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THE ANTICURARE ACTIVITY OF TETRAETHYL-AMMONIUM ION IN THE CAT

BY

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Although tetraethylammonium ion (TEA) was reported to have some anticurare activity in the cat by Rothberger (1902) little attention has been paid to this aspect of its pharmacological activity. However, its anomalous action compared with other simple onium ions at the neuromuscular junction, such as its relative lack of "curariform" activity, its production of fibrillary twitching in voluntary muscle, and its augmentation of the response of frog muscle to indirect stimulation, has been studied in some detail. The literature on these points has been reviewed by Ing (1936), as have the observations of the direct action of TEA on frog nerve. Lorente de No (1948, 1949) also has observed that TEA affects frog nerve activity in that TEA and several related compounds were found to restore conduction to certain types of frog nerve fibres after they had been caused to fail by sodium depletion.

In a study (Kensler, 1949) of the action of TEA on the rat phrenic nerve-diaphragm preparation (Bülbring, 1946), TEA was found to antagonize the curarizing agents *d*-tubocurarine chloride, β-erythroidine hydrochloride, and dihydro-β-erythroidine hydrochloride, and to restore function impaired by a deficiency of calcium ions. TEA also antagonized the action of tubocurarine on the kitten phrenic nerve-diaphragm preparation although Sullivan and Kensler (1950) found it less effective in "replacing" calcium than in the rat preparation. As TEA has recently been observed (Kensler, 1950) to antagonize the curarizing action of 1 : 2 : 3-tri-β-diethyl-aminoethoxy) benzene triethiodide (Flaxedil) and decamethonium iodide (C10) on both the rat and kitten diaphragm preparations, the study of the anticurare activity of TEA has been extended to the cat gastrocnemius muscle preparation *in vivo*.

Acheson and Pereira (1946a) noted that TEA antagonized tubocurarine to some extent in the cat. However, these authors (1946b) state that "TEA over a wide range of doses has no action other than a specific ganglionic blocking action."

METHODS

Cats were anaesthetized first with ether and then with chloralose (ca. 80 mg./kg.) and were prepared for recording the response of the left gastrocnemius muscle to sciatic nerve stimulation using the technique of Bülbring and Burn (1941). The sciatic nerve was stimulated with supra-maximal single shocks of 0.5 msec. duration from a square wave stimulator at rates of 12–18 per minute. Blood pressures were recorded from the carotid artery by means of a mercury manometer.

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The curarizing compounds were injected intra-arterially through a cannula inserted into the central end of the right iliac artery at the bifurcation of the aorta and TEA was injected at this site or intravenously through a cannula inserted into the right external jugular vein. Doses of TEA refer to tetraethylammonium bromide.

RESULTS

In confirmation of the results obtained with isolated phrenic nerve-diaphragm preparations, TEA has been found to antagonize the action of tubocurarine chloride, flaxedil, and to a lesser extent decamethonium iodide in the cat gastrocnemius preparation.

The results of a typical experiment in which the curarizing action of 350 μg. *d*-tubocurarine chloride was antagonized by 4 mg. TEA are shown in Fig. 1. Both compounds were injected intra-arterially. As can be seen in this Figure the anticurare activity of TEA is extremely rapid. Smaller doses of TEA antagonized tubocurare equally rapidly although less completely. The smaller doses (0.5 mg. or less) did not cause a significant lowering of the blood pressure although the higher doses did.

TEA readily antagonized flaxedil, but only the larger amounts (ca. 4 mg.) were active against decamethonium iodide (C10). The antagonism of a C10 block by intra-arterially injected TEA is illustrated in Fig. 2.

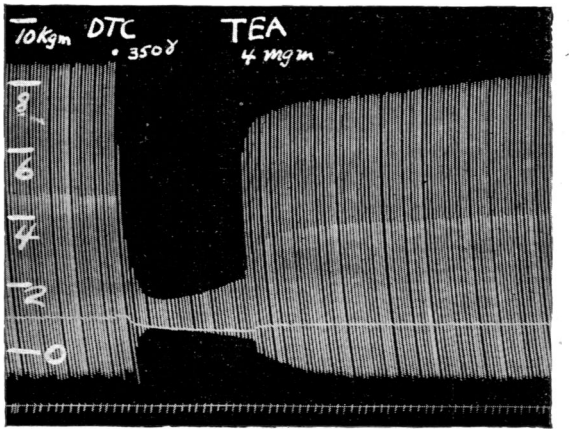

FIG. 1.—Cat. Ether and chloralose. Record of single twitches of left gastrocnemius in response to supramaximal single shocks of 0.5 msec. duration at 12–18 per min. Neuromuscular block produced by 350 μg. *d*-tubocurarine chloride relieved by 4 mg. TEA. Both drugs given intra-arterially. Time marker every 10 sec.

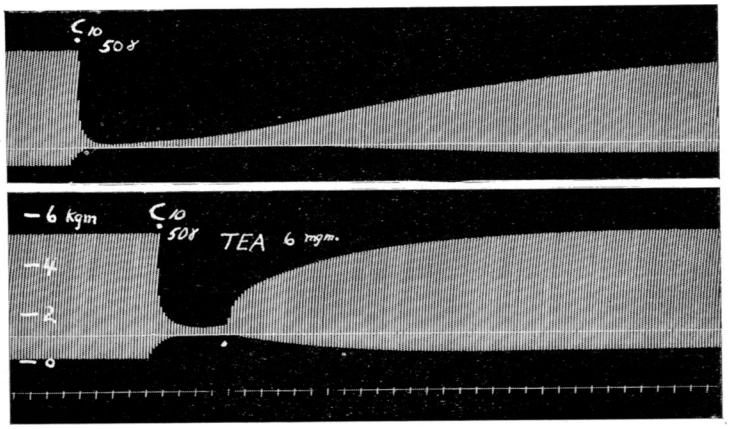

FIG. 2.—Similar preparation to that of Fig. 1. Intra-arterial injections. Top: spontaneous recovery of preparation from neuromuscular block produced by 50 μg. decamethonium iodide. Bottom: an hour earlier 6 mg. TEA restored contractions after a dose of decamethonium iodide (50 μg.). Time marker every 30 sec.

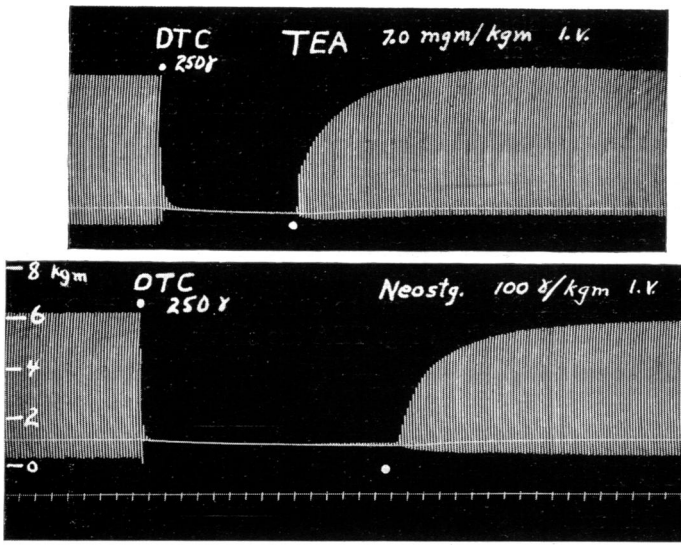

FIG. 3.—Similar preparation to that of Fig. 1. Effect of *intravenous* injections of TEA (7 mg./kg.) and neostigmine (100 μg./kg.) after neuromuscular block produced by 250 μg. tubocurarine chloride. An hour elapsed between the upper and lower recordings. Time marker every 30 sec.

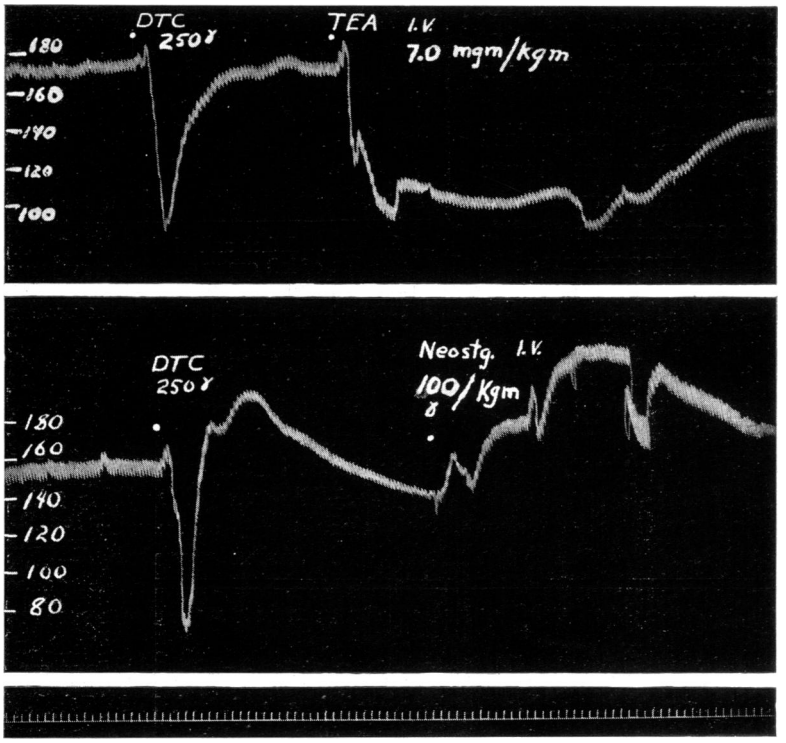

FIG. 4.—Same experiment as in Fig. 3. Blood pressure records obtained at the same time as the gastrocnemius tracings in Fig. 3. Upper: tubocurarine followed by TEA. Lower: tubocurarine followed by neostigmine. Time marker every 10 sec.

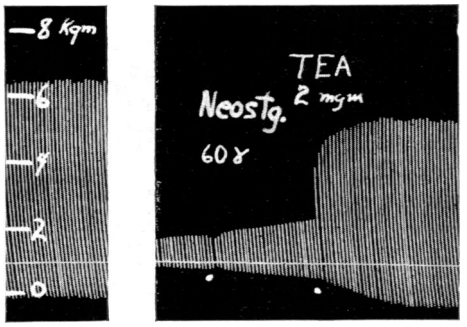

FIG. 5.—Similar preparation to that of Fig. 1. Tubocurarine and neostigmine given alternately until neostigmine failed to antagonize the former; 2 mg. TEA now produced a prompt restoration of the contractions.

Intravenous injection of TEA has also been found to antagonize the action of tubocurarine. The results of such an experiment are shown in Fig. 3; the concurrent blood pressure recordings are shown in Fig. 4. The anticurare action of intravenously administered neostigmine in the same animal is shown in Fig. 3 and the concurrent effect of neostigmine on blood pressure is shown in Fig. 4. It will be noted that the anticurare action of TEA is more rapid than that of neostigmine, although the dose of TEA administered produced a fall in blood pressure whereas neostigmine produced a rise. No quantitative comparison of the anticurare activity of TEA and neostigmine has been made, although on the basis of the work done TEA appears to be less than one hundredth as active as neostigmine on a molar basis.

In a series of experiments (Kensler, 1950) on the rat phrenic nerve-diaphragm preparation it was observed that if the amount of tubocurarine which could be antagonized by increasing amounts of neostigmine was exceeded, TEA still possessed anticurare activity. Conversely, neostigmine was also found to exert anticurare activity when the amount of tubocurarine that could be antagonized by TEA was

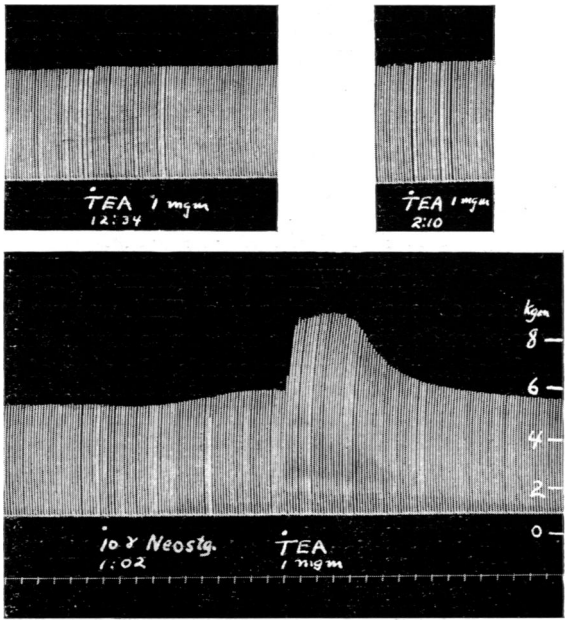

FIG. 6.—Similar preparation to that of Fig. 1. TEA (1 mg.) had scarcely any effect on the contractions of the cat gastrocnemius at 12.34 and 2.10 p.m., but 6 min. after 10 μg. neostigmine at 1.02 p.m. the same dose of TEA (1 mg.) produced a marked increase in the size of the contractions. Time marker every 30 sec.

exceeded. The results of a similar experiment in the cat are shown in Fig. 5. In this experiment partial paralysis was produced by the injection of 250 μg. tubocurarine chloride. This was antagonized by the intra-arterial injection of 60 μg. neostigmine, again blocked with tubocurarine, and antagonized by neostigmine and so on until further injections of neostigmine no longer accelerated recovery from the tubocurarine block. This " titration " was carried out over a period of 58 minutes during which 750 μg. tubocurarine chloride and 260 μg. neostigmine were administered. As is shown in Fig. 5 TEA in this situation still possessed marked anticurare activity.

TEA has no significant anticholinesterase activity (Barlow and Ing, 1948), but it does antagonize C10 which is not antagonized by neostigmine (Paton and Zaimis, 1949) ; these facts indicate that the anticurare activities of TEA and neostigmine are dependent on different mechanisms. However, it was noted that in preparations treated with neostigmine, TEA greatly increased the response of the gastrocnemius muscle to sciatic nerve stimulation. As is shown in Fig. 6 TEA in a dose which by itself produced little or no effect produced a marked increase in muscle response when it was preceded by a dose of neostigmine just sufficient to augment the muscle response slightly.

Discussion

The experiments described show that TEA, in doses commonly used to achieve ganglionic blocking action, has a marked effect on neuromuscular transmission in the cat gastrocnemius muscle ; it will antagonize neuromusclar block produced by tubocurarine, flaxedil, or decamethonium iodide ; it will also augment the response of the muscle to indirect stimulation after small doses of neostigmine have been administered.

In view of the observations of Loeb and Ewald (1916), Cowan and Ing (1933, 1935), and Cowan and Walter (1937) that treatment of frog nerves with TEA (0.01–0.042 M) gives rise to repetitive discharges when they are stimulated with single shocks, one is inclined to explain the results obtained in the cat on this basis. However, there is no evidence that lower concentrations of TEA have this action, and Ing and Wright (1933) have shown that whereas TEA in a concentration of 0.01 M produced augmentation of the response of the frog sartorius muscle to nerve stimulation, a concentration of 0.001 M was without effect. In experiments on the rat diaphragm in which muscle action potentials following nerve stimulation were recorded Vaughan Williams and Kensler (unpublished) were unable to detect any evidence of repetitive firing when TEA was used either to restore function depressed by calcium deficiency or to antagonize curare, although small amounts of neostigmine produced double action potentials. In view of these observations and the fact that the doses of TEA used in the cat produced no observable fasciculation it would appear that the results reported are not due to repetitive nerve responses to single shock stimuli.

Summary

1. Intra-arterially injected tetraethylammonium ion has been found to antagonize the curarizing action of *d*-tubocurarine chloride, 1 : 2 : 3-tri-β-diethylaminoethoxybenzene triethiodide (flaxedil) and to a lesser extent decamethonium iodide in the cat gastrocnemius muscle stimulated through its nerve.

2. Intravenously administered tetraethylammonium ion in doses commonly used to produce ganglionic blocking action has also been found to antagonize *d*-tubocurarine chloride.

3. Tetraethylammonium ion has been shown to restore the contractions of a cat's gastrocnemius even after doses of tubocurarine and neostigmine have been given alternately until the latter drug, in what were initially completely effective doses, ceases to be effective in relieving the neuromuscular block.

4. Doses of tetraethylammonium ion which produced little or no action on the response of the cat gastrocnemius muscle to nerve stimulation produce after small doses of prostigmine a marked augmentation of the response.

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