Brit. J. Pharmacol. (1966), **26**, 218–228.

A COMPARISON BETWEEN THE EFFECTS OF TETRAETHYLAMMONIUM AND TRIETHYL-(3-HYDROXYPHENYL)AMMONIUM ON FROG NEUROMUSCULAR TRANSMISSION

BY

A. S. KUPERMAN* AND M. OKAMOTO

From the Department of Pharmacology, Cornell University Medical College, New York City, U.S.A.

(*Received June* 24, 1965)

The pharmacological effects of certain ethonium ions have been compared in vertebrate nerve-muscle preparations (Kuperman & Okamoto, 1964, 1965). These compounds are of special interest because, despite their exceedingly weak anticholinesterase activities, they are potent facilitatory drugs; that is they increase contractile responses to single maximal nerve shocks (throughout the paper this phenomenon is referred to as " potentiation "), and they antagonize the paralytic effect of tubocurarine. Two ethonium ions, tetraethylammonium (TEA) and triethyl(3-hydroxyphenyl)ammonium (3-OH-PTEA), have been studied in particular detail in the cat, and the results indicate that they have different mechanisms of facilitatory action. These mechanisms can be differentiated by tubocurarine because the neuromuscular actions of 3-OH-PTEA are exceedingly sensitive to suppression by tubocurarine, whereas those of TEA are relatively resistant.

In the present investigation, various effects of the two ethonium ions have been studied more intensively on an isolated frog nerve-muscle preparation. Drug concentrations were precisely controlled under steady-state conditions, and the results were analysed more quantitatively than was possible in previous studies based on *in situ* techniques. Furthermore, some interesting pharmacological differences between the mammalian and amphibian neuromuscular junction have been revealed.

METHODS

All tissues used in these experiments were isolated from the frog (*Rana pipiens*) and kept at room temperature (20 to 22°C) in a buffered Ringer solution (*p*H 7.2) of the following composition (mM): 110.88 NaCl, 2.0 KCl, 1.8 $CaCl_2$, 0.1 NaH_2PO_4 and 2.02 $NaHCO_3$.

Neurally-evoked contraction. The sartorius nerve-muscle preparation was used, and the technique for recording contractile responses produced by single maximal nerve shocks was as described previously (Kuperman & Okamoto, 1964). The nerve shocks, consisting of 0.01 msec rectangular pulses, were applied once every minute through a pair of platinum electrodes. Only one concentration of an ethonium was tested on each preparation. Anticurare activity or contractile potentiation was measured as the maximum increase

* Present address: Department of Physiology and Health, Hunter College of the City University of New York, New York City, U.S.A.

in amplitude, calculated as a percentage of the control, during a 30 min period of drug treatment. In the range of concentrations used, the ethonium ions produced their maximum effects well within the 30 min period.

Directly-evoked contraction. The muscle organ-bath contained 5×10^{-5} g/ml. of tubocurarine. Rectangular pulses of 2 msec duration were applied directly to the sartorius muscle once per min. A platinum electrode plate was placed under the muscle belly and another was placed under the tendon. Stimulus voltage was adjusted to cause a contraction amplitude equal to that produced by a maximal nerve shock in the absence of tubocurarine.

Nerve action potentials. Spontaneous and stimulus-evoked potentials were recorded from isolated sciatic nerves, sheathed or desheathed. For the study of repetitive after-discharge, nerves were mounted in a three-compartment chamber. The centre compartment, 1 cm in length, contained the test solution. A pair of platinum stimulating electrodes was placed on one side of the centre segment of nerve, and recording electrodes were placed on the other side. The nerve under the distal recording electrode was crushed. A rectangular pulse of 0.01 msec duration was delivered once every 2 sec. The stimulus voltage was supramaximal for the rapidly conducting A fibres. The monophasic action potentials were recorded in air with a differential amplifier, displayed on a cathode-ray oscilloscope and photographed.

In order to test for spontaneous activity, recording electrodes were placed on both the treated and untreated segments of nerve. Bipolar recordings in air or mineral oil were made at periodic intervals.

Materials. TEA iodide, 3-OH-PTEA iodide and cetrimonium bromide were obtained from Eastman Kodak. The bromide salt of diethyl(3-hydroxyphenyl)methylammonium (3-OH-DEMPA) was supplied by Hoffman-La Roche. Crystalline (+)-tubocurarine was purchased from Mann Research Laboratories (U.S.A.). All compounds were dissolved in the frog-Ringer solution.

RESULTS

Effects on neurally-evoked contraction in the absence of tubocurarine

The maximum contraction produced by a single nerve volley is potentiated by either 3-OH-PTEA or TEA, and the dose/response regression lines are illustrated in Fig. 1. Within the range of concentrations used for calculating these lines, the response varies

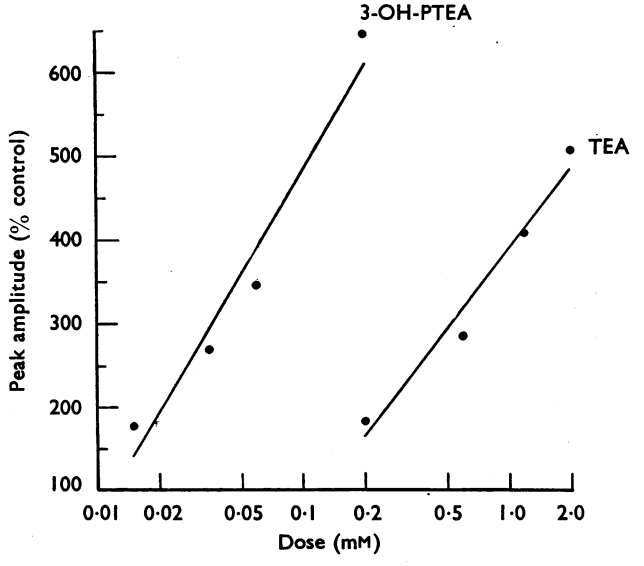

Fig. 1. Relationship between dose (on log scale) and magnitude of potentiation of neurally-evoked contractions. Each point is the average of nine independent observations.

linearly in relation to log dose ($P<0.05$). Beyond this range the linear relationship is lost, and concentrations greater than 5 mM cause depression of the neurally-evoked twitch. Slopes of the regression lines of Fig. 1 are not significantly different ($P>0.2$) but 3-OH-PTEA is about fifteen times more potent than TEA and produces a greater maximum response. In fact, the maximum contractile tension produced by 3-OH-PTEA is equal approximately to the maximum neurally-evoked tetanic tension (200 impulses/sec for 10 sec).

The potentiating action of TEA or 3-OH-PTEA is rapid in onset, but thereafter develops slowly, not reaching its peak until after several contractions (Fig. 2,*a*). With continuous presence of drug in the muscle chamber, the peak response remains steady for 2 hr. Thus we cannot confirm the results of Nastuk & Alving (1959), who showed a significant decline in potentiation by 3-OH-PTEA during a 75 min period. The considerable fluctuations in contraction amplitude which occur during the potentiating action of TEA (Fig. 2) are typical only of the response to maximum potentiating doses of this ion, but not of 3-OH-PTEA. These fluctuations are probably related to the spontaneous contractions going on simultaneously.

In the absence of nerve stimulation or during intervals between neurally-evoked responses, both ions cause spontaneous repetitive contractions (Fig. 2). Note that the repetitive contractions associated with nerve stimulation are frequently as large as the control responses to maximal nerve volleys. Another effect of these ions, also illustrated in Fig. 2,

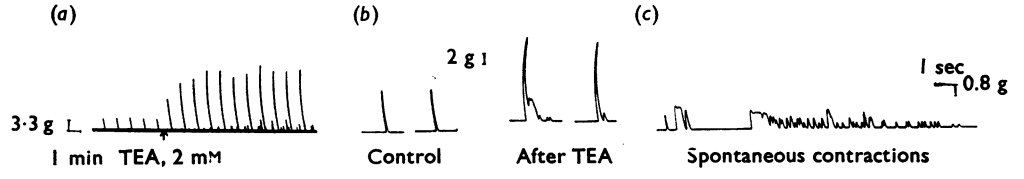

Fig. 2. Typical effects of TEA on frog nerve-muscle preparation. (*a*) Potentiation of neurally-evoked contractions and spontaneous activity. (*b*) Repetitive contractions immediately following potentiated response. (*c*) Spontaneous contractions; note the faster chart speed and higher amplification. TEA concentration is 2 mM in each instance.

is the repetitive contraction occurring during and immediately after the recovery phase of a neurally-evoked response. The threshold concentrations required for these foregoing effects are listed in Table 1. For 3-OH-PTEA they are higher than those concentrations which cause maximum potentiation of the neurally-evoked twitch. For TEA they are the same as those concentrations which produce submaximum potentiation.

Effects on neurally-evoked contraction in the presence of tubocurarine

The effects of 3-OH-PTEA and TEA were studied on nerve-muscle preparations previously treated with paralysing concentrations of tubocurarine. A dose/response regression line for tubocurarine was first established, and 10^{-6} g/ml. (1.43 μM) was selected as the standard concentration for these experiments. In 212 preparations the mean depression produced by 20 min treatment with 10^{-6} g/ml. of tubocurarine was 73%. It is noteworthy that 3-OH-PTEA and TEA not only increase contraction amplitude of the curarized muscle

TABLE 1

ACTIONS OF TRIETHYL(3-HYDROXYPHENYL)AMMONIUM (3-OH-PTEA) AND TETRAETHYLAMMONIUM (TEA) ON THE FROG NERVE-MUSCLE PREPARATION

* 10^{-6} g/ml. tubocurarine; † 5×10^{-5} g/ml. tubocurarine.

Action	Concentration (mM) required for minimum effect	
	3-OH-PTEA	TEA
Potentiation of neurally-evoked contraction	0.015	0.2
Potentiation of neurally-evoked contraction in presence of tubocurarine*	0.2	0.5
Increase in the neurally-evoked contractile response in curarized preparation*	0.015	0.02
Spontaneous contractions	0.5	1.5
Potentiation of directly-stimulated muscle†	1.0	3.0
Repetitive after-discharge in sheathed nerve	10.0	5.0
Repetitive after-discharge in desheathed nerve	1.0	1.0
Repetitive after-discharge in sheathed nerve treated with cetrimonium	5.0	2.0

to control (before curare) level, but also cause potentiation (Fig. 3). The dose/response regression lines for these effects are shown in Fig. 4,*a* (3-OH-PTEA) and Fig. 4,*b* (TEA).

The points shown in Fig. 4 (*a* and *b*) cannot be fitted to a single regression line; the most accurate and best fit of these points is achieved by calculating two linear regression lines ($P<0.05$) which have significantly different slope values ($P<0.01$). It is probably not mere coincidence that the demarcation between the two regression lines, in the case of either 3-OH-PTEA or TEA, occurs at a point on the ordinate which approximates control (before curare) contraction amplitude, for it is likely that different mechanisms and/or sites of ethonium ion action are involved above and below this point. In support of this concept

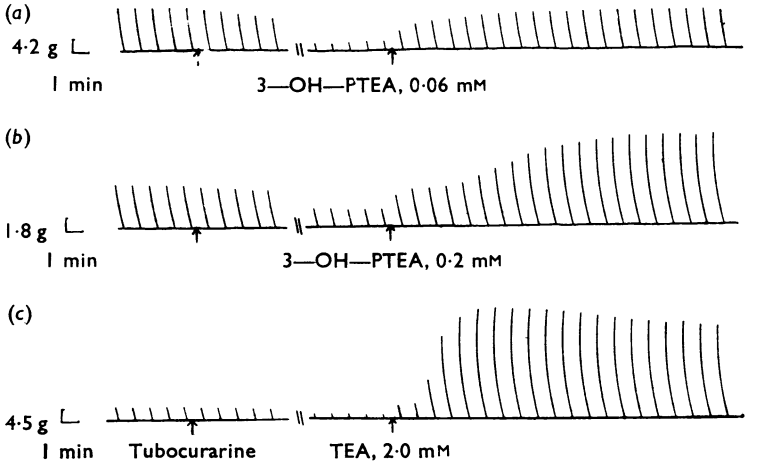

Fig. 3. Typical effects of 3-OH-PTEA and TEA on neurally-evoked contractions in curarized nerve-muscle preparation. Tubocurarine (10^{-6} g/ml.) was given at the first arrow in each tracing. In (*a*) 3-OH-PTEA (0.06 mM), in (*b*) 3-OH-PTEA (0.2 mM) and in (*c*) TEA (2.0 mM) were given at the second arrows. Note the timing of the anticurare effects in the middle and lower tracings; the twitch first increases to control level, then suddenly increases above control level. In each instance contraction amplitude remains at the level shown for at least another hour.

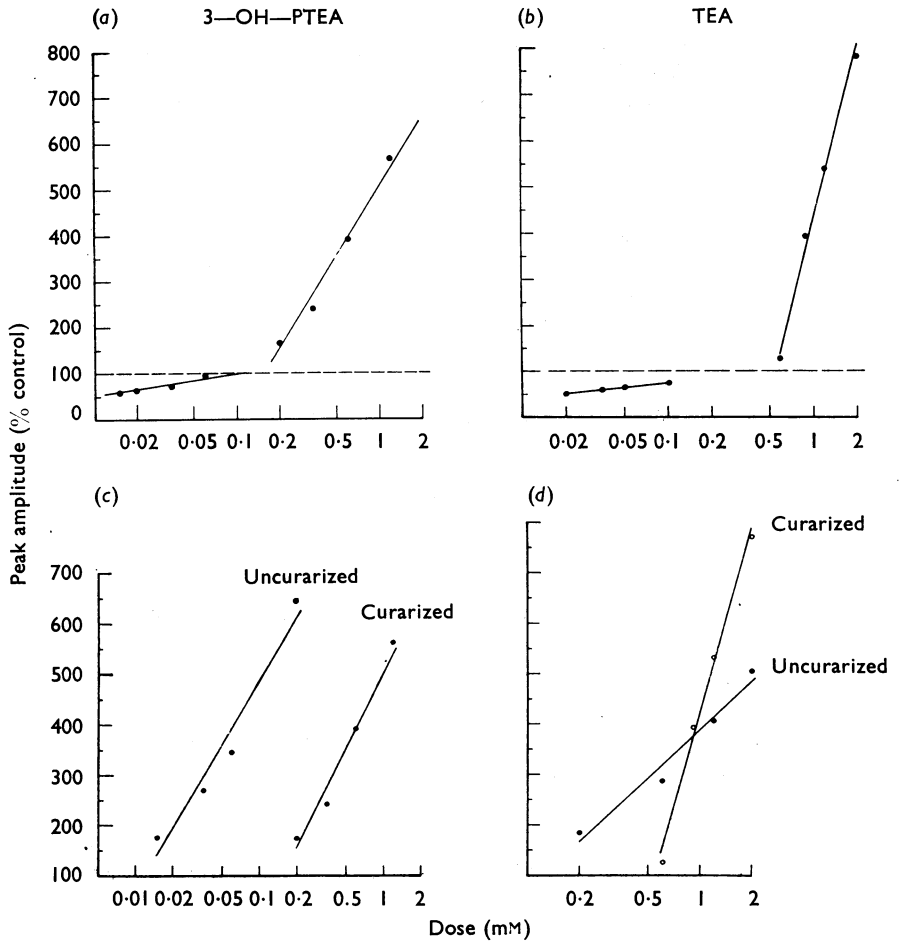

Fig. 4. (*a*) and (*b*): relationship between dose (on log scale) of 3-OH-PTEA (*a*) and TEA (*b*) and increase in amplitude of neurally-evoked contractions in curarized nerve-muscle preparation; each point is mean of nine independent observations. The interrupted line represents the control level before tubocurarine. (*c*) and (*d*): relationship between dose (on log scale) of 3-OH-PTEA (*c*) and TEA (*d*) and magnitude of potentiation of neurally-evoked contractions in presence and absence of tubocurarine. Each point is the mean of nine independent observations.

is an observation often made after the injection of ethonium ions in a concentration which increases the curarized twitch amplitude to control level; subsequent to the initial anticurare action, there is a distinct secondary potentiation of contractile amplitude (Fig. 3). It also seems important to note that the dose/response curves for other anticurare drugs contain two distinct components (Okamoto, 1964).

We have already seen that the dose/response lines for the contraction-potentiating actions of 3-OH-PTEA and TEA in the absence of tubocurarine are parallel. Likewise, the regression lines representing the anticurare actions of these ions below control (before curare) contraction amplitude do not have significantly different slopes ($P>0.1$). However, above control contraction amplitude, the regression lines of Fig. 4,*a* and *b* are not parallel

($P<0.01$). The dose/response lines are redrawn in Fig. 4,*c* and *d*, and clearly show that tubocurarine causes an increased slope of the TEA regression line and an increased maximum potentiating response. In contrast, tubocurarine causes a considerable decrease in potentiating potency of 3-OH-PTEA but has no influence on regression line slope or maximum response. In passing we should also note that tubocurarine completely antagonizes the spontaneous repetitive contractions produced by both ethonium ions.

Direct effects on muscle

The effects on contractile response to direct muscle stimulation were studied in the presence of 5×10^{-5} g/ml. of tubocurarine. This concentration is about ten times that which completely blocks the muscle response to maximal nerve stimuli. Both ethonium ions potentiate directly-developed contraction (Fig. 5). This ethonium ion effect on skeletal muscle deserves further study, but for our purposes the important point is that the threshold

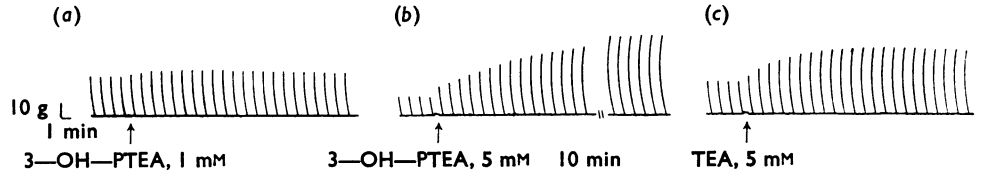

Fig. 5. Typical effects of 3-OH-PTEA (1 mM in *a*; 5 mM in *b*) and TEA (5 mM in *c*) on the directly-evoked contractile response in the presence of 5×10^{-5} g/ml. of tubocurarine. Calibrations, 10 g and 1 min.

concentrations (Table 1) are about five times those needed for minimal potentiation of neurally-evoked contraction in the presence of 10^{-6} g/ml. of tubocurarine. Therefore it is improbable that the direct action contributed significantly, if at all, to the potentiation of the neurally-evoked response in the presence or absence of tubocurarine.

Effects on nerve axons

Under present experimental conditions, the main portion of nerve was not exposed to drug solutions but the intramuscular portions and nonmyelinated axon terminals were. In view of the well-known excitatory effects of TEA on frog peripheral nerve (Cowan & Walter, 1937) and motor axon terminals (Koketsu, 1958), a quantitative evaluation of ethonium ion action on Aα fibres seemed appropriate. Axonal effects may contribute significantly to the potentiation of neurally-evoked contraction and also to the changes in endplate potential reported elsewhere (Koketsu, 1958; Kuperman & Okamoto, 1964).

Both ethonium ions cause repetitive after-discharge in response to stimulation of Aα fibres, and the effect is reversible and dose-dependent. Substitution of one ethyl group by methyl, as in triethylmethylammonium and 3-OH-DEMPA, causes almost complete abolition of this effect. The latter compound occasionally produces a very weak response if suitable concentrations (greater than 50 mM) are applied. The relationship between concentration and magnitude of nerve response to 3-OH-PTEA is illustrated in Fig. 6. The concentrations required to produce a minimal effect, like that seen in the left-hand tracing of Fig. 6, were about 25- and 700-times those required to produce a threshold potentiation of neurally-evoked twitch for TEA and 3-OH-PTEA respectively (Table 1). A maximal

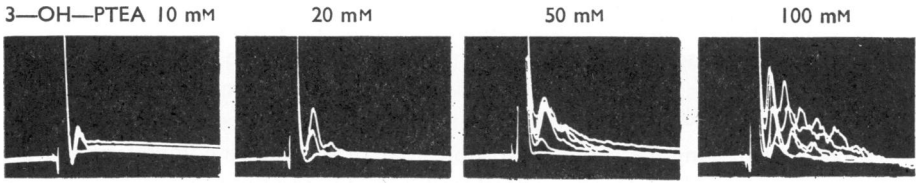

Fig. 6. Repetitive after-discharge in sciatic nerve produced by threshold (10 mM) to maximum (100 mM) concentrations of 3-OH-PTEA. The lower trace in each record is the control; the upper trace is taken after 30 min treatment. Calibration grid, 1 mV and 5 msec.

response, like that seen in the right-hand tracing of Fig. 6, requires 50 mM-TEA and 100 mM-3-OH-PTEA. The great disparity between ethonium ion concentration needed for axonal and neuromuscular effects suggests that the two effects are unrelated. However, the epineural sheath which surrounds the peripheral nerve bundle is probably a barrier to the movement of chemical agents. Accordingly, the ethonium ions were also tested on desheathed nerve and were found to be effective in concentrations of 1 mM or even less. Furthermore, the magnitude of the response, as seen in Fig. 7, was much greater than that obtained with 50- to 100-times higher concentrations in sheathed nerves.

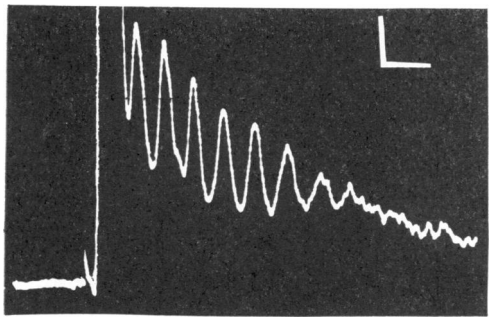

Fig. 7. Effect of 1 mM-TEA on the desheathed sciatic nerve after 1 min treatment. Calibrations, 1 mV and 5 msec.

Another procedure which increases the response of nerve axons to ethonium ions is previous chemical treatment by cationic detergents. These materials are assumed to reduce the effectiveness of certain permeability barriers which impede the passage of drugs to their receptor sites in the axon (Walsh & Deal, 1959). In conjunction with treatment of the sheathed nerve by the cationic surface-acting agent cetrimonium (5 mM), concentrations of ethonium ion required for threshold or maximum effects are reduced by about one-half (Table 1). Prolonged soaking (60 to 90 min) in cetrimonium alone have no effect on the nerve action potential.

In addition to the stimulus-evoked repetitive discharge, both ethonium ions cause spontaneous firing in minimum concentrations of 5 to 10 mM (Fig. 8). The initial response consists of small monophasic potentials (greater than 50 μV) occurring in the treated segment of nerve, and propagated diphasic potentials appear later in both treated and untreated segments. In these experiments the nerve was placed on a gang of fine platinum-iridium

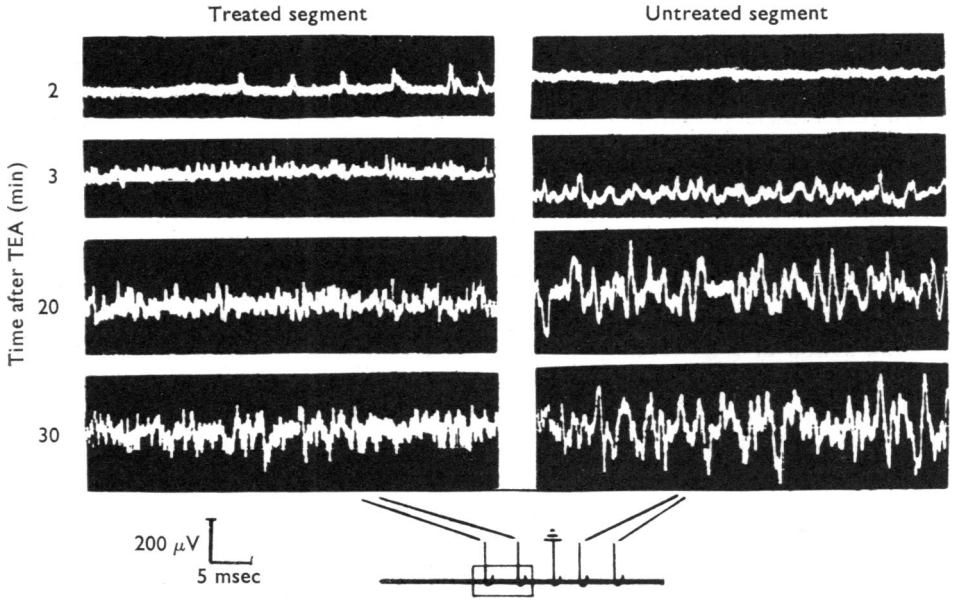

Fig. 8. Spontaneous activity in sciatic nerve produced by 50 mM-TEA, given before the first record. Note nonpropagated potentials in the treated segment after 2 min treatment.

electrodes, with an interelectrode distance of 2 mm; recordings were bipolar. Accordingly the monophasic potentials are assumed to represent local subthreshold responses of the membrane. The speed of onset of these local potentials is dose-dependent. With 50 mM-TEA they appear within 1 min with a frequency of 200 to 500 per sec. The maximum frequency attained is usually about 1,000 per sec regardless of drug concentration but this parameter is time-dependent. The local potentials often appear in rhythmic groups, with an interval of about 50 to 100 msec between each group. After treatment with the same concentrations of TEA as used here, grouped bursts of spontaneous potentials have also been observed in frog sympathetic ganglion cells (Riker, 1964).

DISCUSSION

Both 3-OH-PTEA and TEA have some similar actions on the isolated frog nerve-muscle preparation. These actions are exerted, depending on dose, either on the nerve axon, muscle fibre or neuromuscular junction. A junctional effect is inferred from the potent anticurare and twitch-potentiating actions which are produced by concentrations of ethonium ion much lower than those needed to affect muscle or nerve fibres directly. Furthermore these ions increase the amplitude of the deeply curarized endplate potential (Kuperman & Okamoto, 1964).

If it is assumed that ethonium ions act at the neuromuscular junction, an important problem is to determine whether the site of drug action is pre- or postsynaptic. Although the present experiments were not designed to deal specifically with this problem, the results from the nerve experiments have some relevance, especially when viewed in conjunction with the recent work of Karczmar, Kim & Blaber (1965). These investigators reported

that 3-OH-PTEA produces antidromic repetitive after-discharge in frog ventral root axons, and we have confirmed these findings using either 3-OH-PTEA or TEA (unpublished). Significantly, the concentrations required for this effect in the isolated ventral root-sciatic nerve-sartorius muscle preparation are the same as those which cause repetitive after-discharge in isolated nerve. This suggests that the antidromic response is not the result of a unique pharmacological effect on nonmyelinated motor nerve terminals, but rather is functionally equivalent to the ethonium ion action on myelinated axons. The fact that 3-OH-DEMPA causes a weak and inconsistent repetitive after-discharge either in the isolated nerve or in the " antidromic " preparation (Karczmar *et al.*, 1965) supports this contention.

The weak action of 3-OH-DEMPA on the axon should be compared to its exceedingly potent facilitatory action on neuromuscular transmission; the order of threshold anticurare or twitch-potentiating potency is 3-OH-DEMPA>3-OH-PTEA>TEA (Kuperman & Okamoto, 1964). Quite oppositely, the order of potency to produce a repetitive after-discharge in normal or detergent-treated nerves is TEA>3-OH-PTEA>3-OH-DEMPA. These results indicate the lack of a causal relationship between the axonal effects of the foregoing compounds and their effects on neuromuscular transmission.

The problem of site of drug action has been approached in another way by Koketsu (1959); he made direct electrical recordings from the most distal accessible intramuscular branches of the frog motor axon. The results suggested that TEA augments a negative after-potential in the motor axon terminal, and on this basis Koketsu concluded that TEA causes increased transmitter output. However, we must note that the concentrations required for this axon terminal effect are equivalent to those which cause threshold after-discharge in the isolated nerve and are even greater than those which produce after-discharge in desheathed nerve.

Turning aside from considerations of the site of drug action, we shall now discuss some of the dose/response data. Comparison of the actions of 3-OH-PTEA and TEA in the presence and absence of a paralysing concentration of tubocurarine yields particularly interesting results. The results indicate that the mechanism of 3-OH-PTEA potentiation is very sensitive to depression by tubocurarine whereas the mechanism of TEA potentiation is relatively resistant. This same conclusion was reached on the basis of different kinds of results obtained in a mammalian nerve-muscle preparation (Kuperman & Okamoto, 1965). In fact, the mechanism of 3-OH-PTEA potentiation is even more sensitive to tubocurarine in the mammal than in the frog; 3-OH-PTEA cannot potentiate the contractile response of curarized mammalian muscle.

The parallelism of the 3-OH-PTEA dose/potentiation regression lines obtained in the curarized and noncurarized frog preparations suggests a competitive interaction between 3-OH-PTEA and tubocurarine at some junctional focus. However, the increase in maximum effect of TEA and the increase in slope of the TEA dose/potentiation regression line caused by tubocurarine are difficult to interpret. One explanation relates to the occurrence of spontaneous repetitive contractions caused by submaximum potentiating doses of TEA, but not of 3-OH-PTEA. This spontaneous activity is completely antagonized by tubocurarine. Accordingly, if the assumption is made that spontaneous activity leads to transmitter depletion, an augmentation of the effect of TEA by tubocurarine would be expected. Alternatively, there is the interesting possibility of a direct synergistic interaction between

TEA and tubocurarine. Indeed, tubocurarine frequently enhances the TEA-induced spontaneous discharge in isolated frog nerve (Okamoto, unpublished).

In the noncurarized frog preparation, the dose/potentiation regression lines of 3-OH-PTEA and TEA are parallel. In the mammal, however, the two regression lines have drastically different slopes, and the TEA potentiation is very limited. Despite the parallelism of dose/response curves in the frog, the weight of evidence supports the concept of a dual mechanism of ethonium ion action. In addition to that presented above, this evidence (Kuperman & Okamoto, 1964) is that: the two ethonium ions have different effects on the curarized endplate potential; and the anticurare and potentiating actions of 3-OH-PTEA are much more sensitive to tubocurarine concentration than are the effects of TEA (even in the presence of a completely paralysing concentration of tubocurarine, TEA causes the appearance of a contractile response to nerve stimulation).

The dose/response relationships suggest not only a difference between the mechanisms of action of the two ethonium ions but also a difference between the mechanism of the anticurare and twitch-potentiating actions of each separate ion. All of these mechanisms remain to be explored. The relatively potent anticholinesterase activity of the hydroxyanilinium ion must be considered but other possiblities cannot be overlooked, especially in view of the sensitivity of motor axon terminals to ethonium ion action (Kuperman & Okamoto, 1965). The failure of these ions to produce repetitive after-discharge in frog motor axons in concentrations which have no direct effects on myelinated A fibres seriously limits any attempt to study presynaptic mechanisms in this species. It is possible to record action potentials directly from nonmyelinated motor axon terminals (Koketsu, 1958; Katz & Miledi, 1965) provided tubocurarine or excess magnesium is present in order to prevent muscle contractions and electrode displacement. However, the experiments of Koketsu (1958) show the nonmyelinated terminal in the curarized frog nerve-muscle preparation is no more sensitive to TEA than is the myelinated A fibre. Collier & Exley (1963) reported that TEA increases the amount of acetylcholine released by stimulation of the rat phrenic nerve; but the TEA concentration required is now known to cause repetitive after-discharge in frog myelinated axons. Thus, the capacity of TEA to increase endplate potential amplitude and rate of rise is caused by some effect, presumably presynaptic, which remains to be specified and which is extraordinarily resistant to tubocurarine.

SUMMARY

1. A comparison of certain pharmacologic effects of two ethonium ions, tetraethylammonium (TEA) and triethyl(3-hydroxyphenyl)ammonium (3-OH-PTEA) was made on the frog sciatic nerve-sartorius muscle preparation *in vitro*.

2. Both ions potentiate the neurally-evoked contractile response; the dose/response regression lines are parallel and 3-OH-PTEA is more potent than TEA.

3. In the presence of tubocurarine, both ions cause an increase in amplitude of the neurally-evoked contraction to control level and potentiate contraction above control level; the slopes of the dose/response regression lines above and below control amplitude have significantly different values.

4. In the presence of tubocurarine, the potentiating potency of 3-OH-PTEA is decreased but its ceiling effect remains unchanged. The same concentration of tubocurarine does not

depress the potentiating potency of TEA, increases the slope of its dose/potentiation regression line and increases its maximum effects.

5. Both ions potentiate the contractile response to direct muscle stimulation but the concentrations required are significantly greater than those needed to increase the neurally-evoked response in curarized or noncurarized muscle.

6. Both ions cause repetitive after-discharge and spontaneous firing in Aα fibres of frog sciatic nerve, TEA being more potent than 3-OH-PTEA.

7. We conclude that the two ethonium ions facilitate frog neuromuscular transmission through two different mechanisms; the 3-OH-PTEA mechanism is exceedingly sensitive to tubocurarine whereas the TEA mechanism is relatively resistant.

This investigation was carried out with the aid of U.S. Public Health Service Grant NB-3597. Dr Okamoto was supported by Graduate Research Training Grant 2G-99, U.S. Public Health Service.

REFERENCES

COLLIER, B. & EXLEY, K. A. (1963). Mechanism of the antagonism by tetraethylammonium of neuromuscular block due to d-tubocurarine or calcium deficiency. *Nature (Lond.)*, **199**, 702–703.

COWAN, S. L. & WALTER, W. G. (1937). The effects of tetraethylammonium iodide on the electrical response and the accommodation of nerve. *J. Physiol. (Lond.)*, **91**, 101–126.

KARCZMAR, A. G., KIM, K. C. & BLABER, L. C. (1965). Pharmacological actions of oxamides and hydroxyanilinium compounds at frog neuromyal junction. *J. Pharmacol. exp. Ther.*, **147**, 350–359.

KATZ, B. & MILEDI, R. (1965). Propagation of electrical activity in motor nerve terminals. *Proc. roy. Soc. B*, **161**, 453–482.

KOKETSU, K. (1958). Action of tetraethylammonium chloride on neuromuscular transmission in frogs. *Amer. J. Physiol.*, **193**, 213–218.

KUPERMAN, A. S. & OKAMOTO, M. (1964). The relationship between anticurare activity and time course of the endplate potential; a structure-activity approach. *Brit. J. Pharmacol.*, **23**, 575–591.

KUPERMAN, A. S. & OKAMOTO, M. (1965). A comparison of the effects of some ethonium ions and their structural analogues on neuromuscular transmission in the cat. *Brit. J. Pharmacol.*, **24**, 223–239.

NASTUK, W. L. & ALVING, B. O. (1959). Further study of 3-hydroxyphenyldimethylethylammonium (edrophonium) and its closely related analogues with respect to activity at the neuromuscular junction. *Biochem. Pharmacol.*, **1**, 307–322.

OKAMOTO, M. (1964). *The Effects of Ethonium Ions on Neuromuscular Transmission in the Frog.* Ph.D. thesis at Cornell University, New York City, U.S.A.

RIKER, W. K. (1964). Effects of tetraethylammonium chloride on electrical activities of frog sympathetic ganglion cells. *J. Pharmacol. exp. Ther.*, **145**, 317–325.

WALSH, R. R. & DEAL, S. E. (1959). Reversible conduction block produced by lipid-insoluble quaternary ammonium ions in cetyltrimethylammonium ion-treated nerves. *Amer. J. Physiol.*, **197**, 547–550.