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ACTIONS OF MECAMYLAMINE, DIMECAMINE, PEMPIDINE AND THEIR TWO QUATERNARY METHO-SALTS AT THE NEUROMUSCULAR JUNCTION

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Mecamylamine, dimecamine and pempidine differed in neuromuscular-blocking activity on the isolated phrenic nerve-diaphragm preparation of the rat from the corresponding methiodides by a factor of less than two. It was concluded that the active component of each amine was the cation acting extracellularly. The finding that the neuromuscular-blocking activity of mecamylamine at *p*H 6.7 was similar to the activity at *p*H 7.7 did not refute this conclusion. Mecamylamine, dimecamine methiodide, dimecamine and pempidine, at concentrations insufficient to cause block, could increase the twitch response of the rat diaphragm; the ability to do this increased in the above order. With pempidine (the most active compound) this effect, on the isolated sartorius muscle of the frog, was a direct action. During steady partial block by each of the compounds, the responses to brief tetanic stimulation, to neostigmine and to an increase in calcium concentration were similar to those observed during block by tubocurarine. From indirect evidence, pempidine methiodide appeared both to enhance the release of acetylcholine from motor-nerve terminals and to cause postsynaptic block.

The ganglion-blocking amines mecamylamine and pempidine each exist in solution in two molecular forms, the free base and the cation. Either form may have pharmacological activity. It is not known to what extent each form contributes to the blocking activity of mecamylamine and pempidine at ganglia and at the neuromuscular junction.

The pK_a of mecamylamine is about 11.4 (Stone, Torchiana, Navarro & Beyer, 1956) and that of pempidine is similar, being 11.25 at 30° C (Hall, 1957), therefore the concentration of the cationic form at a physiological *p*H will be greater than that of the free base by a factor of approximately 10^4. It is therefore unlikely that the free base alone is the active form if the compounds act in the extracellular space. However, mecamylamine and pempidine pass readily into cells (Milne, Rowe, Somers, Muehrcke & Crawford, 1957; Payne & Rowe, 1957; Harington, Kincaid-Smith & Milne, 1958; Muggleton & Reading, 1959) and it is possible that they act there (Bennett, Tyler & Zaimis, 1957; Spinks & Young, 1958; Spinks, Young, Farrington & Dunlop, 1958), although the evidence in favour of an intracellular action is inconclusive (Payne & Rowe, 1957; Corne & Edge, 1958; Spinks *et al.*, 1958).

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In this paper, we have attempted to clarify this point by comparing the neuromuscular-blocking activity of each amine with that of the corresponding quarternary metho-salt, which can exist as the cation only and which may be assumed not to pass readily across cell membranes. The blocking activities were similar, and it was concluded that, at the neuromuscular junction, the amines act extracellularly in the form of their cations. Other effects of the amines and their quaternary metho-salts at the neuromuscular junction are also described.

METHODS

Most experiments were made on the isolated phrenic nerve-diaphragm preparation of the rat (Bülbring, 1946). The muscle was set up in a 50 ml. organ-bath at 37° C in a Krebs-bicarbonate solution of the following composition (mM/l.): Na 144; K 5.9; Ca 2.54; Mg 1.2; Cl 129; HCO_3 25; H_2PO_4 1.2; SO_4 1.2; with glucose 2 g/l. The solution was bubbled vigorously with a mixture of 5% carbon dioxide and 95% oxygen. The nerve was sucked into a capillary electrode in the bath (Furshpan & Potter, 1959) and stimulated supramaximally through an isolating transformer with rectangular pulses of about 250 μsec duration. A light steel-spring torsion lever was used to record the twitches, and under-swing was prevented by an adjustable stop.

Some experiments were carried out at room temperature with the isolated sartorius nerve-muscle preparation of the frog (*Rana temporaria*), suspended in Ringer solution of the following composition (mM/l.); Na 105; K 2.0; Ca 2.0; Mg 1.0; Cl 88; HCO_3 25; with glucose 2 g/l., bubbled with a mixture of 5% carbon dioxide and 95% oxygen. To allow both direct and indirect stimulation, the preparation was set up so that the pelvic bone and a short length of the nerve-free part of the muscle were exposed above the surface of the solution, the exposed portion being kept moist by the spray from bubbling the solution. Direct stimulation was made between a nickel–chromium wire threaded through the pelvic bone and an electrode in the solution, with rectangular pulses of about 4 msec duration and about 1 V intensity which gave maximal twitches equal to those obtained with nerve stimulation.

The chick biventer cervicis muscle preparation (Ginsborg & Warriner, 1960) was used as a test object for acetylcholine-like stimulant activity.

Stimulus frequency. The usual frequency of stimulation was 8 shocks/min. The frequency dependence of neuromuscular block was observed at steady levels of block by recording at 30 sec intervals trains of ten twitches at each of five or six frequencies up to 2 shocks/sec (Blackman, 1963).

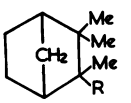
Measurement of neuromuscular-blocking activity. By adding a drug to the organ-bath in increments at 20 min intervals, two or three levels of steady partial neuromuscular block of between 30 and 70% were obtained. The molar concentration of drug required to produce a steady 50% reduction in the muscle twitch (EC50) was then estimated graphically. For a given preparation, the log concentration/response curves for different drugs were approximately parallel. EC50 values were therefore compared by drawing parallel lines through the points for each drug. Some drugs increased the twitch response before causing block and allowance was made for this (see Results).

The total volume of solution containing the added drug (dissolved in 0.9% saline) was usually less than 1 ml. A steady level of block was approached within 10 to 15 min of addition of the drug to the bath and only on a few occasions was it necessary to extend the equilibration time beyond the usual 20 min. Preparations were washed three times during the first 30 min of a wash period of not less than 1 hr before any further estimate of blocking activity was made.

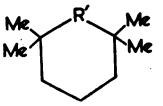
The effect of *p*H was studied by using two modified Krebs solutions of *p*H approximately 6.7 and 7.7 (at 37° C) containing 4 and 40 mM-sodium bicarbonate solution respectively (Barlow & Hamilton, 1962).

The following drugs were used: mecamylamine (I; 2,2,3-trimethy(-3-methylaminonorborane) hydrochloride; dimecamine (II; 3-dimethylamino-2,2,3-trimethylnorborane) hydrobromide, and

its methiodide (III; 2,2,3-trimethyl-3-trimethylammonionorborane iodide); and pempidine (IV; 1,2,2,6,6-pentamethylpiperidine) hydrogen tartrate, and its methiodide (V; 1,1,2,2,6,6-hexamethylpiperidinium iodide). Dimecamine methiodide was made from dimecamine. The melting point of the methiodide was 189°C (compare with 187°C found by Vejdelek and Protiva, 1959).



I, R=NHMe
II, R=NMe_2
III, R=N^+Me_3



IV, R'=NMe
V, R'=N^+Me_2

RESULTS

Neuromuscular-blocking activity

Some of the compounds increased the muscle twitch response to nerve stimulation before they produced neuromuscular block. This effect was considered before comparisons were made of the neuromuscular-blocking activities of the compounds.

Increased twitch response. In the rat diaphragm, block by mecamylamine was usually preceded by a small increase, about 5 to 8%, in the twitch response. Payne (1957) noted a similar increase in the twitch of the cat tibialis anterior muscle with mecamylamine. In the rat diaphragm the effect was best seen with threshold blocking concentrations and was maximal about 20 min after the addition of the drug. Pempidine methiodide did not increase the twitch response, but the remaining three compounds did, being more active than mecamylamine. Near-threshold blocking concentrations of dimecamine increased the twitch response by 15 to 20%. Dimecamine methiodide increased the response by about 10%, and pempidine increased it by 20 to 40%. For comparison, the increase produced by 2×10^{-3}M-tetraethylammonium solution in the rat diaphragm was 5 to 10%. On washing the preparation after neuromuscular block by the effective compounds a phase of increased twitch response was always observed before the twitch returned to control size in 30 to 60 min.

The nature of the increased twitch response was studied using the frog sartorius preparation, since satisfactory maximal direct stimulation was more easily obtained with it than with the rat diaphragm preparation. In one experiment with the sartorius preparation, pempidine (2×10^{-4}M) produced a 20% increase in the twitch response to nerve stimulation within 30 min. Block ensued when the concentration was raised to 3×10^{-4}M. In a second experiment (Fig. 1), in which the muscle was stimulated alternately through the nerve and directly, pempidine increased the twitch by a direct action on the muscle.

Comparison of neuromuscular-blocking activities. The estimates in Table 1 of the concentration of each drug required to produce a steady 50% reduction in the twitch response to nerve stimulation (EC50) were made on the assumption that the increased twitch response produced by threshold blocking concentrations of some of the compounds was the result of a direct action on the muscle, as had been shown (above) for pempidine. To allow for this effect, the preparation was first exposed to a threshold blocking concentration of the drug for about 20 min before the concentration was raised to a blocking level. The maximal twitch height observed was taken as the control for estimating the percentage block produced later. Because at the higher blocking concentrations the response of the muscle to direct stimulation would

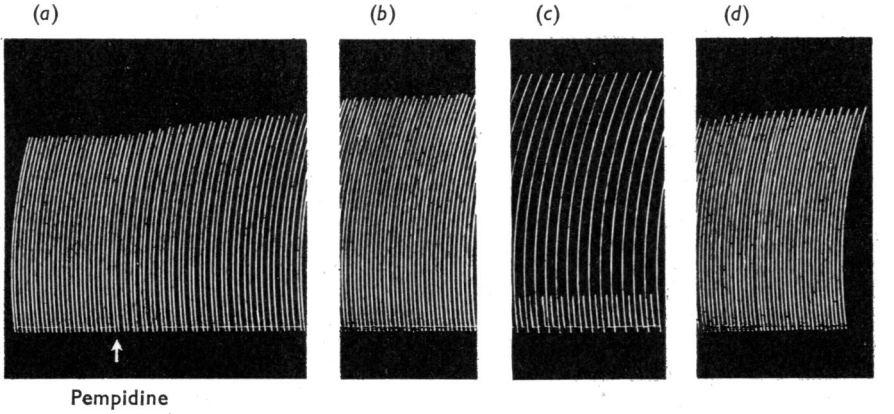


Fig. 1. Effect of pempidine on twitches of a frog sartorius muscle stimulated alternately through the nerve and directly at approximately 8 shocks/min. (*a*), pempidine (10^{-4} M) was added at the arrow; (*b*), responses 10 min later; (*c*), 30 min later after the pempidine concentration had been increased to 3×10^{-4} M (at 15 min after *b*) to give almost complete neuromuscular block; (*d*), responses 30 min after washing.

presumably have been greater than the control twitch response (see Fig. 1), this procedure was likely to underestimate the blocking activity. However, the steepness of the observed log concentration response curves would ensure that the error from this cause in the estimate of the EC50 was small.

Table 1 shows that the blocking activities of the amines and their respective quaternary metho-salts differed from each other by a factor of less than two. The difference in the postsynaptic blocking potencies of pempidine and pempidine methiodide may be less than is indicated in Table 1 since, as is reported below, pempidine methiodide probably enhances the release of acetylcholine, which would reduce its effective blocking activity.

The activity of mecamylamine given in Table 1 is similar to that found by Spinks *et al.* (1958) in the rat diaphragm. These authors reported a very slow recovery from block on washing out the mecamylamine, but we always observed rapid and complete

TABLE 1

RELATIVE NEUROMUSCULAR-BLOCKING ACTIVITIES OF MECAMYLAMINE, DIMECAMINE, PEMPIDINE AND THEIR QUATERNARY METHO-SALTS IN THE RAT DIAPHRAGM

Results were obtained from four diaphragm preparations. Each EC50 value is the mean of two separate estimations of the molar concentration of drug required to give a steady 50% reduction of the twitch response

Drug	Molar EC50 ($\times 10^{-4}$)	Equipotent molar ratio (mecamylamine =1)
Mecamylamine hydrochloride	2·2	1·0
Dimecamine hydrobromide	2·8	1·3
Dimecamine methiodide	1·7	0·8
Pempidine hydrogen tartrate	8	3·6
Pempidine methiodide	14	6·4

recovery following washout even after prolonged periods of steady partial block. Recovery from block on washing out the other compounds was rapid and complete also.

The blocking activity of the compounds as a group was not high. For comparison, the mean EC50 for (+)-tubocurarine chloride in four similar experiments was 1.2×10^{-6}M, and that for decamethonium bromide in nine similar experiments was 8.4×10^{-5}M.

Effect of p*H.* Assuming that only the uncharged forms of mecamylamine and the other amines are able to pass across cell membranes, a change in *p*H of one unit in the physiological range should produce little change in the extracellular concentration of the cationic form, but an approximately tenfold change in both the extracellular and intracellular concentrations of the free base and in the intracellular concentration of the cation. A corresponding tenfold change in blocking activity would be inconsistent with the possibility suggested by the experiments already described that the activity of the amines is due to the cations acting extracellularly.

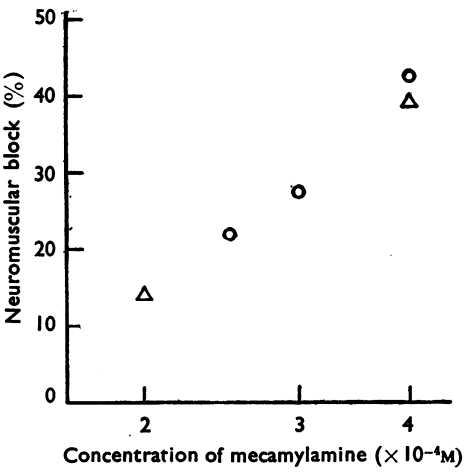


Fig. 2. Effect of *p*H on the blocking activity of mecamylamine in a rat diaphragm. The preparation was exposed to each concentration of drug (abscissa) for 5 min and washed for 15 min. Blocking activity (ordinate, % of control) was determined first at *p*H 7.7 (circles) and then at *p*H 6.7 (triangles). See text for details.

Fig. 2 shows that the blocking activity of mecamylamine in the rat diaphragm was little altered by a *p*H change (one unit) from 7·7 to 6·7. In carrying out this experiment it was found, with the solution of lower *p*H, that the amplitude of the twitch response declined slowly to about half of the control level in 2 to 3 hr. To allow for this change, the diaphragm was exposed at each *p*H to a blocking concentration of the drug for 5 min and then washed for 15 min with solution of the same *p*H before changing to the other solution. This experiment allowed the time course of the decline in the twitch at the lower *p*H to be estimated and a correction to be applied to the calculation of the percentage block. The decline in the twitch was slowly reversed on returning the preparation to the solution of higher *p*H.

It is possible that changes in the preparation due to the alteration of *p*H (such as have been described by Castillo, Nelson & Sanchez, 1962) may have masked a large change in the activity of mecamylamine. A rough check on the effect of the *p*H change on the preparation was therefore made by using the procedure described above to determine the effect of *p*H on the blocking activity of tubocurarine. The EC50 at *p*H 7.7 was 3.1×10^{-6}M and at *p*H 6.7 it was 2.5×10^{-6}M. This change in activity of tubocurarine is in the same direction as that in the frog rectus muscle preparation on reducing the *p*H (Kalow, 1954). Kalow (1954) attributed the change to depression of the ionization of a phenolic hydroxy group to give a more active form of tubocurarine. This effect presumably accounts for at least part of the change in the activity of tubocurarine observed in the rat diaphragm and it may be inferred that any change in the sensitivity of the preparation produced by altering the *p*H was not sufficient to mask a large change in the blocking activity of mecamylamine.

Nature of the block

Effect of a brief tetanus. During steady partial block by each of the compounds, tetanic stimulation of the nerve (for 10 sec at 60 shocks/sec) produced a brief non-sustained contraction followed by a period of enhanced twitch response. These responses were similar to those observed during block by tubocurarine.

Effect of calcium and neostigmine. Increasing the calcium concentration to 5 mM during partial block by any of the compounds produced a sustained reversal of block. A similar response to an increase in calcium concentration was observed during block by tubocurarine. Neostigmine reversed block by mecamylamine and the other compounds.

Effect of stimulus frequency. Indirect evidence that a blocking drug has an effect on release of acetylcholine in addition to any postsynaptic blocking action may be obtained by comparing the "frequency dependence" of the block produced by the drug with that produced by a compound (such as tubocurarine) which may be assumed to have no effect on acetylcholine release (Blackman, 1963). Comparisons of this kind were made with mecamylamine, pempidine and their derivatives.

As with tubocurarine (Blackman, 1963), there was an approximately linear relation between the stimulus frequency and the percentage block produced by each compound in the rat diaphragm. The frequency dependence of block by mecamylamine was similar to the frequency dependence of block by tubocurarine at several levels of block (Fig. 3). The frequency dependence of block by dimecamine and pempidine was also similar to that of block by tubocurarine when comparisons were made at a steady level of approximately 30% of block (Fig. 3). However, the frequency dependence of block by the two quaternary derivatives, dimecamine methiodide and pempidine methiodide was greater than that of block by tubocurarine (Fig. 3 *b* and *c*). The difference was sufficiently great with pempidine methiodide to suggest that, in addition to its blocking action, the drug might enhance the release of acetylcholine from motor-nerve terminals. This conclusion was supported by the finding that pempidine methiodide had an "anti-curare" action (Fig. 4). On the other hand, no concentration of dimecamine methiodide was found which antagonized the action of tubocurarine.

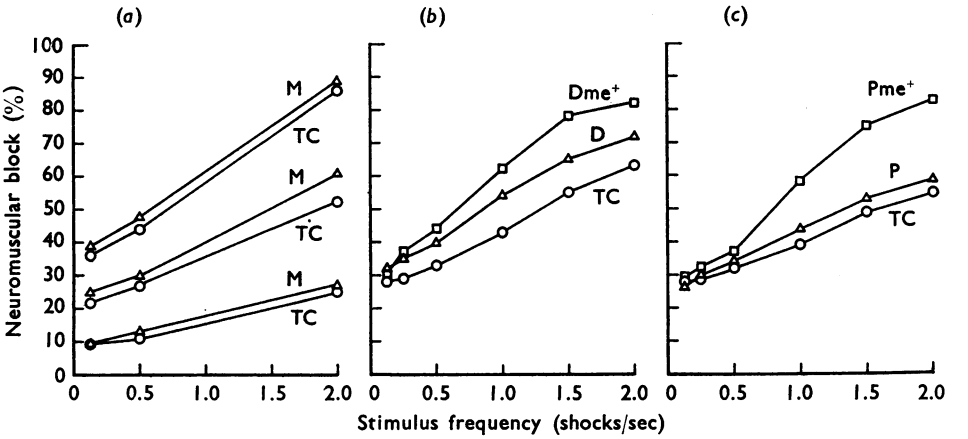


Fig. 3. Effect of stimulus frequency on neuromuscular block in three rat diaphragm preparations. (*a*), effect of stimulus frequency at different levels of block by tubocurarine (approximately 10^{-6} M, TC) and mecamylamine (approximately 3×10^{-4} M, M); (*b*), effect of stimulus frequency during steady partial block by tubocurarine (approximately 10^{-6} M), dimecamine (approximately 3×10^{-4} M, D), and dimecamine methiodide (approximately 2×10^{-4} M, Dme^+); (*c*), effect of stimulus frequency during steady partial block by tubocurarine (approximately 10^{-6} M), pempidine (approximately 9×10^{-4} M) and pempidine methiodide (approximately 1.2×10^{-3} M, Pme^+).

Contracture of avian muscle. A slowly developing but moderate contracture of the chick biventer cervicis muscle was observed with 5×10^{-4}M-dimecamine methiodide solution. A small reduction in the twitch response was also observed. Similar concentrations of dimecamine and pempidine produced only slowly developing and very small contractures. Pempidine methiodide and mecamylamine were inactive. The activity of dimecamine methiodide was about one-third of that of tetramethylammonium in one preparation.

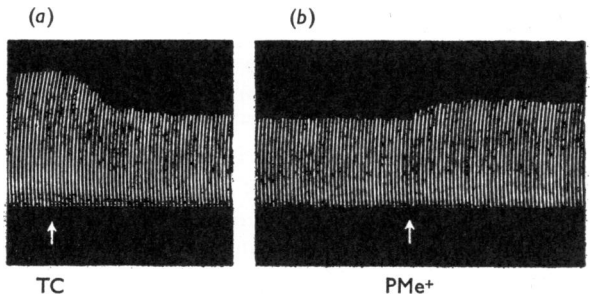


Fig. 4. Rat diaphragm preparation, stimulus frequency 8 shocks/min. (*a*), tubocurarine (approximately 10^{-6} M) was added at TC; (*b*) 20 min later, during steady partial neuromuscular block, pempidine methiodide (2×10^{-4} M) was added at PMe^+.

DISCUSSION

Our chief finding is that the amines, mecamylamine, dimecamine and pempidine, differ in neuromuscular-blocking activity from their corresponding quaternary metho-salts by a factor of less than two. This result has led us, on the basis of at least two assumptions, to conclude that the active form of each amine is the cation acting extracellularly. The two assumptions are : that the quaternary metho-salts do not pass readily across cell membranes ; and that the cationic form of each amine has, because of structural similarity, blocking activity close to that of its quaternary metho-salt.

It is reasonable to expect the quaternary compounds, because of their complete ionization, to be largely confined to the extracellular space. For pempidine methiodide there is indirect evidence for an extracellular distribution (Spinks & Young, 1958 ; Spinks *et al.*, 1958) : when the drug was given intravenously into the cat, the duration of its ganglion blocking action was brief compared with the duration of action of its parent amine pempidine which is widely distributed throughout the body compartments (Harington *et al.* 1958 ; Muggleton & Reading, 1959).

It is not a necessary consequence of the structural similarity of the quaternary metho-salts and the cations of the corresponding amines that they should have closely similar blocking activities. However, if this were not so, it would be necessary in order to account for the general similarity in the activities of the amines and their quaternary metho-salts, to postulate an extremely fortuitous relationship between the activities of the quaternary compounds, which we must assume to act extracellularly, and of the amines, whether they are thought to act mainly as the free base outside the cell or as the free base or the cation or both within the cell or within the membrane. It is simplest to conclude that the active form is the cation acting extracellularly.

The lack of effect of *p*H on the blocking activity of mecamylamine adds support to this conclusion, but only to the extent that it makes it unlikely that the blocking activity depends on the concentration of the free base. Whether the active form was the free base acting in the extracellular space, or the free base or the cation or both acting within the cell or the cell membrane, an approximately tenfold change in blocking activity should have followed a change in *p*H of one unit. In spite of the fact that the state of the rat diaphragm itself must have been altered by the change in bicarbonate concentration and *p*H (Creese, 1950, 1951 ; Harris, 1953 ; Rogers & Fenn, 1957 ; Castillo *et al.*, 1962), the alteration could not have been so great (to judge by the response to tubocurarine) as to mask a tenfold change in the blocking activity of mecamylamine.

Experiments by Payne & Rowe (1957) provide further evidence for an extracellular site of action of mecamylamine. They found in the cat, that, after a large dose of mecamylamine, an increase in inhaled carbon dioxide concentration increased the plasma concentration of mecamylamine, reduced the blood pressure and increased neuromuscular block. Evidently, with the lowering of blood *p*H produced by the carbon dioxide, there was a shift of mecamylamine from cells into the extracellular space where it acted.

As regards the mode of action of the compounds, our results can indicate little more than that the neuromuscular-blocking action of the amines and their quaternary metho-salts was postsynaptic and probably not due to depolarization. Although block by the compounds could not be distinguished from that produced by tubocurarine, it must not be concluded that the compounds were acting as competitive blocking agents. Van Rossum & Ariëns (1959) obtained evidence from experiments on the frog rectus abdominis preparation that mecamylamine had a predominantly non-competitive action. Our results do not exclude such an action. The finding that the stimulus frequency dependence of the block produced by the compounds was equal to or greater than that of the block produced by tubocurarine excludes the possibility that the block, which is frequency independent of stimulus frequency, was presynaptic like that due to magnesium and makes it unlikely that the block produced by some of the compounds was accompanied by depolarization (Blackman, 1963). The slowly developing contracture produced in the chick biventer cervicis preparation by dimecamine methiodide and, to a lesser extent, by dimecamine and pempidine, suggests that they had some depolarizing activity in this preparation, although it could not have been more than a ten-thousandth of the activity of decamethonium (Ginsborg & Warriner, 1960). If a similar ratio of activity applies to the rat diaphragm, depolarization is unlikely to be an important factor in the action of these compounds on this preparation. No evidence of depolarizing activity was found by Stone *et al.* (1956) or by Corne & Edge (1958) when they treated the frog rectus abdominis preparation with large doses of mecamylamine or pempidine. We did not test the compounds for anticholinesterase activity, but Corne & Edge (1958) have reported that 2.1×10^{-2}M-pempidine solution was needed to produce 50% inhibition of the activity of erythrocyte acetylcholinesterase : neostigmine was 10^6-times more active.

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