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INTERACTION BETWEEN FACILITATING AND DEPOLARIZING DRUGS AT THE NEUROMYAL JUNCTION OF THE CAT[1, 2]

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ABSTRACT

BLABER, L. C. AND A. G. KARCZMAR: Interaction between facilitating and depolarizing drugs at the neuromyal junction of the cat. J. Pharmac. exp. Ther. **156: 55–62, 1967.** A number of oxamides and other facilitating drugs have been examined for their ability to change the action of depolarizing drugs at the neuromyal junction from augmentation of the twitch followed by blockade to that of only augmentation ("reversal"). It has been observed that each of the drugs producing reversal possessed both facilitating and curaremimetic properties and that reversal was produced by the dose at which the two antagonistic properties were approximately in equilibrium. The pre- and postsynaptic actions of the depolarizing and the reversal-producing drugs have been discussed and a possible mechanism of action for reversal suggested.

In 1957 Karczmar reported that the oxamide methoxyambenonium (WIN 8078) exhibited the unusual property of being able to antagonize both *d*-tubocurarine (*d*-TBC) and decamethonium bromide (C_{10}) blockade of skeletal neuromyal transmission in cats. Subsequently, Blaber (1960) suggested that the antagonism of C_{10} was due to the curaremimetic properties which methoxyambenonium exhibits at dose levels higher than those producing the facilitating effects which are responsible for the *d*-TBC antagonism.

Karczmar (1957) also reported that the same compound produced the "reversal" of depolarizing blocking drugs; in the course of reversal the usual effect of depolarizing agents, consisting of augmentation of the twitch and of subsequent neuromyal blockade, was converted to that of only augmentation.

It was decided to examine this effect further to see if the reversal phenomenon could be explained by the known actions of the drugs in question. Oxamides closely related to methoxyambenonium have been used in this study, as well as facilitatory drugs differing from oxamides in their chemical structures.

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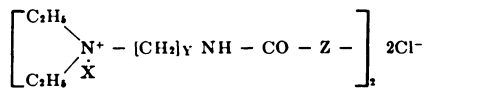
[2] A preliminary communication of the work was presented in Fedn Proc. Fedn Am. Socs exp. Biol. **23: 543, 1964.**

METHODS. A total of 60 cats, anesthetized with chloralose (80 mg/kg i.v.) were used throughout the experiments. Shielded platinum electrodes were placed on the tibial nerve and the sciatic nerve ligated in the popliteal space. The limb was set up in a horizontal position and fixed rigidly. Twitches of the tibialis anterior muscle, recorded on an Offner Dynograph with a Statham force transducer, were excited once every 10 sec by rectangular pulses to the tibial nerve of 50-μsec duration and twice the strength required to evoke a maximal twitch. The blood pressure and muscle temperature were monitored; the latter was maintained at 37°C by means of heat lamps.

Drugs were injected i.v. or close-arterially (c.a.). For close-arterial injections the modified (Blaber, 1960) technique of Brown (1938) was used; the popliteal artery was occluded peripherally to the posterior tibialis artery and the sural artery.

Effective dose ranges of the facilitatory compounds have been established with regard to potentiation of the twitch response to indirect stimulation, neuromyal blockade, antagonism of *d*-TBC, as well as to reversal of action of succinylcholine (SCh). Generally, facilitatory drugs were administered c.a.; SCh was given i.v. The dose ranges for each specific effect were determined by administering the drugs in question, at 30- to 45-min intervals, in logarithmic dose increments. Three to five cats were used for each drug; at least six dose levels were evaluated in each cat. The same

TABLE 1



Compound[a]	Structure X	Y	Structure Z
WIN 3286	Benzyl	2	
WIN 8077	2-Chlorobenzyl	2	
WIN 8078	2-Methoxybenzyl	2	
WIN 8626	2-Chlorobenzyl	3	
WIN 12305	2-Chlorobenzyl	2	$(CH_2)_3$
WIN 8627	2-Chlorobenzyl	2	CH_2

[a] Also used was WIN 12423, an asymmetrical compound N,N-bis(diethylaminoethyl)oxamide 2-chlorobenzyl chloride.

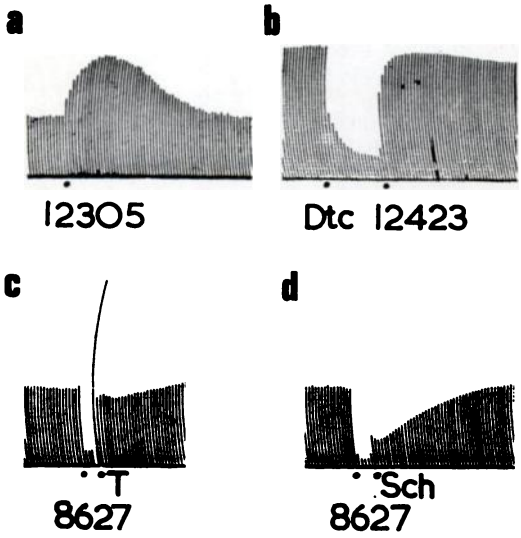


FIG. 1. The facilitatory and curaremimetic effects of oxamides. a, Cat, 2.5 kg; b, cat, 2.85 kg; c and d, cat, 3.6 kg. Maximal twitches of the anterior tibialis muscle were elicited once every 10 sec. At 12305, 80 μg of WIN 12305; at 12423, 5 μg of WIN 12423; at 8627, 500 μg of WIN 8627; and at Sch, 15 μg of succinylcholine chloride were injected c.a. At Dtc, 750 μg of *d*-tubocurarine were injected i.v., and at T the nerve was stimulated at 100/sec for 5 sec.

series of experiments were used to determine twitch augmenting and blocking doses.

The method employed for recording ventral root potential was described previously (Blaber and Bowman, 1963a); the muscle twitch was recorded simultaneously with ventral root and muscle potentials.

The drugs used were chlorides of the oxamides, WIN 8078, ambenonium (WIN 8077), WIN 3286, WIN 12305, WIN 8627, WIN 8626 and WIN 12423; neostigmine bromide, tetraethylammonium chloride (TEA), 3-hydroxyphenyl-triethylammonium iodide (3-OH PTEA), C_{10}, SCh and *d*-TBC were also employed. All doses were expressed in terms of salts. The chemical structures of oxamides are given in table 1.

RESULTS. *Facilitation.* The ability of a compound to produce facilitation was determined by its ability to augment the twitch amplitude and to antagonize paralysis produced by *d*-TBC.

All of the compounds studied, including the oxamides, 3-OH PTEA and TEA, produced antagonism of *d*-TBC; all of these compounds also augmented the twitch except for WIN 8078 and WIN 8627. Examples of facilitatory effects of oxamides are shown in figure 1, a and b; the effective facilitatory dose ranges are given in figure 2. As can be seen, the first effective doses of the compounds in question varied widely. WIN 8077 and WIN 3286 augmented the twitch in c.a. doses of less than 1 μg, while a dose of 500 μg was necessary in the case of TEA (fig. 2). Anticurare potency also differed markedly from compound to compound. While the sequence of potencies was similar for all compounds with regard to both anti-*d*-TBC and twitch-augmenting action, the dose ratio for these two effects varied. In fact, these actions seemed occasionally independent, as in the case of WIN 8078, a potent anti-*d*-TBC compound, for which a twitch-augmenting action was rarely observed. It should be added that the facilitatory actions, whenever obtained, were not always of similar duration or magnitude, being particularly short-lived in the case of 3-OH PTEA and weak in that of TEA (*cf.* also next section).

After the first observable facilitation, increasing the dose produced, up to an optimum, increased facilitation. Further increase in dose produced, sequentially, less facilitation and then blockade.

Neuromyal blockade. Neuromyal blockade was produced with sufficient doses of all the compounds tested. Except for the paralysis induced by neostigmine, all compounds produced blockade resembling that of *d*-TBC. Thus, the paralysis was relieved by tetanic stimulation of the nerve and by close-arterial administration of depolarizing blocking drugs

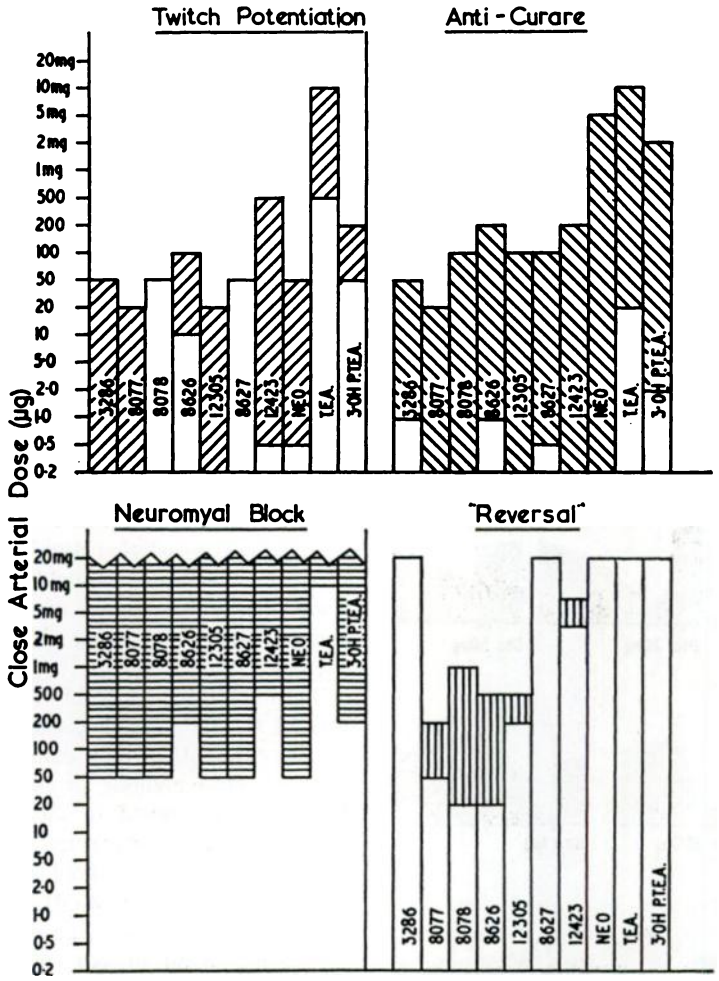


FIG. 2. Comparison of the effectiveness of oxamides and certain other drugs in eliciting twitch potentiation, anticurare action, neuromyal blockade and reversal. The hatched portions of the histograms represent the dose ranges in micrograms at which the compounds administered close-arterially produced the various responses. The open bars indicate dose ranges at which the compounds in question were found ineffective in producing the pertinent response.

(fig. 1, c and d). However, the paralysis produced by the oxamides was not relieved or only weakly relieved by the administration of neostigmine or edrophonium; therefore, the neuromyal blockade due to oxamides more closely resembled that caused by benzoquinonium (Bowman, 1958; Karczmar, 1961).

The effective dose ranges of all compounds capable of neuromyal block are given in figure 2. As can be seen, the first effective blocking doses differed between 50 µg c.a. (WIN 3286, WIN 8077, WIN 12305, WIN 8627 or neostigmine) and 10 mg c.a. (TEA).

Reversal. Reversal occurred with five of the compounds studied when they were administered c.a. 1 min previous to the depolarizing blocking drug; for all of these compounds, reversal was produced close to the dose where facilitation was superceded by neuromyal blockade (*cf.* fig. 2). An example of reversal produced by an oxamide, WIN 8626, is given in figure 3). The optimal reversal was produced by a dose which was intermediate between the extremes of the effective reversal-producing dose range This dose did not necessarily coincide with the dose producing the first observable blockade.

Those compounds with which reversal could not be observed were WIN 3286, WIN 8627, neostigmine, 3-OH PTEA and TEA, admin-

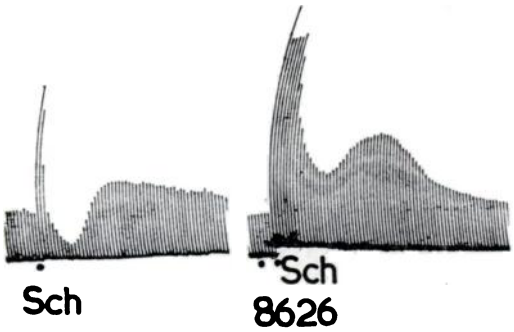


FIG. 3. The reversal of the response of succinylcholine chloride by WIN 8626. Cat, 2.0 kg. Maximal twitches of the anterior tibialis muscle were elicited once every 10 sec. At Sch, 45 μg of succinylcholine chloride were injected i.v.; at 8626, 50 μg of WIN 8626 were injected c.a.

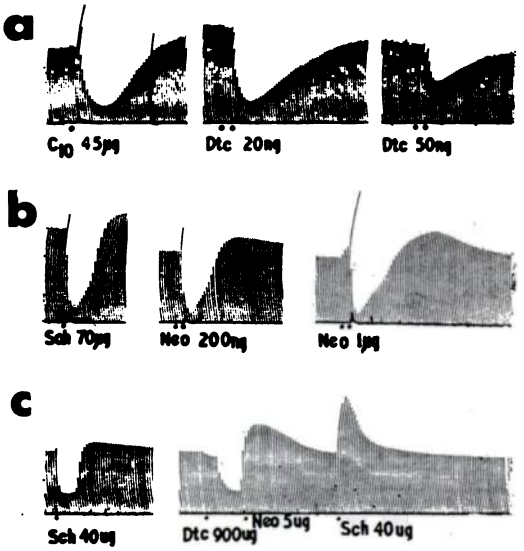


FIG. 4. The interaction between a depolarizing drug and (a) a curaremimetic drug, (b) a facilitatory drug and (c) a combination of a curaremimetic and a facilitatory drug. a, Cat, 2.1 kg; b, cat, 4.25 kg; c, cat, 2.7 kg. Maximal twitches of the anterior tibialis were elicited once every 10 sec. In a, at C_{10}, decamethonium bromide was injected i.v.; at Dtc, *d*-tubocurarine was injected c.a. followed 1 min later by 45 μg of decamethonium bromide i.v. In b, at Sch, succinylcholine chloride was injected i.v.; at Neo, neostigmine bromide was injected c.a. followed 1 min later by 70 μg of succinylcholine chloride i.v. In c, at Sch, succinylcholine chloride and, at Dtc, *d*-tubocurarine was injected i.v., and, at Neo, neostigmine bromide was injected c.a. a, b and c are 3 separate experiments, the time interval between panels was 1½ hr in a and 1 hr in b and c.

istered c.a. WIN 3286 had weak and transient actions in producing both facilitation and blockade. In low doses, it produced very little effect on the response to the depolarizing drugs; high doses (100–200 μg c.a.) produced some reduction of the blocking action of the depolarizing drugs.

WIN 8627 had a weak but prolonged facilitatory action, which could be observed generally at doses at which neuromuscular blockade was also produced. In these cases, a short blockade was followed by facilitation, which at higher doses (500 μg c.a.) was marked and prolonged.

Small doses of neostigmine (5–200 ng c.a.) potentiated the initial stimulant effect of the depolarizing blocking drugs without affecting significantly the depth of blockade; when used at higher doses (0.5–5 μg c.a.) neostigmine rapidly converted the initial stimulation by SCh or C_{10} into a blockade which was deeper than that of the control dose (fig. 4b). Reversal was never seen with neostigmine. It should be stressed that the blocking action of neostigmine did not resemble that of *d*-TBC, since tetanic stimulation of the nerve, acetylcholine and depolarizing drugs all deepened the block.

3-OH PTEA and TEA are not chemically related to the oxamides, which they resemble to the extent that they exhibit both facilitating and curaremimetic properties. These compounds were studied to ascertain whether or not the oxamide structure was necessary to produce reversal. It should be stressed that facilitatory effect of 3-OH PTEA was very transient (100 μg c.a. produced twitch augmentation for 30–40 sec). In order to increase the duration of action, this drug was given i.v.; under these circumstances reversal could be obtained (fig. 5). TEA exhibited very weak facilitating properties, and reversal was never obtained with this drug, since possibly, at the doses required, the curaremimetic effect of this

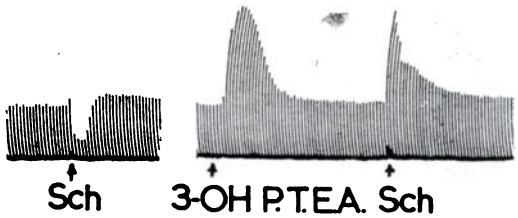


FIG. 5. The interaction between 3-OH PTEA and a depolarizing drug. Cat, 2.0 kg. Maximal twitches of the anterior tibialis muscle were elicited once every 10 sec. At Sch, 45 μg of succinylcholine chloride were injected i.v. and at 3-OH PTEA 5 mg of 3-hydroxyphenyl-triethylammonium iodide were injected i.v.

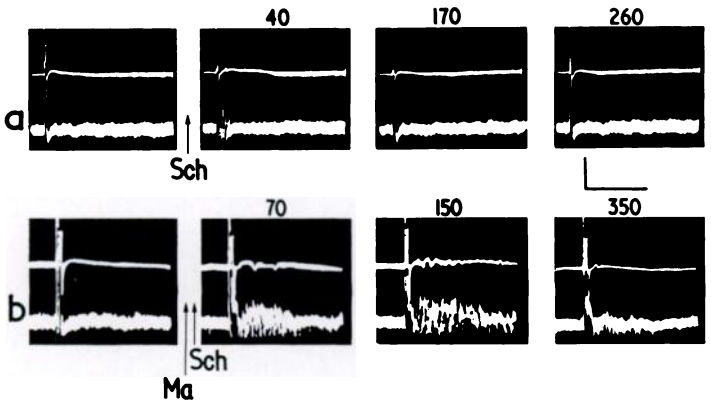


FIG. 6. Cat, 2.75 kg. Muscle action potentials were recorded with a concentric needle electrode (upper beam) and nerve action potentials were recorded antidromically from the ventral root (lower beam) in response to stimulation of the motor nerve with single supramaximal shocks once every 10 sec. a, Repetitive firing and antidromic discharges produced by 60 μg of succinylcholine chloride (Sch) i.v.; b, repetitive firing and antidromic discharges produced by 20 μg of methoxyambenonium (Ma) c.a. followed 2 min later by 60 μg of succinylcholine chloride i.v. The figures above the panels denote the time in seconds after the succinylcholine chloride injection. One hour elapsed between a and b. Time calibration: a, 80 msec; b, 40 msec. Voltage calibration: a, muscle 10 mV, nerve 100 μV; b, muscle 10 mV, nerve 40 μV.

compound overshadowed its facilitating properties.

Since a combination of facilitating and curaremimetic properties was exhibited by those drugs which were capable of producing reversal, a combination of *d*-TBC and neostigmine was administered before the injection of a depolarizing drug, the drug combination possibly possessing the properties of a single agent such as an oxamide. Small doses of *d*-TBC (5–20 ng c.a.) given immediately before the depolarizing blocking drug first of all reduced the early facilitatory effect of the latter without appreciably changing the depth of blockade; as the dose of *d*-TBC was increased (50 ng–1 μg c.a.), the depth of the depolarizing blockade was decreased (fig. 4a). The combination of *d*-TBC and neostigmine was then administered; in these experiments, 50% blockade with *d*-TBC was antagonized by neostigmine and the depolarizing drug administered when the muscle twitch returned to control amplitude. Under these conditions, the reversal was readily obtained (fig. 4c).

Production of antidromic potentials in the ventral root. SCh was employed in these experiments because of its short duration of action. SCh, 40 to 100 μg i.v., produced antidromic discharges in the ventral root after orthodromic stimulation of the nerve. The discharges occurred during the stimulant phase of the drug action upon muscle twitch and could be observed for a period of 1 to 2 min. The antidromic discharges became progressively less as the blocking action of the depolarizing drug increased. Small doses of SCh (10–40 μg i.v.) which produced only augmentation of muscle twitch produced antidromic discharges after orthodromic stimulation throughout the duration of the stimulant action of the drug. WIN 8078 produced no antidromic discharge when administered c.a., alone, confirming the results of Blaber and Bowman (1963a). However, in the course of reversal, induced by the administration of WIN 8078 followed by SCh, antidromic discharges could be readily demonstrated upon orthodromic stimulation (fig. 6). These discharges lasted for a period of 8 to 10 min; hence their duration was 5 to 10 times longer than that of discharges due to SCh alone (see above).

DISCUSSION. Five compounds (fig. 2) converted the classical pattern of action of depolarizing agents at the neuromyal junction, consisting of short-lived facilitatory and more pronounced blocking effects, into an action consisting of only twitch-potentiating effect; this constitutes reversal (Karczmar, 1957, 1961). The five compounds in question possessed facilitatory (twitch-potentiating and anticurare) as well as competitive blocking properties. Moreover, in the presence of WIN 8078, the most potent of reversal-producing compounds, the number of stimuli accompa-

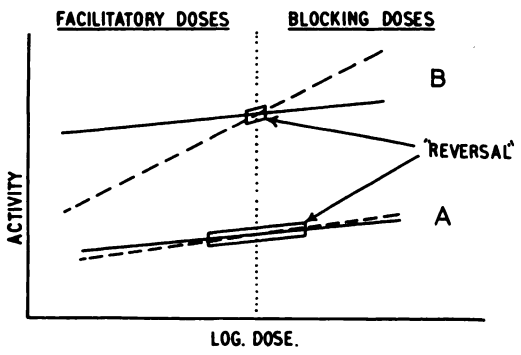


FIG. 7. Hypothetical dose-response curves for two compounds A and B exhibiting both facilitating and blocking properties at the neuromyal junction: ——, curve for facilitatory effects; – – –, curve for blocking effects.

nied by SCh-induced antidromic firing was greater than that of stimuli causing the repetitive firing with SCh alone. It may be suggested that the interplay between these various activities leads to reversal, and that particularly the combination of curaremimetic and facilitatory action is important in this context. During reversal, the facilitatory action of depolarizing compounds may be added on to that of the oxamide or hydroxyanilinium drugs; at the same time, the curaremimetic effect of the oxamides and the hydroxyaniliniums prevented undue depolarization at the endplate by SCh or by C_{10}, this effect corresponding to the antagonism between *d*-TBC and depolarizing drugs, demonstrated first by Dallemagne and Philippot (1952). The net result of the interplay between these two actions should be prolonged facilitatory action, provided these actions are at equilibrium. This point was illustrated in the experiment in which instead of oxamides, *i.e.*, drugs endowed with both curaremimetic and facilitatory action within similar dose range, neostigmine, which showed no blocking action within the facilitatory range, was combined with *d*-TBC to produce reversal of SCh. In this experiment, the combined effects of *d*-TBC and of neostigmine were balanced at the time of injection of the depolarizing agent, the twitch being at the control height.

The compounds which did produce reversal had widely differing dose ranges. WIN 8078 produced some degree of reversal when administered in c.a. doses of between 20 μg and 1.0 mg, whereas WIN 12305 produced reversal in c.a. doses of 200 to 500 μg. This can be explained if it is assumed that for a particular compound the dose-response curve for facilitation is different from that for the curaremimetic action. Thus, taking two extreme cases (fig. 7), compound A with dose-response curves of similar slopes exhibits a wide range for reversal, whereas compound B with widely differing dose-response curve slopes has a very narrow range for reversal. Compounds A and B may represent WIN 8078 and WIN 12305, respectively. The near equality of facilitatory and blocking actions required for the reversal would explain at the same time the lack of twitch potentiation by WIN 8078, which otherwise would appear surprising as this compound is a potent *d*-TBC antagonist. Yet, it is more difficult to produce twitch potentiation than to antagonize *d*-TBC; in the presence of the latter the activation of a minimal number of units is sufficient to restore transmission, *i.e.*, the safety factor of transmission blocked by *d*-TBC is lower than that which obtains in the case of supramaximal twitch response.

Reversal was observed to occur at or about the dose level at which the neuromuscular blocking properties were first observed; optimal reversal did not occur at this dose but presumably at the dose level where the two opposing effects were in equilibrium; this depended upon the relative extent of each effect, its time course and kinetics. In the case of those compounds (WIN 3286 and WIN 8627) which exhibited weak facilitatory and/or weak blocking action, reversal was not seen, presumably since the facilitatory and blocking properties could not be in equilibrium at any dose. Moreover, WIN 8627 exhibited potent blocking action of prolonged duration, and perhaps it could not produce reversal, since in its particular case the equilibrium between the facilitatory and blocking actions did not persist for a sufficient time period.

The nerve terminal, as possibly involved in reversal, deserves special comment. Several oxamides (Blaber and Bowman, 1963a,b; Karczmar *et al.*, 1965) and hydroxyaniliniums (Riker *et al.*, 1957; Werner, 1961) have been shown to have an action at the motor nerve terminal. The mechanism of action of the

depolarizing drugs has previously been thought to be solely at the endplate, but recent publications have stressed actions at the motor nerve terminal (Edwards and Ikeda, 1962; Segawa *et al.*, 1965; Kato and Fujimori, 1965; Standaert and Adams, 1965; Wikinski *et al.*, 1965). The present results have also shown that SCh produces antidromic firing in the motor nerve after orthodromic stimulation during facilitation, supporting the results of Standaert and Adams (1965), and that antidromic firing can be observed during reversal. It is possible, therefore, that the depolarizing drugs produced at least part of their facilitation by nerve terminal action; this would explain why low doses of *d*-TBC which are known to abolish the antidromic firing associated with nerve terminal action (Riker *et al.*, 1957; Werner, 1961; Blaber and Bowman, 1963a,b) selectively prevented facilitation by the depolarizing drugs.

WIN 8078 seemed in these, as well as in earlier experiments, incapable of producing antidromic firing in the motor nerve (Blaber and Bowman, 1963a; Karczmar *et al.*, 1965); however, after this drug, the number of orthodromic stimuli after which antidromic firing was observed in the presence of SCh increased markedly. Neostigmine, capable of augmenting the stimulant effect of depolarizing drugs (Zaimis, 1951) also exhibits nerve terminal actions (Masland and Wigton, 1940; Blaber, 1963; Blaber and Bowman, 1963a). Thus, the nerve terminal action of combinations of SCh with oxamides and hydroxyaniliniums may have contributed to the reversal in conditions in which the motor endplate was protected from blocking actions of the depolarizers. It should be remembered that the blocking effect of curaremimetic drugs may be more intense at the nerve terminal than at the endplate (Werner, 1961; Blaber and Bowman, 1963a; Standaert, 1964). It has to be assumed, therefore, that the facilitatory action of the oxamides or hydroxyaniliniums in combination with SCh was sufficient to overcome the blocking action of the reversal-producing drugs at the nerve terminal.

The foregoing hypothesis, that the reversal of the action of depolarizing drugs by certain compounds depends upon a combination of facilitatory and curaremimetic action, could not be applied automatically to all the drugs studied. Failure to obtain reversal with certain compounds has already been mentioned and could be formally explained by the "equilibrium" theory. Certain additional difficulties encountered in explaining the action of WIN 8087 were also discussed.

Altogether, the hypothesis in question is based on a number of assumptions as well as upon a vectoral approach to the problem, and other explanations may be possible. Karczmar (*cf.* Karczmar, 1957; Karczmar *et al.*, 1961, 1965; *cf.* also Koelle *et al.*, 1963) suggested on the basis of the results obtained with the frog neuromyal junction that the reversal by oxamides and hydroxyaniliniums may be based upon their unique property of augmenting depolarization and endplate potential without prolonging it (sensitization); conventional reversible anticholinesterases such as physostigmine and neostigmine strikingly prolong depolarization and the endplate potential and do not produce reversal. Although the results obtained on the frog do not apply directly to the cat, it may be that this sensitizing action of reversal-producing agents is also of importance in the present context, since no evidence was obtained in these experiments to indicate that such an action does not contribute to the reversal obtained in the cat.

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