clearly implicated. From these facts it has been possible, in some instances, to reach new conclusions regarding drug action on the pre-junctional structure. Hopefully, these considerations have led to an integrated and provocative account of the pharmacology of motor nerve terminals, as this is currently known.

Detection of Drug Action on Motor Nerve Terminals by Means of Pre-Junctional Monitoring

Background.—When Masland & Wigton (142) and Feng & Li (69, 70) described a method of antidromic monitoring to detect the activity of motor nerve terminals in the cat, their reports represented the first records of the selective action of certain drugs on these structures. This lead was not pursued until 16 years later when the technique was recognized as a means of resolving the pharmacology of facilitatory drugs (171).

The principle of the method is simply that when a physostigmine-like drug is administered, a post stimulus repetition arises from motor nerve terminals and can be detected by recording from appropriate ventral root filaments. This signals that the drug has acted on the nerve endings. Feng & Li (69) likened this to a post-tetanic potentiation (PTP), wherein twitch augmentation is due to repetitive discharges arising from motor nerve terminals that are invaded only by single impulses. However, this crucial finding was overlooked for an ancient and common reason: it did not fit with the general belief. At this time, the cholinergic theory of neuromuscular transmission had been virtually established. The notion that physostigmine, or a like drug, might act directly and uniquely on motor nerve terminals was antithetical since its assigned role was to inhibit post-junctional cholinesterase (ChE) and so allow neurally liberated acetylcholine (ACh) to cumulate.

A direct facilitatory drug action on neuromuscular function was described in 1946, when Riker & Wescoe (175) demonstrated that neostigmine, a quaternary ammonium ion, can directly depolarize neuromuscular junctions causing excitation independently from ChE inhibition. They sought to relate the characteristic twitch potentiation to this action, but further study by others (86, 148, 188) made it clear that there is no correlation. Nevertheless, Riker, Wescoe & Brothers (177) and Riker & Wescoe (176) were impressed that some direct action of neostigmine might be re-

sponsible for twitch potentiation. To attenuate the anti-ChE potency of neostigmine, the carbamate moiety of the molecule was removed and the 3-hydroxyphenyltrimethylammonium ion (3-OH PTMA) studied. This proved to have strong twitch potentiating and anti-curare activities despite the fact that its anti-ChE potency was approximately 1/100th that of neostigmine (176, 224). However, as with neostigmine, a neuromuscular blocking action remained prominent. Study of related hydroxyanilinium ions (171) showed that 3-hydroxyphenyltriethylammonium ion (3-OH PTEA) produces a striking twitch potentiation and a good anti-curare effect without, however, releasing excitatory effects that have been traditionally ascribed to ACh. Contraction, fasciculations, and neuromuscular block do not occur. As an anti-ChE, 3-OH PTEA is equivalent to edrophonium and 3-OH PTMA.

3-OH PTEA proved to be an important discovery, because it presented a seeming contradiction of what ChE inhibition should do. Obviously, excitatory effects with neostigmine-like drugs are a methonium ion function and are attributable to the direct effects of the particular drug.

Because of the impressive synchrony of the 3-OH PTEA stimulus-bound repetition in a population of muscle fibers responding to indirect stimulation, the motor nerve terminals seemed a likely site of its action. Moreover, if this proved to be the case for 3-OH PTEA, it very likely would apply to the action of other facilitatory drugs.

Antidromic monitoring revealed that both 3-OH PTEA and 3-OH PTMA cause motor nerve terminals to react repetitively to single impulse invasion (171). This is referred to as post-drug repetition (PDR). The later development of the "matched pair" technique (see below) proved that the terminals are in fact the primary site of these facilitatory drug actions, and that the repetition which appears in the muscle is a consequence of the repetitive discharge of the terminals. Thus, the fact that these drugs evoke twitch potentiation in indirectly stimulated mammalian muscle furnishes presumptive evidence of action on the terminals. For a thorough discussion of twitch potentiation as a signal of facilitatory drug action and the several examples of dissociation of this action from ChE inhibition, the reader is referred to the review by Werner & Kuperman (223).

An accurate and concise account of the pharmacologic findings and thought that have led to an appreciation of the presynaptic site of action of physostigmine-like drugs appears in a recent monograph by Triggle (210).

Motor nerve terminal as a site of facilitatory drug action.—The value of the antidromic technique as a means of detecting drug action on motor nerve terminals has since been appreciated by others who have employed the method in the cat (13, 14, 16, 40, 41, 112, 128, 156, 157, 158, 165, 169, 171, 173, 191–93, 195, 197, 211, 219, 220–22). These investigations encompass work with motor nerves to gastrocnemius, soleus, and tibialis muscles. Still others (4, 35–37, 166, 214) have adapted the *in vitro* preparation of rat phrenic nerve-diaphragm for the purpose.

A primary goal in the Cornell laboratory has been to develop and refine the technique not only for quantitative purposes, but also to ascertain surely whether motor nerve terminals exposed to facilitatory drugs are responsible for the well-known repetitive response of the muscle, and, in turn, twitch potentiation. The important contributions of Werner (220), and Standaert (191, 193) accomplished this in the development of the "matched pair" technique. From Standaert's work it became certain that the repetitive discharges recorded at ventral root fibers originate from motor nerve terminals, and that the muscle response is obligatory.

Because of its significance, the "matched pair" *in situ* method merits brief description. It is shown schematically in Figure 1. The soleus muscle in the cat is exposed. A ventral root, usually L7, is disconnected at its origin. Careful teasing enables location of a fine filament containing a single motor axon which innervates surface fibers of the muscle. With this arrangement, stimulation (Sn) of the axon causes a visible contraction. It is then possible to place a microelectrode (Rm) in a junctional region of the motor unit.

A stimulus applied to the motor nerve, either in the periphery or at the filament, causes both orthodromic and antidromic propagation. Therefore, in the control circumstance, the electrodes in the muscle (Rm) and on the motor axon (Rn) record the expected single nerve and muscle action potentials, illustrated respectively in the upper and lower traces in inset A of Figure 1. However, conditioning the motor nerve terminal with a facilitatory drug, e.g., neostigmine, or with high-frequency stimulation, leaves the terminal in an altered state, such that the next single impulse to invade it overly activates the terminal membrane and initiates an after discharge

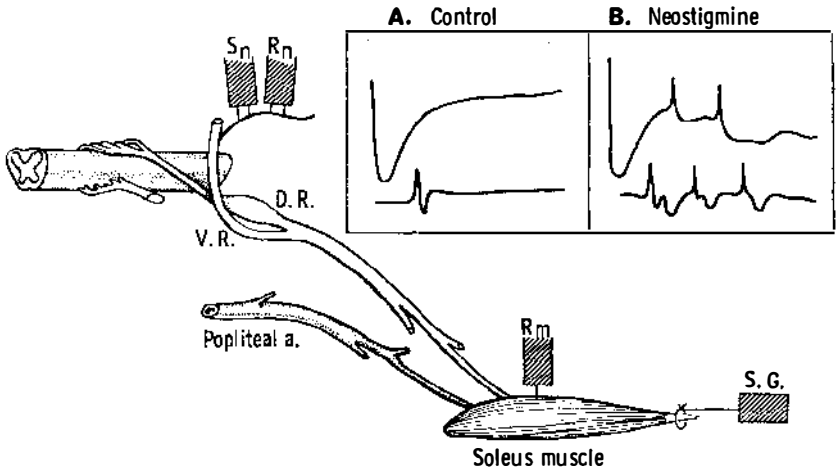

FIG. 1. "Matched pair" technique for monitoring related nerve terminal and muscle responses in the cat. See text for description.

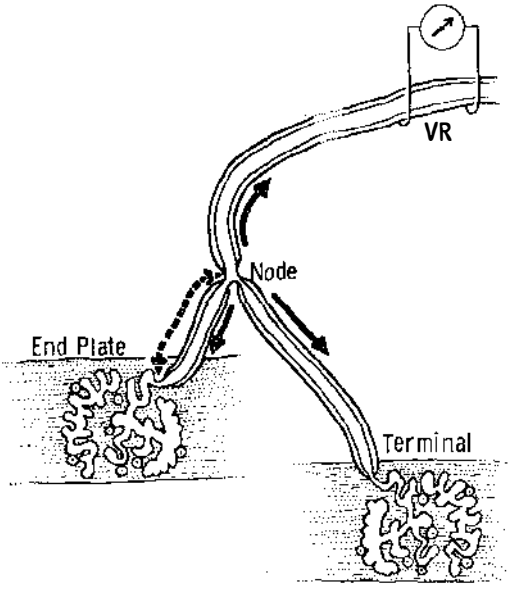

FIG. 2. Scheme for generation of PDR or PTR by motor nerve terminals. See text for description.

(PDR or PTR). This is also propagated ortho- and antidromically and is pictured in inset B as it appears in nerve (upper trace) and muscle (lower trace). The neurally recorded repetition identifies the activity of motor nerve terminals. Note that for each repetitive muscle discharge there is a precedent nerve discharge in a fixed temporal relationship. This is always the case, regardless of whether the terminals have been conditioned with high-frequency stimulation or by facilitatory drug. Thus, the matched nerve and muscle elements establish that the facilitatory drug action responsible for the characteristic muscle repetitive discharge and twitch potentiation arises from motor nerve terminals.

Figure 2 shows the way in which the conditioned terminal causes repetitive discharge of motor nerve. Interaction between the terminal and the last node of Ranvier re-excites the axon, and impulses propagate from that point both ortho- and antidromically. The interaction continues as long as the terminal is conditioned above a threshold level for stimulating the adjacent axon. The antidromic propagation is, therefore, a convenient means of detecting this motor nerve terminal activity.

The "matched pair" technique dismisses an early concern that repetition might originate in muscle and activate motor nerve terminals in a retrograde fashion. Werner (222) has made it clear that unusual circumstances are required to effect retrograde transmission. Other experiments have eliminated muscle as a source of the neural repetition. Barstad (4) and

Randic & Straughan (166) cut the muscle fibers of the rat diaphragm transversely, leaving the terminal innervation intact. Despite the muscle injury, facilitatory drugs continue to cause antidromic repetitive discharges, since the terminals are functional.

The advantages of antidromic monitoring are several. First, recording is from the cell in which the activity arises. Therefore, evaluation of drug activity is directly obtained, in contrast with post-junctional monitoring, wherein drug action on two cells may confuse the issue. Secondly, the preparation is set up *in vivo* with the critical structures undisturbed. Thus, a normal circulation provides proper oxygenation, nutrient, electrolytes, and pH; temperature is easily maintained. Further, intravascular administration resembles the way in which drugs reach the neuromuscular junction clinically.

The cat soleus nerve-muscle preparation has an intrinsic advantage. As suspected by Feng & Li (69, 70) and later proved by Standaert (191, 193) these nerve terminals, in contrast with those in fast muscle, are capable of generating repetition following tetanic conditioning. This aids isolation of a single functional motor axon because it is a reproducible means of activating a motor nerve terminal for identification. Motor nerve terminals to fast muscles do not show this property, but it must be emphasized that facilitatory drugs activate the terminals in these nerves just as they do in slow muscles (4, 13, 14, 16, 35–37, 166, 171, 173, 211).

By means of the antidromic technique, it has now been recognized that the facilitatory effects of other common drugs such as neostigmine, edrophonium, physostigmine, di-isopropyl fluorophosphate, galanthamine, ambenonium, etc. are all attributable to a similar direct conditioning action on motor nerve terminals (4, 13, 14, 105, 166, 168, 169, 172, 196, 211, 220).

Mechanism of facilitatory drug action.—A mechanism by which the facilitatory effect arises was proposed by Werner (220). Using antidromic monitoring, he probed drug-treated terminals with appropriately spaced conditioning and testing volleys. From this he learned that the post-drug time course of terminal excitability exhibits a pattern consistent with the development and maintenance of significantly large negative and positive afterpotentials. He postulated that because of this, facilitatory drugs enhance the capacity of the unmyelinated terminal to interact with the main axon (cf. Fig. 2).

Standaert (191, 193) substantially advanced this idea with his demonstration of the pattern, nature, and time course of soleus PTP. An augmented negative afterpotential in the unmyelinated segment of the axon, at a point close to the myelinated portion, was seen to be the trigger for the generation of post-tetanic repetition (PTR). This is essentially the mechanism of facilitatory drug action on motor nerve terminals (172, 196).

In a different way, Hubbard & Schmidt (97) and Hubbard, Schmidt & Yokota (98) confirmed these findings. They tested the excitability cycle of rat phrenic nerve endings *in vitro* following repeated stimulation and after

neostigmine. A series of conditioning impulses raised the threshold necessary to stimulate the terminals directly, showing that hyperpolarization occurred. A subsequent single stimulus evoked an enlarged presynaptic spike. The augmented presynaptic spike would have a relatively increased negative afterpotential and so this PTP mechanism is like that described by Standaert (191, 193) in the cat *in vivo*. Neostigmine appears to differ only in that it is a more direct means of enlarging negative afterpotential. Hubbard, Schmidt & Yokota (98) found that the drug modified the time course of terminal excitability consistent with a pattern of an increased amplitude and duration of negative afterpotential.

Koelle (120) has suggested that facilitatory drug action on motor nerve terminals involves ChE inhibition, with cumulated ACh reacting on the terminal. This proposal meets formidable obstacles. Certain anti-ChE's, e.g., methyoxyambenonium (12, 14, 16, 126, 223), are not facilitatory drugs and do not potentiate twitch or evoke stimulus-bound repetitive discharge from motor nerve terminals. Conversely, substances that are not significant anti-ChE's nevertheless can potentiate twitch and initiate strong stimulus-bound repetition from motor nerve terminals; some examples are succinylcholine (SCh) (195) and choline (14). Finally, recent studies of ACh on neuromuscular transmission are incompatible with this idea. Ciani & Edwards (39); Hubbard, Schmidt & Yokota (98); Riker (169) and Citrin (41) in different ways have disclosed that ACh mainly diminishes rather than facilitates motor nerve terminal function.

Riker (169) and Citrin (41) have shown that exogenous ACh over a wide range of doses does not evoke a PDR comparable in intensity or duration with that seen after facilitatory drugs. The ACh PDR is feeble and brief. Other strong depolarizers such as decamethonium (C10) and phenyltrimethylammonium ion (PTMA) exhibit the same abbreviated PDR pattern (153). Secondly, ACh causes neuromuscular block in exactly the dose range in which it initiates weak PDR (41, 169). Therefore, the poor facilitatory action of ACh is obliterated by a blocking action. Moreover, these same doses of ACh suppress not only PDR but also PTR, a fact which directly discloses the depressant action of ACh on the mammalian terminals (see discussion of depressant drugs). These data are inconsistent with the idea of ACh mediating facilitatory drug action, especially when one recalls the strong uncomplicated twitch potentiation caused by 3-OH PTEA (171).

To retain ACh as a mediator of facilitatory drug action, Hubbard, Schmidt & Yokota (98) offer a revised ACh feedback theory. In this, released ACh is thought to leak back to the distal Ranvier node and there initiate PDR. This still does not fit the facts. It requires ACh per se to be a good facilitatory drug, which it isn't. Moreover, it leads to a contradiction: ACh depresses motor nerve terminals, as Ciani & Edwards (39), Hubbard, Schmidt & Yokota (98), Riker (169), and Citrin (41) describe. Thus, assuming that ACh did initiate PDR at the node, the repetition would be extinguished in the terminal. This is not consistent with the course of facilita-

tory drug action; PDR is vigorous and enduring, providing that the drug itself is not exerting a depolarizing action. Lastly, it is unlikely that two portions of the unmyelinated axon will react differently to a depolarizing ion, and there are ample references to depolarization of unmyelinated axon and terminal by ACh and related drugs (1, 52–54, 98, 101, 111, 169, 195). Thus, there is as yet no sufficient reason to believe that facilitatory drugs exhibit other than a direct action on motor nerve terminals.

The importance of the negative afterpotential in the development of facilitatory drug action is illustrated by veratrum alkaloids. Their application to nerve prolongs negative afterpotential and leads to repetitive discharge (186). A similar action is demonstrable with the physostigmine-like drugs, providing they are applied to unmyelinated axon (52a). As Dettbarn points out, one would not expect the terminal portion of the axon to react differently from another part, if the drug has access to the membrane.

The facilitatory effects of veratrum alkaloids differ from those of the physostigmine-like drugs. The veratrum alkaloids are ubiquitous in their actions on excitable tissues, and selectivity by dose is difficult to achieve. Flacke (71) and Hofmann (87) reported that the primary twitch-potentiating action of veratrum alkaloids in the cat and in man results from muscle repetition and not from nerve repetition. Okamoto (153), using veratridine, has confirmed this in "matched pair" nerve-muscle preparations of the cat soleus. She defined a twitch-potentiating range of dose as one which regularly produces muscle repetition in response to single indirect stimuli without generating repetition in the matched nerve. Only when the dose of veratridine was raised approximately five times, were antidromic nerve repetitions seen; these could in turn propagate muscle action potentials. Here, then, is a clear-cut post-junctional facilitation, in which it may also be noted that the motor nerve terminals are not activated in a retrograde manner, despite intense muscle repetition.

As veratridine dose is increased, the motor nerve generally is affected. Feng & Li (70) showed that cutting the nerve at its entry into the muscle, or administering curare, does not abolish the antidromically recorded PDR. This contrasts sharply with the physostigmine-like PDR which is eliminated by these procedures. Therefore the veratrum alkaloids facilitate neuromuscular transmission, first by a post- and secondly by a presynaptic action at multiple loci. The latter, however, differs from the action of the physostigmine-like drugs since it is not specifically related to the unmyelinated terminal.

Motor nerve terminal as a site of depolarizing drug action.—The antidromic method as first employed by Masland & Wigton (142) was not concerned with stimulus-bound PDR but rather with the initiation of discharges in the absence of stimulation. They found that the intra-arterial injection of ACh caused a shower of antidromic discharges. Since that time, others (40, 41, 54, 111, 153, 169, 195) have observed a similar phenomenon with ACh, SCh, K, C10, PTMA, and tetramethylammonium (TMA). The

consensus is that the discharge reflects an initial depolarization of the unmyelinated terminals. The interpretation accords with the directly observed depolarizing effect of these and other quaternary ammonium ions on a variety of unmyelinated nerves (1, 2, 52, 53, 56, 57).

This discharge follows within 2 sec of a close arterial injection and the burst usually subsides within 30 sec. The intensity is variable but it is less than the maximum PDR evoked by a facilitatory drug.

Although Kato & Fujimori (111) were inclined to attribute part of the SCh effect to retrograde activation of motor nerve terminals by muscle, the more extensive study of Standaert & Adams (195) disavows this. The "matched pair" technique has been invaluable in establishing that when SCh, ACh, and K directly excite motor nerve terminal, there follows a correlated muscle response (40, 41, 169, 195). In the case of ACh, this has particular significance. The finding that the motor nerve terminal is highly sensitive raises important questions requiring further study: (*a*) How can depolarization of the subsynaptic membrane by exogenous drug be distinguished as a phenomenon independent from a pre-junctional depolarization? (*b*) Does the response to exogenous ACh have any physiologic relevance?

Although the "matched pair" technique unequivocally relates nerve terminal activity and muscle response, the question of a post-junctional reactivity to these depolarizing substances is not addressed by this technique. For example, the recordings of Standaert & Adams (195) with SCh, and Riker (169) with ACh, disclose that muscle action potentials are much more regularly evoked than are the nerve potentials. The impression is that the post-junctional membrane has been directly depolarized independently. However, both investigators furnish indirect evidence to indicate that the muscle responses were emanating from terminals but that antidromic propagation was blocked. Their argument is based on the known depressant actions of SCh and ACh on motor nerve terminals (39, 60, 98, 169, 195). Katz & Miledi (113) encountered a similar phenomenon while directly stimulating unmyelinated intramuscular axons in frog. Nevertheless, the data of Standaert & Adams (195) and Riker (169) are not inconsistent with the possibility of a post-junctional depolarization of mammalian muscle by intra-arterially injected SCh or ACh.

The reactivity of motor nerve terminals to facilitatory and depolarizing drugs has prompted some to dismiss these events as reflecting merely that the terminals have "cholinoceptive" properties; the implication being that these drug effects are side effects, unimportant in the "real" scheme of things. It is necessary, therefore, to reply that this view is without substance. First, the term "cholinoceptive," like so much other terminology, says nothing other than that the structure responds to ACh, SCh, and like ions, which was known at the outset. Secondly, the demonstrable pharmacologic facts speak for themselves: (*a*) ACh and related quaternary ammonium ions excite motor nerve terminals, causing them to transmit and the muscle to respond; and (*b*) facilitatory drugs condition motor nerve termi-

nals so that a single impulse initiates a repetitive discharge which is transmitted to the muscle. The site and sequence of these events account entirely for the well known end effects of these substances.

Amphibian responses.—Drug-induced antidromic discharges in amphibian motor nerve have been demonstrated by several investigators (7, 8, 58, 59, 101, 107, 129). The methodology is essentially as in the mammalian preparation. Most commonly, gastrocnemius, tibialis, and sartorius muscles are used; the ninth and tenth ventral roots are disconnected centrally for recording antidromic activity. An attraction of the amphibian preparation is that it can be arranged *in vitro:* the muscle with its nerve connections and spinal cord segment is isolated and mounted in a chamber; drug is introduced directly into the bathing medium.

An SCh-evoked antidromic discharge, unrelated to stimulation and comparable with this type of response as seen in the cat, has been reported (101). The discharge was brisk and short lived; it was terminated by increasing the dose, suggesting that both excitation and block develop prejunctionally. From other work (see below) it is possible that this depolarization might have arisen from anywhere along the lengthy intramuscular course of the unmyelinated terminal axon in the frog. Therefore, the significance of the action may be different from that in the cat, in which only the unmyelinated junctional terminal is affected.

A significant difference between the amphibian and mammalian preparations is the responsiveness to facilitatory drugs. Dun & Feng (58, 59) observed that the amphibian preparation does not generate antidromic discharges in response to physostigmine. Nevertheless, the facilitatory action of physostigmine is clearly evidenced by a good twitch potentiation. Similar findings have been made for edrophonium (148, 152), ambenonium, and neostigmine (107). However, the amphibian preparation does exhibit antidromic discharges after exposure to veratrum alkaloids or guanidine, (58, 59). In the amphibian, as in the mammal, the antidromic discharge set off by veratrum drugs or guanidine probably originate from any of several points; these include fine nerve terminals, intramuscular nerve branches, and peripheral trunks.

Although the amphibian doesn't develop antidromic discharges following exposure to physostigmine, neostigmine, edrophonium, ambenonium, or 3-OH PTMA, PDR does appear after 3-OH PTEA, PTEA, and tetraethylammonium (TEA) treatment. However, in the amphibian direct axonal effects of ethonium ions have long been known. In 1914 Marshall (137) observed that the stimulus threshold of motor fibers in frog sciatic nerve decreases after injection of TEA. Loeb & Ewald (135) observed skeletal muscle fasciculations after TEA application to the nerve. Cowan & Walter (45) made an extensive analysis of TEA effects in isolated frog nerve. TEA was seen to produce a lowering of thresholds for excitability and after-discharge in A-fibers. Okamoto & Kuperman (155) confirmed and extended this to include PTEA and 3-OH PTEA. The dose required to pro-

voke antidromic discharge is always higher than that needed to cause twitch potentiation (129). This is distinctly in contrast to the mammalian system in which these events have the same dose-response relationship. Kuperman & Okamoto (129) showed that local application of TEA to frog sciatic nerve induces after-discharge, as well as spontaneous discharges. In view of these findings, TEA and other ethonium ions (129) may cause in the frog an antidromic phenomenon having a different origin from that which obtains in the cat. Accordingly, Karczmar, Kim & Blaber (107) found 3-OH PTEA caused a stimulus-related after-discharge at the ventral root monitor after single stimulation of frog peripheral nerve. Beaulieu, Frank & Inoue (8) observed a similar result with TEA. Kuperman & Okamoto (129) confirmed these findings but pointed out that the concentrations necessary are the same as those which cause after-discharge in isolated nerve. There is little doubt that this antidromically recorded event in frog is prejunctional but it is not comparable with the presynaptic action of facilitatory drugs in the cat. Ethonium ion acting anywhere along the frog intramuscular nerve distribution can trigger the occurrence (129, 164). The cat motor axon, on the other hand, is generally unresponsive to PTEA, 3-OH PTEA, and TEA. Therefore it may be incorrect to conclude that the effects of ethonium ions in amphibia reflect a selective facilitatory action on the junctional terminal.

Structure-activity data reinforce the difference between frog and mammalian responses to ethonium ions. The methonium and ethonium hydroxyaniliniums evoke PDR from mammalian motor nerve terminals, but only the ethonium ions, 3-OH PTEA, and 3-hydroxydiethylmethylammonium (3-OH DEMA), cause the antidromic phenomenon in amphibia. Moreover, the less ethylated 3-OH DEMA is less potent than 3-OH PTEA. Apparently the effect of these facilitatory drugs in the frog is attributable to an ethonium ion action and not to an hydroxyanilinium action as it is in the cat. Hydroxylation is not essential for the effect in the frog, and the effectiveness of PTEA in this species suggests a different mechanism. Additionally, Payton & Shand (164) have noted that gallamine, a neuromuscular blocking ethonium ion, like TEA, causes a stimulus-bound antidromic discharge.

Kuperman & Okamoto (129) have evidence to support the idea that ethonium ions exaggerate local oscillatory behavior of nerve membrane leading to propagated activity. An ethonium ion action such as this on intramuscular axonal branches could account for the orthodromic and antridromic propagation seen by Karczmar, Kim & Blaber (107), Kuperman & Okamoto (129), and Beaulieu, Frank & Inoue (8). This action resembles and may in fact be identical with that seen after Ca diminution (7, 24, 45, 154). In view of these data, the unique position of 3-OH PTEA and 3-OH DEMA among the facilitatory drugs may be explained. Although they produce an antidromic phenomenon in both cat and frog, not only are the sites of this PDR generation different but so also may be the mechanism.

Drug interaction at the terminals.—For mammalian neuromuscular transmission, Brown (26) discovered that ACh could be made to potentiate

the single indirect twitch response, much as physostigmine does, if a small dose of physostigmine is previously given. Since ACh alone could not do this, the obvious interpretation, at that time, was that ChE inhibition allows more ACh to reach and to survive at the end plate. There are now sufficient pharmacologic reasons to doubt this interpretation. In the early context, physostigmine or whatever anti-ChE is used, plays nothing more than a passive role. However, the evidence in foregoing sections shows clearly that the characteristic facilitatory effect of physostigmine-like drugs is due to an action on motor nerve terminals. Moreover, the well known ACh effects on neuromuscular transmission can be accounted for entirely by demonstrated actions on these terminals (169). It is therefore likely that subthreshold doses of physostigmine-like drugs and ACh interact at motor nerve terminals, to cause a strong facilitatory response.

Moreoever, Citrin (41, 42) learned that neostigmine, in a dose too small to initiate twitch potentiation and PDR from motor nerve terminals, causes a subsequent ACh dose to release a vigorous PDR and a sizeable potentiation. The effects were comparable in intensity and duration with what would have occurred after a single effective neostigmine dose. The dose-response concept is crucial here in that the ACh dose used was one which, when given alone, produces a considerable neuromuscular block followed by a minimal, fleeting twitch increase and PDR. It is hardly likely, therefore, that neostigmine is permitting even more ACh to act.

The striking neostigmine-ACh post-drug repetition indicates that mammalian motor nerve terminals are the site of this interaction and dose-response consideration indicates that ChE inhibition does not explain the phenomenon. This latter point requires discussion, since Karczmar (105) believes it necessary to inactivate ChE if "true" ACh effects are to be seen. What must not be overlooked are: (*a*) ACh dose-response, and (*b*) that the neostigmine-ACh response is qualitatively different from the response to ACh alone. ACh by itself in any amount cannot duplicate the effects seen when it is administered after a facilitatory drug. This, unlike sensitization of the frog rectus muscle to ACh (86), is a qualitatively different response.

The physostigmine-ACh effect is essentially like the neostigmine-TEA effect: TEA does not cause potentiation of the mammalian twitch unless it is administered after pretreatment with a physostigmine-like drug (119). The experiments of Citrin (41, 42) offer another example. Potassium chloride injected intra-arterially, like ACh, causes at best a weak and fleeting post drug repetition. Following a small conditioning dose of neostigmine, K leads to the appearance of a strong and continued stimulus-bound repetition. Citrin (41) has advanced cogent reasons why ChE inhibition does not explain this. In each of these instances pretreatment with facilitatory drug has qualitatively changed response.

The experiments of Blaber & Karczmar (16) support the idea that interaction between a facilitatory drug and a depolarizing ion is not a potentiation but rather a qualitatively different response. Using antidromic moni-

toring in the cat, they showed that methoxyambenonium greatly intensifies and prolongs the PDR evoked by SCh. The intensity and duration of the SCh post-drug repetition following methoxyambenonium could not have been duplicated by any SCh dose given alone. Larger SCh doses would have depressed the response of the motor nerve terminal and caused neuromuscular block (16, 195). It could scarcely be maintained that the "true" actions of SCh are not in evidence unless ChE is inhibited. The circumstance with ACh is no different.

The neostigmine-ACh effect can be reproduced without employing an anti-ChE drug. Citrin (41) has shown that tetanic conditioning, like facilitatory drugs, can cause motor nerve terminals to respond to either ACh or K with a strong post-drug repetition. The priming action in these circumstances is undoubtedly on motor nerve terminals and ChE inhibition is not a factor.

Directly related to the physostigmine-ACh type interaction is the "reversal" phenomenon recently studied by Karczmar (106). This effect was described originally by Zaimis (226) who found that blocking doses of TMA and C10 could be made to potentiate the single twitch in the cat, if a small prior dose of neostigmine or physostigmine were given. Since that time Karczmar (104) and Blaber & Karczmar (16, 17) have examined a series of *bis* quaternary ammonium oxamide compounds having, in varying degree, the properties of twitch potentiation, neuromuscular block, antagonism of curare, and "reversal" action. They concluded that facilitatory and curarimimetic actions are essential ingredients in the conversion of depolarization block to facilitation. On the basis of antidromic recordings in the cat, they decided that the "reversal" interaction occurs at the motor nerve terminals. The conclusions of Citrin (41) regarding "reversal," or in fact interaction, concur with these.

Facilitatory drug PDR can be enhanced by prior high-frequency conditioning (220). This would hyperpolarize terminals and further exaggerate the augmented negative afterpotential initiated by a facilitatory drug. Epinephrine and norepinephrine enhance twitch potentiation and PDR caused by facilitatory drugs (14, 20, 27, 28). Available evidence (20, 84) indicates that the catecholamines may hyperpolarize the terminals, and it may be that because of this they simulate the conditioning effect of high-frequency stimulation. Interestingly, the conditioning effect of catecholamines is not exhibited by isoproterenol and is reversed by α-adrenergic blocking drugs (20). Since Krnjevic & Miledi (124)have shown epinephrine to act at motor nerve terminals, and in view of the pre-junctional action of neostigmine, there is at least little doubt that motor nerve terminals are the site of this interaction. Bowman & Raper (20) reach the same conclusion and offer additional evidence.

Motor nerve terminals as a site of depressant drug action.—Standaert's (191, 193) recent study of PTP of cat soleus muscle has furnished a new and valuable means of assessing drug action on motor nerve terminals *in*

vivo. Brief high-frequency stimulation of soleus nerve regularly causes each subsequent response to a single stimulation to become repetitive. Using the "matched pair" technique, Standaert (191, 193) learned that this PTR originates in the motor nerve terminals and is then transmitted to the muscle, causing PTP.

The advantages of this technique are: (*a*) post-tetanic repetition or potentiation, or both, disclose motor nerve terminals as they function *in situ;* (*b*) the method is an even more direct probe of motor nerve terminal function than is PDR or the related twitch potentiation, since whatever uncertainty is connected with drug activation is avoided; (*c*) the fact that PTR is stable and reproducible in single neural elements for long periods enables quantification of drug effects; PTP, likewise is stable and reproducible.

An obvious application of PTR or PTP, or both, is in the analysis of drugs depressing neuromuscular transmission. Thus, if a neuromuscular blocker suppresses PTR, motor nerve terminals are clearly identified as a site of action. Futhermore, dose-response quantification not only allows drug comparisons but also furnishes insight into the relative importance of effect on terminal as against other possible blocking sites.

Standaert (192), applying this to *d*-tubocurarine, learned that PTR and PTP are progressively suppressed and finally obliterated by increasing doses. It is impressive that PTR and PTP were abolished by a curare dose that exhibits only a minimal blocking effect on neuromuscular transmission.

The curare antagonism of PTP and PTR undoubtedly relates to the curare suppression of twitch potentiation and PDR caused by facilitatory drugs (13, 14, 36, 69, 70, 142, 166, 174). Since the two phenomena, PTR and PDR, are essentially the same, involving a prolongation of terminal after-negativity, the indication is strong that a prominent curare action is to curtail this after-potential. This is supported by Hubbard (94) and Hubbard, Schmidt & Yokota (98), who stimulated intramuscular nerves close to the terminal in rat diaphragm and found that curare shortened the supranormal period. Assuming, then, that PTR suppression reflects this action of curare, the neuromuscular blocking action remains to be explained. Because curare dosage greater than that necessary to abolish PTR initiates a progressive block of transmission, Standaert (192) and Riker & Standaert (172) have suggested that with higher doses a decrease in terminal action-potential occurs. This would lead to neuromuscular block from a pre-junctional site of action. Evidence from other kinds of experiments has accumulated in recent years to substantiate a significant curare action on motor nerve terminals (5, 134, 145).

Recognition of the fact that curare depresses excitability of motor nerve terminals does not deny that the drug may also act post-junctionally. However, it does encourage a re-examination of the evidence for post-junctional block. The fact that curare prevents ACh activation of mammalian skeletal muscle is not an adequate argument for a post-junctional action, since it has been demonstrated that intra-arterial ACh in the cat excites motor nerve

terminals, causing them to transmit (169). Since there is evidence for pre-junctional actions of curare and ACh, the curare antagonism of ACh may well be pre-junctional.

The evidence for post-junctional block by curare rests on *in vitro* experiments chiefly with amphibian nerve-muscle preparations. However, frog and mammalian junctions may differ with regard to curare action, and other prominent differences between cat and frog terminals have been cited. The recent experiments of Katz & Miledi (113) in which extracellular recording was made directly from distal unmyelinated terminals in the frog, indicate that *d*-tubocurarine rapidly blocks the end-plate potential (EPP) and that propagation in the terminals is unchanged. Nevertheless, Katz & Miledi (113), using curare concentration in excess of that needed for transmission block, noted in some instances a slow reduction in terminal spike amplitude and, in one experiment, a reversible block.

A direct approach such as this is most desirable and should be fruitful in deciding questions of drug actions on frog terminals. Gissen, Karis & Nastuk (81), in what appears to be one experiment, recorded similarly from frog terminal and end-plate, and found that microperfusion of halothane in a curare-treated preparation, progressively reduced the EPP without affecting the terminal action-potential. They concluded that halothane block of transmission in frog is a result of a post-junctional depression. However, other data in their report seem to suggest otherwise (see below).

Direct recording from intramuscular nerves in a mammalian preparation has been made by Hubbard & Schmidt (97) and Chang, Chen & Cheng (35, 36). These workers have placed a microelectrode extracellularly in the region of the nerve-muscle junction of rat diaphragm, and after stimulation of the nerve proximally have recorded a small diphasic potential. The extremely critical positioning of the electrode, and the precedence of the small potential to the EPP, suggest that it is pre-junctional.

A blocking dose of curare (36) was found not to affect this potential. This implies that curare is without a pre-junctional action in the rat. However, Song (190), in electron microscopic studies of this junction in longitudinal section has disclosed that phrenic nerve axons are myelinated virtually to their termination in the synaptic gutter, which in turn is covered by the large Schwann cell. Couteaux (44) emphasizes this anatomy, pointing out that these cells cover the synaptic gutters, and "separate entirely the terminal axoplasm from the extracellular medium." Accordingly, direct pre-junctional recording in the rat is very likely from myelinated nerve ending, in which case, curare, a quaternary ammonium ion, would not be expected to exert an effect.

For drugs resembling curare, Werner (219) concluded that gallamine depresses motor nerve terminals of the cat because it abolishes the PDR initiated from that site by facilitatory drugs. Blaber & Bowman (13, 14) on the basis of similar data decided that benzoquinonium has a pre-junctional action. Likewise, Chang, Chen & Cheng (35) found that two other quater-

nary ammonium ions having neuromuscular blocking properties, guanethidine and bretylium, abolish PDR from motor nerve terminals.

Hexamethonium (C6) (218) prevents edrophonium-induced twitch potentiation in cat tibialis muscle that is indirectly stimulated. On the basis of the reviewers' experience there can be little doubt that this represents blockade of a PDR from motor nerve terminals. Certainly the result is consistent with the known comparable effect of many other quaternary ammonium ions. Volle (218) finds, however, that C6 does not prevent or reduce a PTP produced in the same muscle and believes the findings discrepant. The fact is that they are not. It has already been emphasized that facilitatory drug PDR can be evoked from all motor nerve terminals. In contrast, PTR arises only from terminals in slow muscle (e.g., cat soleus); PTP occurring in cat tibialis muscle is not associated with PTR and represents an altered behavior of the contractile mechanism (181, 193). It is anticipated that C6 would suppress PTR from soleus terminals.

To evoke myogenic PTP via nerve stimulation, a sustained repetitive transmission is required. Drugs which impair tetanus maintenance would therefore prevent this PTP. Segawa, Kojima & Takagi (182–184) showed this to be the case for the fast muscle of rat diaphragm by using increasing doses of curare, SCh, C6, atropine, Mg, and Ca until tetanus maintenance was compromised. In this regard it may be noted that in Volle's experiment (218) the C6 dosage did not impair the tetanic stimulation, and his result is expected. With regard to the other drugs examined by Segawa, Kojima & Takagi (182–184), it remains to be determined by more direct methods whether the effects are pre-junctional.

Related to curare suppression of motor nerve terminal PTR, is the similar effect of excess Mg (166, 225) or Ca depletion by EDTA (159). In these cases, PTR suppression accords with the recognition of the pre-junctional influences of these ions. It is not likely that PTR as a pre-junctional indicator would in one instance (Mg) prove reliable and in another (curare) unreliable. It seems rather that the Mg effect re-enforces the significance of the curare effect.

From the time that Bovet et al. (18) and Burns & Paton (29) called attention to the neuromuscular blocking action of depolarizing quaternary ammonium ions, most subsequent studies have focused on the relationship of this block to post-junctional depolarization (for review see 105, 146, 147, 207). What has emerged is that neuromuscular block due to these ions is not simply correlated with changes in post-junctional membrane potential (82). Therefore, it is suggested that a desensitization of the post-junctional membrane progresses while membrane potential recovers. The interpretation of this circumstance has been that post-junctional receptors are inactivated by excessive amounts of ACh or ACh-like drugs. However, this dissociation of post-junctional depolarization and neuromuscular block could reflect a pre- and post-junctional action of the depolarizing drugs. In support of this are those studies which show that mammalian motor nerve terminals

are depressed by the depolarizing ions so far examined: SCh (*72, 73,* 195), ACh (41, 98, 169), K (40, 41), and C10 (153, 180).

It isn't surprising that the unmyelinated motor nerve terminal is susceptible to quaternary ammonium ions and certain other drugs; in this respect it is not different from other unmyelinated axons. Douglas & Ritchie (57) have demonstrated that ACh directly excites the endings of cutaneous nonmyelinated fibers in the cat. What appear to be common effects of ACh and related nicotinic ions on unmyelinated mammalian and crustacean axons are the lowering of the resting potential and the reduction of action-potential amplitude (1, 2, 49, 50, 52, 53, 178). Dettbarn (51) points out that there seems to be no essential difference in the effects of these drugs, whether they act on the axonal membrane of the terminal or elsewhere on the axon.

Curare also acts on unmyelinated axons. Dettbarn (50, 52a) and Ritchie (178) saw that it, like other quaternary ammonium ions, diminishes the action-potential amplitude in a lobster axon and in unmyelinated rabbit vagus. In addition, Ritchie discovered that curare causes a small depolarization of these nerves. Also, a curare conduction block in myelinated peripheral nerve axon occurs at the unmyelinated Ranvier node (49). A relationship of these axonal effects to a curare action on mammalian motor nerve terminal is seen not only from the curare suppression of PTR (192) but also from experiments (98) which show that curare can first raise and then lower the threshold of motor nerve terminal to direct stimulation. All of these data support Standaert's (192) proposal that curare might establish transmission block by decreasing the amplitude of the terminal action potential.

Although the evidence for curare depression of motor nerve terminals is abundant, the evidence for a pre-junctional anticurare action of facilitatory drugs is less satisfying because it is less direct. However, there are two principal considerations which argue for a pre-junctional anti-curare action: (*a*) post-tetanic decurarization complements, exactly as it should, the demonstrable curare suppression of PTP and PTR; and (*b*) the antagonism of facilitatory drugs to curare complements the curare action to suppress the twitch potentiation and PDR initiated by these drugs. This is, nevertheless, presumptive because the antagonism of motor nerve terminal repetition by curare is so intense that it is impossible to restore either PDR or PTR following even small curare doses. Thus, tetanic conditioning or facilitatory drug will reinstate transmission, but not repetition and twitch potentiation. This strongly suggests that there are two curare dose-response relationships developing at motor nerve terminals. The one which now appears to be established is to reduce the negative after-potential, and the other, which is speculative, is to reduce action-potential amplitude in the terminal. It is the latter which would be involved in an easily reversible mutual antagonism with a facilitatory drug and the former, which seems to be irreversible.

The neural depressant properties of procaine and related substances make these drugs prime candidates for study on motor nerve terminals.

Riker et al. (173) abolished the post-drug repetition attributable to 3-OH PTEA with procaine; Ueda et al. (211) similarly erased galanthamine PDR. In an extensive research, Usubiaga & Standaert (213) showed that procaine, lidocaine, tetracaine, and dibucaine, as a function of dosage, suppress PTR and PTP in cat soleus nerve and muscle. In this respect, the relative potencies of these drugs on motor nerve terminal correspond, as one would expect, to their relative potencies in blocking peripheral nerve. Further, with each drug, the small diameter terminal is the first target, since the doses causing full disappearance of PTP are without effect on conduction in the myelinated axon.

The common susceptibility of peripheral axons and motor nerve terminals to other types of neurotropic depressants further indicates that the terminal responds pharmacologically like the rest of the axon. Toman (209) found that repetitive stimulation of frog sciatic nerve conditions the fibers so that subsequent single excitations are each followed by an after discharge; this effect is essentially no different from that in the *in vivo* antidromic method of monitoring the activity of motor nerve terminals. A good example of the relationship between PTR in isolated peripheral nerve, and of PTR evoked from motor nerve terminals *in situ,* is furnished by the effects of diphenylhydantoin and barbiturates. Diphenylhydantoin and phenobarbital, in concentrations equivalent to those that would obtain systemically during therapy, abolish the *in vitro* PTR in frog nerve (209). This significant neural depression is revealed even more relevantly by barbiturate and diphenylhydantoin suppression of PDR (173, 211) and of PTR (161, 165) from motor nerve terminals of the cat. Raines & Standaert (165) have compared the repetitive discharges of seizures with the experimentally induced PTR of the terminal and conclude that PTR suppression by diphenylhydantoin reflects essentially its anti-epileptic action. It is odd, therefore, to note the tendency to disregard the strong depressant action of small curare doses on mammalian terminals, simply because the finding does not conform to a stereotype; this pharmacologic fact could be important.

It is known that general anesthetics depress axonal and synaptic functions; references to early literature on this may be found in the review by Toman (209). The motor nerve terminal should be no exception. Accordingly, Riker et al. (173) and Van Poznak (215–217) have reported that PDR and PTR are sensitive to suppression either by cyclopropane or ether. The effect appears following the inhalation of amounts of anesthetic agent that produce minimal clinical effects. It was concluded that the motor nerve terminals are highly susceptible to these neurotropic depressants. This agrees well with the interpretation of synaptic vulnerability to anesthetics (131).

Recent studies have been directed to the effects of ether and halothane on frog post-junctional membrane (81, 109, 110). Although the data of these experiments indicate that these anesthetics prevent the depolarization of the post-junctional membrane by carbamylcholine, they do not make a

good case for this as an important action. To oppose this post-junctional depolarization, it is apparently necessary to expose the preparation for a considerable time to a concentration (5 per cent) of ether—almost twice that which completely blocks transmission (110). Moreover, it seems that even this high concentration of ether is not always effective; the carbamylcholine dose may depolarize as much as 22 mV, despite the ether (109). Obviously, the action of ether to oppose post-junctional membrane depolarization by carbamylcholine occurs at concentrations well in excess of those found by Van Poznak (217) to depress motor nerve terminals in the cat. The findings, however, may reflect species differences.

Like ether, halothane reduces the CCh depolarization of frog post-junctional membrane (81). However, the concentration of halothane required is exceedingly high (5 per cent); 1.5 per cent in Ringer's solution causes complete neuromuscular block and 4 per cent blocks axonal conduction. From these data the post-junctional effect of halothane appears irrelevant and difficult to reconcile with other experiments in the same report (see above) which suggest that the post-junctional membrane is the vulnerable point.

The utility of PTR suppression in evaluating drugs that depress excitable cells continues to extend beyond the confines of neuromuscular transmission. A recent significant application has been in a study of anti-arrhythmic drugs (132, 179, 197, 198). These investigators have found for pronethalol, bretylium, propranolol, diphenylhydantoin, procaine, and quinidine, an excellent correlation between antagonism of a digitalis-induced arrhythmia and capacity to suppress the PTR of motor nerve terminals. The systemic dose ranges for each of these effects are identical. In view of this, the authors suggest that the anti-arrhythmic actions relate to depression of intra-cardiac nerve endings. Standaert & Roberts (197) and Levitt et al. (132) furnish strong evidence to correlate pronethalol suppresion of digitalis arrhythmia with neural depression and not with β-adrenergic blockade. Neither this conclusion nor pronethalol suppression of PTR is surprising in view of the fact that this drug is a local anesthetic twice as potent as procaine (185). Levitt et al. (132) have studied diphenylhydantoin and further emphasize the susceptibility of intra-cardiac nerves. This drug has no α-adrenergic blocking action; it does not depress the myocardium; it is however, depressant to nerve terminals and presumably for this reason is effective as an anti-arrhythmic.

The importance of integrity of the motor nerve terminal for the development of either PTR or PDR has been conclusively shown by Okamoto & Riker (156–158). They succeeded in producing an early, subtle damage of motor nerve terminals of the cat by a short-term denervation. When the motor nerve was sectioned 48 hr prior to experiment, indirect twitch response was mainly little affected but facilitatory drug potentiation of twitch and PTP were grossly reduced or absent. Therefore, both PDR and PTR were either absent or feeble; in the case of tetanic conditioning a post-tetanic depression often appears, signalling the poor recuperation of the ter-

minals. The fasciculatory response to physostigmine also disappears, as Langley & Kato (130) and Reid (167) earlier learned; this, too, points to motor nerve terminal, since Riker (170) has shown that fasciculations released by physostigmine-like drugs originate from there. These nerve terminal deficits constitute the earliest functional sign of denervation. Accordingly, Song (189) has observed in rat neuromuscular junction that the earliest degenerative change involves the unmyelinated terminals.

This pattern of injury to the motor nerve terminal by short-term denervation accords with the common effect of all of the neurotropic depressant drugs described herein, in which PDR or PTR is the first function to be lost as dose is increased. Therefore, the significance of PTR or PDR suppression as an index of terminal vulnerability to drugs appears to be considerable.

Especially relevant to the short-term denervation results are the findings of Desmedt (46–48) in myasthenia gravis. Following rundown of twitch and action-potential by recurrent low-rate stimulation of motor nerve, high-frequency stimulation is succeeded by an abnormally small PTP. Immediately thereafter a post-tetanic depression appears and continues for many minutes, gradually returning to the pre-tetanic control. These results led Desmedt to conclude that the myasthenic lesion is pre-junctional. Therefore, the dramatic reversal that the physostigmine-like drugs effect in myasthenia gravis is perhaps eloquent testimony that these facilitatory drugs act specifically on motor nerve terminals.

Detection of Drug Action on Motor Nerve Terminals by Means of Post-junctional Monitoring

Pre-junctional failure.—A significant means of detecting function in the motor nerve terminal is the presynaptic failure method developed by Krnjevic & Miledi (123–125). Observations were made from rat nerve-diaphragm *in vitro* in a normal physiologic salt solution and from rat gracilis *in situ*. The intermittent failure of EPP during a train of indirect stimuli pointed to pre-junctional impairment, since the occurrence of spontaneous miniature end-plate potentials (MEPP) indicated that the post-junctional membrane was intact. Moreover, during repetitive stimulation, multiple recordings from different end plates in the same unit revealed independent behaviors. This enabled failure to be assigned to distal points in the terminal.

Factors which were found to provoke failure of the motor nerve terminal (125) include stimulus frequency, temperature, hypoxia, and catelectrotonus. Stimulation at a frequency of 50 per sec is associated with a considerable failure rate *in situ* and *in vitro*. The lower the frequency the less the failure, but even at 20 to 30 per sec *in vivo*, failures occur. *In vitro* preparations fail even more at lower frequencies of stimulation.

Oxygen lack leads to a reversible pre-junctional failure, at a speed dependent on the rate of oxygen deprivation: nitrogen equilibration causes total failure in less than a minute. The effects of anoxia and recovery from

it could be repeatedly demonstrated, as Hubbard & Loyning (96) have more recently shown using spontaneous MEPP frequency as an index of terminal function.

By the intermittent failure method, Krnjevic & Miledi (124) found for rat neuromuscular junctions *in vitro* and *in vivo,* that presynaptic function could be restored with epinephrine. Thus, an important epinephrine action is located at motor nerve terminals and is reminiscent of the salutary effect of sympathomimetic drugs in myasthenia gravis, wherein a terminal defect seems to be the focus (48). Bowman & Raper (20) from a different experimental basis also conclude that the catecholamines exert important actions on motor nerve terminals.

In the opinion of the present authors an apparently incidental pharmacologic observation made by Krnjevic & Miledi (125) invites discussion. In their study of presynaptic failure, Krnjevic & Miledi noted that when neuromuscular transmission is reduced by either Mg or curare, *in vitro* or *in situ,* much higher frequencies of stimulation are required (100 or more per sec) to produce transmission failure than in the absence of these substances. Moreover, the profound ability of temperature change to enhance failure is lost. These and the control data reveal that Mg and curare are acting on motor nerve terminals. The direction of this action may be thought at first to be opposite to what one would expect, but other considerations provide a suitable explanation. The adverse effects of anoxia, stimulus frequency, cooling, and catelectrotonus indicate that intermittent presynaptic failure arises from a reduction of the terminal membrane potential. In the case of repetitive stimulation, the terminal membrane potential would be lowered by summation of negativity. This is borne out by the prevention of presynaptic failure through anodal polarization. Curare and Mg act similarly and this in effect concurs precisely with the demonstrated actions of curare and Mg to suppress the PTR of motor nerve terminals (192, 225). It appears that curare prevents depolarization of the terminal following repetitive stimulation.

In addition, this curare action to prevent presynaptic failure and to "regularize" EPP amplitude demonstrates that a pre-junctional curare action can exert an important influence on a post-junctionally recorded amplitude.

Roberts & Thesleff (180) have recently employed the presynaptic failure method *in vivo* to detect a pre-junctional action of C10. They found that C10 in threshold doses for neuromuscular block causes an intermittent failure characteristic of a presynaptic defect. It is surprising that this excellent method of Krnjevic & Miledi (125) has not been used more in the analysis of drug action.

Unquestionably related to this is the failure method of del Castillo & Katz (31). These investigators, using frog nerve-muscle in a high-Mg low-Ca bath, reduced the EPP response to the MEPP level and found that random failure to indirect stimulation occurred. They were able to relate

the Poisson distribution of this failure incidence to quantal estimation obtained by direct measurement of EPP/MEPP. The interesting aspect of this is that it provided a means of determining quantal content independently of amplitude measurement. This approach has been used by Edwards & Ikeda (60), Ciani & Edwards (39), Hubbard, Schmidt & Yokota (98) and Christ & Blaber (38) to detect, respectively, the effects of SCh, ACh, and benzoquinonium on motor nerve terminals. Adoption of this method probably accounts for the success of these workers in disclosing susceptibility of the terminal to these drugs. A principal concern, however, is that transmission is reduced to an absolute minimum by alteration of ionic composition. On the basis of other reports cited elsewhere in this review it seems questionable to seek to determine the site of drug action in a preparation which is so drastically modified. The real purpose of the method, namely, to analyze the quantal composition of the EPP, reasonably employed the unique action of Mg to reduce EPP amplitude to MEPP size. Mg poisoning as a basis in the location of drug action seems less clear.

MEPP frequency.—A familiar and widely used method of assessing drug action on both amphibian and mammalian motor nerve terminals stems from the discovery by Fatt & Katz (68) of spontaneous subthreshold activity. The nerve terminal source of this discharge was supported by the findings that denervation, local anesthetics, (68) and botulinus toxin (25, 206) abolish it. Since MEPPs are occurring randomly, the frequency of the event provides an obvious parameter. In fact, cumulative experience now indicates that MEPP frequency is a highly reliable indicator of the actions of ions and drugs on motor nerve terminals.

An increase in MEPP frequency appears to be either directly or indirectly related to depolarization of the motor nerve terminals. Del Castillo & Katz (32), Liley (133), Katz & Miledi (113), and Hubbard & Willis (99) have found for amphibian and mammalian preparations that either catelectrotonus applied to a terminal axon or rising K concentration increases the frequency of the discharge. Furukawa, Furukawa & Takagi (75) showed that NH_4 acts similarly.

Other less direct indications of the relationship between depolarization of the motor nerve terminal and MEPP frequency are the effects of repetitive stimulation, hypoxia, veratrine, and certain toxins. A sharp but brief increase in MEPP frequency immediately follows repetitive stimulation (23, 93). Hypoxia causes a large, abrupt, but short-lived increase, which accords with the known depolarizing effect of oxygen deprivation (96). Related to the influence of hypoxia are the effects of 2,4-dinitrophenol (DNP). Kraatz & Trautwein (122) and Beani, Bianchi & Ledda (6) observed that this metabolic poison increases frequency. They reversed the dinitrophenol effect by hyperpolarizing the nerve endings, which suggested that this drug leads to nerve terminal depolarization. Veratrine causes a considerable increase in MEPP release from frog (75), rat (88, 89), and normal and myasthenic human motor nerve terminals (91). This is not surprising when

one considers that the action of veratrine on nerve is to retard repolarization by increasing Na permeability (187, 212). During the early phases of the action of tetanus toxin (163) and saxitoxin (103) MEPP frequency is accelerated. This probably reflects membrane damage. Perhaps related to membrane permeability is the effect of the aliphatic alcohols, methyl, ethyl, and propyl, to increase the frequency of MEPP discharge (77, 100, 151).

Thus, there seems little doubt that an uncomplicated depolarization of motor nerve terminals will be signaled by an increase in MEPP frequency. This relationship affords opportunity to re-emphasize an important distinction made initially by Nastuk & Alexander (148) and extended by Riker et al. (171); namely, that depolarizing and facilitatory actions at the neuromuscular junction are separate and different. Accordingly, physostigmine-like drugs would not be expected to increase MEPP frequency unless they exert an independent depolarizing action. The study of Blaber & Christ (15) shows that the facilitatory drugs neostigmine and edrophonium, at critical doses, will cause a slight increase in MEPP frequency in cat nerve terminal. This probably relates to the small depolarizing action of these quaternary ions and confirms earlier reports (21, 91) in which occasional slight increases in frequency were noted after neostigmine. The facilitatory drug ambenonium is not depolarizing and causes no increase in MEPP frequency. The frog does not show an increase in MEPP frequency after neostigmine (68, 75), and this may reflect a species difference. Thus, it is apparent that MEPP frequency is not a suitable means of detecting facilitatory drug action on motor nerve terminals.

Guanidine increases MEPP frequency slightly in mammalian nerve-muscle preparations (89, 91); this may be due to a small depolarizing effect of guanidium ion. The frog does not show this response (75, 160) and again, as in the case of neostigmine, this may be a matter of species.

MEPP frequency as a sign of depolarization of the motor nerve terminal may be thought to be a useful means of monitoring such an action of ACh, SCh, C10, etc. Hubbard, Schmidt & Yokota (98) perceived this, as did Blaber & Christ later (15). However, neither group could detect, over wide concentration ranges, any clear effect of ACh on MEPP frequency, recorded respectively in rat phrenic nerve-diaphragm and cat tenuissimus preparations in normal Krebs-Ringer solution. Hubbard, Schmidt & Yokota (98) found that ACh often caused an apparent decrease in MEPP frequency but discerned that this was due to a fall in MEPP amplitude. They attributed the amplitude decrease to the well described blocking effect of ACh (118). Similarly, Roberts & Thesleff (180) noted that C10 decreases MEPP amplitude without change in frequency in the rat *in vivo;* Ito (101) found the same with SCh in the frog. It is quite likely that other related quaternary ammonium ions would have an equivalent effect, and thus preclude detection of any effect on frequency.

The extensive studies of Thesleff (202–205) and Axellson & Thesleff (3) have adequately demonstrated for frog, rat, rabbit, guinea pig, and cat

that conditioning doses of SCh, C10, and nicotine, as well as of ACh, very rapidly block the depolarizing action of subsequent ACh doses. Thus, the potential usefulness of MEPP frequency as a means of revealing depolarization of the motor nerve terminal by quaternary ammonium ions is probably aborted by the quick onset of the concurrent blocking action. Dose and rate of application may be critical in disclosing a quaternary ammonium ion effect on frequency. In *in vitro* experiments the time required to change the bathing solution may be important. By the time the recording is again begun, whatever brief effect these ions may have had is probably over. Moreover, the usual presence of a high Mg concentration or curare would be antagonistic to a pre-junctional depolarizing effect. A dose-response study in unpoisoned preparations, beginning with small doses, is needed. Also application by iontophoretic or intravascular administration in *in vivo* preparations would be desirable. The *in vivo* antidromic method is advantageous because it enables close and rapid arterial administration (40, 41, 169, 195); the brief excitatory effect is caught before the terminal is depressed.

Although Ca does not directly depolarize motor nerve terminals, its presence is essential if terminal depolarization is to release transmission (114). Recently, Katz & Miledi (115, 116) have demonstrated this in a most impressive fashion. It was learned that in a Ca-deficient medium, Ca iontophoretically released onto frog nerve terminal must precede a stimulus directly applied to the terminal, if transmission is to occur. In light of this, MEPP recording will be adversely affected unless appropriate Ca is present. Closely related to the experiment of Katz & Miledi (115, 116) is the earlier experiment of Elmquist & Feldman (61), who applied Ca both iontophoretically and in the bath, to motor nerve terminal of the rat in a Ca-deficient medium. In each instance, the result was to restore a grossly diminished spontaneous activity to normal. In a similar vein, Hubbard (92) found that the enormous augmentation of MEPP frequency ordinarily caused by high K does not occur unless Ca is present. Similarly, the post-tetanic increase in MEPP frequency is greatly attenuated in the absence of CA (143). Not surprisingly, therefore, Ca reduction causes MEPP frequency to fall both in frog and mammalian species (21, 61, 79, 92, 95). And, accordingly, restoration of Ca is associated with a recovery of the rate.

Curiously, MEPP discharge, though greatly reduced in frequency, continues despite extreme Ca deficiency (92, 95). Elmquist & Feldman (61) have suggested that intracellular stores of Ca can supply sufficient ion to maintain this activity. However, the more recent work of Hubbard, Jones & Landau (95) indicates that the residual MEPP discharge is truly Ca-independent.

The effect of high Ca concentrations is probably determined by the level of the ongoing discharge. If this is high, elevated Ca tends to depress (61, 62, 79); if it is in the normal range, increasing Ca enhances the discharge, as Hubbard, Jones & Landau (95) point out. The magnitude of the Ca effect is small, however, and not comparable with that of K. Moreover, there

is a species difference. The frog terminal, as del Castillo & Katz (30) observed, does not respond to increased Ca.

The necessity for Ca, if transmission is to occur, could provide an important basis for detecting the action of drugs that may substitute for Ca. For related ions, Elmquist & Feldman (61) have shown that Sr and Ba effectively replace Ca in the maintenance of the MEPP discharge, and Hubbard, Jones & Landau (95) have reported that Mg will also serve this purpose to a limited extent. Thus, all of the alkaline earths support spontaneous activity.

Since cardiac glycosides are presumed to inhibit Na extrusion (83), it is reasonable to ask whether motor nerve terminals may in this way be depolarized indirectly. Birks (11) reported MEPP frequency to increase after exposure to digoxin, but Gage (78), using rat nerve-diaphragm, shows that this effect is due to the alcohol solvent. To avoid this, Gage employed ouabain and saw no effect on MEPP frequency, but his experiments were conducted in a medium containing 11m*M* Mg. Quite contrary to his experience, however, are the data of Elmquist & Feldman (61) who also used the rat and found that ouabain greatly increases MEPP frequency, albeit in the presence of EDTA. These significant differences emphasize the uncertainty of drug evaluation in *in vitro* preparations which are pre-poisoned with Mg; equally, verification of the ouabain effect requires a control. Elmquist & Feldman (61) went on to conclude that ouabain acts by mobilizing membrane-bound Ca, as Govier & Holland (85) described for this drug's action on heart muscle. However, the dose used by Elmquist & Feldman (61) was huge and the proposed effect may not be relevant to any therapeutic action of the glycoside.

Caffeine can increase the MEPP frequency rate about seven fold in rat nerve-diaphragm preparations (61). The effect is equivalent to the increment Hubbard, Jones & Landau (95) report with raising Ca to 10 m*M*. This supports Elmquist & Feldman's (61) suggestion that caffeine frees tissue-bound Ca, as Bianchi (10) showed with skeletal muscle.

The catecholamines, epinephrine and norepinephrine, are like Ca in that they increase MEPP frequency but do not depolarize (102, 124). The mechanism of this pre-junctional effect is not yet understood.

Since an increase in MEPP frequency can indicate depolarization of motor nerve terminals, it is reasonable to ask whether the drug depression of nerve terminal excitability would be manifested by a decrease in this frequency. This question arose in relation to Mg early in the studies of the subthreshold phenomena. Liley (133) described a dose-response relationship for the suppressing action of Mg on spontaneous discharge augmented by K. He further demonstrated that Mg inhibits the exaggerated discharge caused by cathodal current. Like Mg, excess Ca opposes a K-induced acceleration (61). Also consistent with the antagonism of depolarizing influences on motor nerve terminals is the recent finding of Hubbard & Loyning (96) that Mg largely prevents the MEPP frequency increase in response to hy-

poxia. It would be useful to know whether other drugs thought to depress motor nerve terminals will oppose K acceleration.

Some examples of the utility of this approach are afforded by recent work with neomycin, tetanus toxin, and tetrodotoxin. Neomycin is known to block neuromuscular transmission (43), and Elmkuist & Josefsson (65) have disclosed its presynaptic site of action by showing it to inhibit K-accelerated MEPP discharge. In a recent study of tetanus toxin, Parsons, Hofmann & Feigen (163), using rat nerve-diaphragm or mouse intercostal muscle, drove spontaneous discharges to high rates with either K or NH_4. Pre-incubation with tetanus toxin largely opposed this, thereby disclosing its effect on the terminal. Equally noteworthy is the opposite result. In tetrodotoxin paralysis, K acceleration is not inhibited (63). It should also be mentioned that the myasthenic motor nerve terminal responds normally to high K (64).

In review, the device of raising spontaneous discharge with K or NH_4 has not been employed as often as it should be for the evaluation of drugs suspected to be depressant to the nerve terminal.

EPP and MEPP amplitude.—From the pharmacologic standpoint, it is not always clear that either simple recording of MEPP and EPP amplitudes, or quantal analysis based on these is a sure indicator of site or mechanism of drug action. In this approach, the post-junctionally recorded event is the last in a chain, wherein prior points can be affected by drug. Since a drug may act pre-junctionally, post-junctionally or at both sites, the amplitude of the post-junctional potential will be a resultant of drug action wherever that occurs in the sequence.

Curare furnishes an example of the difficulty encountered in this regard. This drug decreases EPP and MEPP amplitudes, as many have observed. By itself the result is no more indicative of site of action than is twitch depression. But by reference to the traditional view of curare as a post-junctional blocker, based chiefly on prevention of ACh depolarization, the effect on end plate recordings is assumed to be post-junctional. However, the fact that curare antagonizes ACh does not preclude the pre-junctional locus as the site of this interaction; good evidence exists to show that both curare and ACh act on motor nerve terminals (41, 169, 192, 196).

The problem of site of drug action may be further obscured by using EPP and MEPP amplitudes to calculate quantal content. Although the quantal content can be calculated independently of amplitude measurements (31), it does not necessarily follow that post-junctional amplitudes recorded in the presence of a drug surely designate the involvement of one or both loci. In the case of curare both amplitudes are depressed concurrently as dose is increased and so the ratio, $EPP_{amp}/MEPP_{amp}$, which is essentially the measure of quantal content, is unaltered (9, 138, 139). The conclusion is that because quantal content is unchanged, the post-junctional membrane is the site of action and, it is tacitly implied, the pre-junctional site is unaffected. A similar result has been ascertained for C10 (19) and this repre-

TABLE I

EFFECTS OF NEUROMUSCULAR BLOCKING DRUGS

Drugs	MEPP Amplitude	EPP Amplitude	Quantal Content[b]	PTR	PDR
Curare	↓ a	↓ a	0(9, 22, 138)	↓ (192)	↓ (36, 174, 219)
ClO	↓ (180)	↓ (180)	[0]	↓ (153)	—
SCh	↓ (101)	↓ (60)	↓ (60)	↓ (195)	—
Mg	↓ a	↓ a	↓ a	↓ (225	↓ (166)
Ca depletion	0 ↓ (55, 68, 143)	↓ (55, 68)	↓ a	↓ (159)	—
Procaine	↓ (68)	↓ (74, 136)	0(136)	↓ (213)	↓ (173)
Tetrodotoxin	0(63, 117, 150)	↓ (76, 117, 149)	[↓]	↓ (153) —	—
Benzoquinonium	↓ (38)	↓ (38)	↓ (38)		↓ (14)
HC-3	↓ (66, 67, 208)	↓ (66, 67, 140, 141, 208)	0 ↓ (66, 67, 140, 141, 208)	↓ (227)	—
Guanethidine	↓ (35, 90)	↓ (35)	[0]	—	↓ (35)
ACh	↑ ↓ (34, 94)	↑ (33)	↓0(39, 94)	↓ (169)	—
Cobra venom	↓ (37)	↓ (37)	[0]	—	↓ (37)
Ether	↓ (109)	↓ (109)	[0]	↓ (215)	—
Botulinum toxin	0(25)	↓ (25, 206)	[↓]	↓ (194)	—

[a] Well known.

[b] Brackets indicate a presumed quantal content based on reported changes in EPP and MEPP amplitudes.

sents a case in which other lines of study indicate a pre-junctional action (see section on Pre-junctional failure). What must be kept in mind is that the pattern of the curare effect becomes an index of a post-junctional action more because of traditional reference than because of any explicit demonstration.

On this basis, other blocking drugs causing a similar proportionate reduction of EPP and MEPP amplitudes have been assigned as post-junctional blockers. Conversely, disproportionate changes in EPP and MEPP amplitudes will lead to increased or decreased values for quantal content and hence influence decisions on site of action. For each of these possible outcomes, other findings described below and summarized in Table I, indicate that quantal measurement per se does not adequately describe locus of action; it may in fact be misleading. That this has been recognized is evidenced indirectly in recent literature in which ancillary techniques are used to verify site of action. The hypothesis and demonstrations of quantized transmission are not the issue; the adequacy of the method for evaluation of drug locus is the concern.

Substances in addition to curare that have been reported to reduce both EPP and MEPP amplitudes are listed in Table I. Other blocking drugs were not included, because for each of the listed substances, information is also available on its effect on post-stimulus repetition generated by mammalian motor nerve terminals. However, the sample is sufficiently representative to enable comparison of effects on end plate amplitudes with either

suppression of PTR or PDR. It will be seen at a glance that nearly all amplitudes are depressed, as one would expect, and that either PTR or PDR is without exception suppressed by these blocking drugs. This latter discloses that the mammalian motor nerve terminal, for each of these drugs, is a site of a depressant action. On the other hand, the diminished MEPP and EPP amplitudes are not decisive with respect to locating the action. Furthermore, calculation of quantal content from these does not resolve the problem, as the mixed results show.

Pre-junctional effects for many of the substances listed in Table I have been established by various other means. The essential role of Ca in the release of transmission by nerve terminal has been unequivocally demonstrated by the skillful experiments of Katz & Miledi (114, 115). The pre-junctional effect of Mg was disclosed by the appearance of intermittent failures in post-junctional response to indirect stimulation (31). For botulinus toxin, Brooks (25) showed that repetitive stimulation transiently restores MEPP frequency after depression by this poison. Tetrodotoxin in the frog blocks propagation in nerve and muscle but does not interfere with spontaneous MEPPs (76). Moreover, Katz & Miledi (117) found that 100 times the paralyzing dose in the frog does not prevent MEPP release by electrotonic depolarization of the terminals. Strangely, doses of tetrodotoxin too small to affect conduction in either nerve or muscle in the cat are sufficient to abolish the PTR originating from the terminals (153).

The effects of tetrodotoxin, Ca depletion, Mg, and botulinus toxin are similar in their effect on the pre-junctional mechanism. This has been summarized well by Thesleff & Quastel (207). It is known that changes in terminal membrane potential develop independently of Ca but that transmission occurrence following terminal depolarization is entirely dependent on its presence (114, 115). This has led to the idea of a depolarization-release coupling system which is linked by Ca and disrupted by tetrodotoxin, Mg, and botulinus toxin. It seems therefore that in the cat the release of PTR by interaction of a nerve terminal and adjacent axon involves a similar coupling of depolarization and activation, except of course that in this instance a transmitter is not implicated. Certainly when judged by PTR suppression, the motor nerve terminal of the cat is exceedingly sensitive to Mg (225, 226), Ca depletion (159), and tetrodotoxin (153); botulinus toxin (194) was not quantitatively studied.

It may also be noted from Table I, with respect to Mg, Ca depletion, tetrodotoxin and botulinus toxin, that quantal content is reduced. In these instances quantal calculation points pre-junctionally and does so because of the peculiar disproportionate action of these drugs on EPP and MEPP amplitudes. In the case of the quaternary ammonium ions, SCh, ACh, C10, HC-3, guanethidine, benzoquinonium, and not least, curare, quantal content is overall not indicative. If the so-called depolarizing blockers are separated, viz., ACh, C10, and SCh, the tendency is for a decrease in quantal content. This accords with the previously cited clear indications of a pre-junc-

TABLE II

EFFECTS OF DRUGS FACILITATING NEUROMUSCULAR TRANSMISSION

Drugs	MEPP Amplitude	EPP Amplitude	Quantal Content[b]	PDR
Neostigmine	↑ a	↑ a	[↑]	+(13, 166)
Physostigmine	—	↑ a	—	+(69)
Edrophonium	0(15)	↑ (15, 127, 148)	[↑]	+(13, 169)
Ambenonium	↑ (15)	↑ (15)	[↑]	+(13)
Methoxyambenonium	0(15)	↑ (15, 108)	[↑]	0(14, 16)
SCh	↓ (101)	↓ (60)	↓ (60)	+(16, 195)
ACh	↑ ↓ (34, 98)	↑ (33)	↓ 0(39, 98)	+(41, 169)
K	↓ (133)	—	↑ (39, 162, 201)	+(40, 41)
TEA	↓ (164)	↑ (108, 121, 127, 199, 200)	[↑]	0(128)
Guanidine	0↓ (87, 160)	↑ (89, 160)	↑ (87, 89, 160)	+(70)
Veratrine	↑ 0(87, 88, 91)	↓ (87)	↑ 0(87, 89)	+(70)
Epinephrine	0(124)	↑ (124)	[↑]	0(14)

[a] Well known.

[b] Brackets indicate a presumed quantal content based on reported changes in EPP and MEPP amplitudes.

tional action of each. For the nondepolarizing blockers the results are not decisive but tend towards no change in quantal content. If other similar blockers are added to this list, viz., procaine, cobra venom, and ether, the pattern is that of curare. The veracity of this implication is questionable, primarily on the basis of the consistent effects of all of the substances in Table I on PTR and PDR.

Of facilitatory compounds, the physostigmine-like drugs are most important. They have been traditionally regarded as enlarging post-junctional response primarily because of their known anti-ChE property. Thus, when it was found (68) that these drugs augment both EPP and MEPP amplitudes, this was generally accepted as a post-junctional effect, essentially a counterpart to the curare effect. Those facilitatory drugs which have been tested by quantal measurement and for PDR production are listed in Table II. Here it is evident that quantal measurements point mainly to a pre-junctional locus. The PDR effects are quite irregular. However, on further examination, the differences point up the respective significances of the two methods in the evaluation of facilitatory drugs.

Until recently no quantal analysis has been reported for the physostigmine-like drugs. Therefore, the findings of Blaber & Christ (15) are highly significant. For cat tenuissimus *in vitro* in a normal physiologic solution it was found for neostigmine, edrophonium, and ambenonium that threshold concentrations for increasing MEPP and EPP amplitudes differed. For neostigmine, 10 times more drug was required to elevate the MEPP amplitude; for ambenonium 100 times more drug was necessary and, for edrophonium, MEPP amplitude was unchanged over a 100-fold range of dose which progressively elevated EPP amplitude. These precise dose-response

studies in a circumstance uncomplicated by the presence of blocking drugs indicate that these facilitatory drugs raise the quantal content. The finding of Hubbard, Schmidt & Yokota (98) that neostigmine slightly decreases it was made in a Mg-rich solution and may reflect synergism of depressant actions to which Freeman (72) has called attention.

Since many of the physostigmine-like drugs are quaternary ammonium ions, direct depression of neuromuscular transmission as a function of dose is to be anticipated. Whether this is pre- or post-junctional, the biphasic actions will suffice to cloud quantal measurement. The result of Blaber & Christ (15) probably was influenced by their attention to dose and avoidance of other blocking drugs.

The possible influence of Mg on quantal calculation for drug evaluation may be appreciated from the studies of Muchnik & Gage (144) and Ginsborg (80). The former found bromide to reduce the quantal content in frog neuromuscular experiments in which Mg-block was employed; Ginsborg ascertained that bromide, replacing up to 95 per cent of Cl, does not alter quantal content in a frog ganglion transmitting in a normal solution. Ginsborg cogently concludes that a presynaptic site of bromide is not a general phenomenon, and expresses concern for a possible Mg bias in the neuromuscular experiments.

SCh and ACh, because of their biphasic actions, pose a difficult problem for post-junctional measurements. In each instance, quantal content decreases (39, 60, 98), pointing to a pre-junctional depressant action. This concurs with the potent PTR suppression observed in the cat preparation (169, 195). However SCh, in small dose in mammalian species, is a strong facilitatory drug (195); ACh is weak (169). In the cat, post-drug repetition by SCh can be produced at will, without block, since these two dose-response curves separate (195). For ACh, these dose-response curves are concurrent and the facilitatory effect is only caught in transit. Although quantal studies have not detected these facilitatory actions, it is conceivable that they would if attention were given to dose-response and if neuromuscular blocking drugs were omitted from the bathing solutions. Nevertheless, the difficulty will be compounded by whatever post-junctional depolarization these ions cause. It may not be possible to disentangle biphasic actions and multiple loci when recording post-junctionally.

For the remaining drugs in Table II, with the exception of K, the antidromic monitoring method has been of no value in detecting their actions on mammalian motor nerve terminals. It is possible, however, that drug interaction would aid in uncovering the responsiveness of motor nerve terminals by the PDR method. For example, Kensler (119) pretreated a cat nerve-muscle preparation with a small dose of neostigmine, after which TEA released a sizeable twitch potentiation. This probably reflects the PDR of motor nerve terminal, in which case similar pre-conditioning of the terminal would render it susceptible to drugs having TEA-like actions. Also, methoxyambenonium (16) and norepinephrine (14) have been discerned to

act on motor nerve terminals because of their action to enhance facilitatory drug PDR.

The veratrum drugs are a special case in view of their limited selectivity. PDR does arise but the cat motor nerve is generally involved (70). The nerve terminals in rat and man are known to be included in this veratrine action, since MEPP frequency is increased by these alkaloids (87, 89, 91). In the frog, a similar effect is manifest (75).

Other methods have successfully disclosed the pre-junctional actions of TEA and guanidine. Koketsu (121), recording from intramuscular axon in the frog, observed that TEA lengthens the negative after-potential; Beaulieu, Frank & Inoue (8) and Dun & Feng (59) found TEA and guanidine, to cause stimulus-bound antidromic discharges to develop in frog intramuscular axon (see Section on Amphibian responses). The action of guanidine on mammalian motor nerve terminals is evident from an increased frequency of spontaneous MEPPs (87, 89). In the frog, Otsuka & Endo (160) observed a guanidine action on the terminals to release "giant" or multiquantal MEPPs.

In sum, the facilitatory actions of the drugs in Table II, with the exception of ACh and SCh, may be said to be consistently revealed by quantal measurement but not by PDR. It is noteworthy that all of these drugs are anti-curare. Therefore, EPP and MEPP amplitudes, during transmission block, are certain to be increased, whether the antagonism occurs pre- or post-junctionally. Further, the fact that quantal content may increase, strictly implies only that the EPP amplitude is relatively more augmented than the MEPP; it suggests, but does not necessarily signal a pre- or post-junctional site. Moreover, the reversal of neuromuscular block, which the increase in quantal content depicts, does not denote a particular facilitatory mechanism, as witnessed by the number of mechanisms which the drugs in Table II represent.

In contrast, PDR provoked by physostigmine-like drugs is a distinctive facilitatory action, essentially characteristic for that group; SCh and ACh are chemical congeners to the physostigmine-like drugs and hence exhibit the typical effect.

A major portion of this review has been devoted to a documentation of the site and mechanism of this unusual facilitation. Therefore, it is crucial to recognize that the PDR technique reveals a single specific type of facilitatory drug action occurring only in mammalian preparations.

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