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THE EFFECT OF TWO ETHYLENEDIAMINES ON NEUROMUSCULAR TRANSMISSION AND ON STRIATED MUSCLE

BY

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An increasing number of substances are known to exert a neuromuscular blocking action either in a curare-like or a decamethonium-like manner. The criteria for distinguishing these modes of action have been considered by Burns and Paton (1951). Other substances, such as procaine (Jaco and Wood, 1944) and botulinum toxin (Burgen, Dickens, and Zatman, 1949), cause paralysis by interfering with the release of acetylcholine at the motor end-plate.

The purpose of this report is to describe the activity of two ethylenediamines on neuromuscular transmission and on striated muscle. Both are weak antihistamine and anti-adrenaline agents, potent local anaesthetics, and central stimulants (Tonks, 1953). They are *N'*-benzyl-*N'*-2-naphthyl-*N*-dimethylethylenediamine (T3) and the *N'*-ethyl derivative (T7). Their formulae are $C_{10}H_7.N'R.(CH_2)_2.N(CH_3)_2$ where R is benzyl ($C_6H_5.CH_2$–) in T3 and ethyl in T7. Their synthesis has been reported by Chapman, James, and Williams (1952).

METHODS

Mammalian Nerve-Muscle Preparations

(*a*) Wistar rats of 300–400 g. wt. were anaesthetized with pentobarbitone sodium 50 mg./kg. or urethane 400 mg./kg. intraperitoneally. The trachea and jugular vein were cannulated. A cannula was inserted into the left femoral artery, directed centrally, from which intra-arterial injection of the right limb was effected. This limb was secured to a rigid horizontal bar by loops around the femur and lower part of the limb. The tendon of the gastrocnemius was exposed, tied, cut, and connected to an isometric lever.

(*b*) Cats were anaesthetized with chloralose 60 mg./kg. intravenously. The trachea, the external jugular vein and the posterior mesenteric artery were cannulated. The external iliac artery to the opposite limb and the terminal branches of the aorta were ligated. The anterior tibial artery was prepared for close-arterial injection and the tibialis anticus tendon connected to an isometric lever.

In both species the muscles were stimulated directly, or via the cut sciatic nerve, with supramaximal induction-coil break shocks through shielded nickel electrodes. The rate of stimulation—usually 8/min.—was regulated by a Lewis's Rotary Contact. Tetanizing square-wave stimuli were delivered from an electronic stimulator at 100/sec., 5 V., and 0.50 msec. duration.

Avian Striated Muscle

Groups of week-old chicks were injected via the brachial vein with the ethylenediamines, gallamine, decamethonium (C10), leptazol, and with mixtures of these.

Amphibian Striated Muscle

In addition to the isolated frog rectus abdominis, experiments were performed with pairs of isolated gastrocnemii. The muscles were secured by hook electrodes connected in series to the secondary terminals of an induction coil. Supramaximal stimuli were applied at the rate of 10/min. The " make " shocks were short-circuited. The muscles were in separate 10 ml. baths and attached to isometric levers. Equimolar amounts of T3 and T7, C10 and monoiodoacetic acid were added, a control muscle being left free from drug in each case.

Compounds were injected in equimolar amounts into the ventral lymph sacs of groups of intact frogs and the effects observed.

Action on Cholinesterase

Warburg manometers were used to determine the effect of the ethylenediamines on cholinesterase. Washed, laked rabbit red cells and acetyl-β-methylcholine (0.027M) and rabbit plasma with benzoyl choline (0.0055M) were used as sources of, and substrates for, true and pseudo-cholinesterase, respectively. Before mixing enzyme and substrate, air was removed from the flasks by bubbling through nitrogen and carbon dioxide (5%) for 30 min. at 37° C. The substrate and ethylenediamine (if any) were placed in the side arms of the flask, so that they were brought into simultaneous contact with the enzyme on shaking. The manometers were read at 10 min. intervals for two hours.

RESULTS

Mammalian Nerve-Muscle Preparation

In the cat and the rat intra-arterial injection of the ethylenediamines caused a transient increase

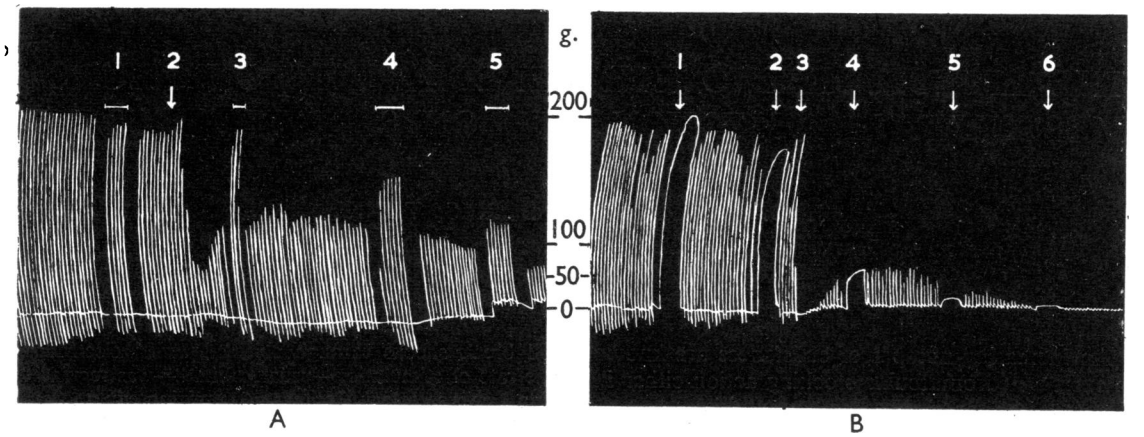

FIG. 1(A).—Cat, chloralose, 2.7 kg. Isometric contractions of tibialis anticus stimulated supramaximally 8/min. via the sciatic nerve or directly as at 1, 3, 4, and 5. At 2, close-arterial injection of T7, 3 mg./kg., inhibits indirect stimulation before direct. (B) Cat, chloralose, 3.0 kg. The same as (A). At 1, 2, 4, 5, and 6 tetanizing stimuli to nerve. At 3, T7 3 mg./kg. by close-arterial injection. Though tension is well sustained during a tetanus, the magnitude of the tetanus as well as twitch responses are diminished.

of the twitch tension followed by a depression in muscles indirectly stimulated at a rate of 8/min. With small doses of these substances (0.8 mg./kg.) recovery from the initial paralysis took place, but with larger doses (3 mg./kg.) recovery was incomplete (Fig. 1a). During the early stage of paralysis direct stimulation of the muscle at the same rate produced a normal response. If large doses of the ethylenediamines were administered this response was later diminished (Fig. 1a). The muscle tension was well-sustained during a tetanus, but the magnitude of the response to subsequent single and tetanizing stimuli was decreased (Fig. 1b). The rapid close-arterial injection of 1–3 mg./kg. produced a twitch in the resting tibialis anticus muscle of the cat. The neuromuscular blocking action of these ethylenediamines sums with that of C10. This is shown in Fig. 2, where injections of C10 and T7 were made singly and together at intervals of 45 min. in the cat.

The paralysis caused by the ethylenediamines was not relieved by neostigmine, adrenaline, or C6 in maximal doses. However, if a small dose (0.08 mg./kg.) of gallamine—which had no effect on the normal response to indirect stimulation—preceded the injection of the ethylenediamine (0.8 mg./kg.), the paralysis caused by the latter was not as severe as when given alone (see Fig. 3). The paradoxical effect of neostigmine on muscle tension during slow and rapid indirect stimulation is well known. This drug increases muscle tension during slow stimulation, whereas depression follows on rapid stimulation. The ethylenediamine (Fig. 4a), C10 (4b), and procaine (4c) readily antagonized the neostigmine-potentiation; but, unlike procaine, the ethylenediamine and C10 failed to

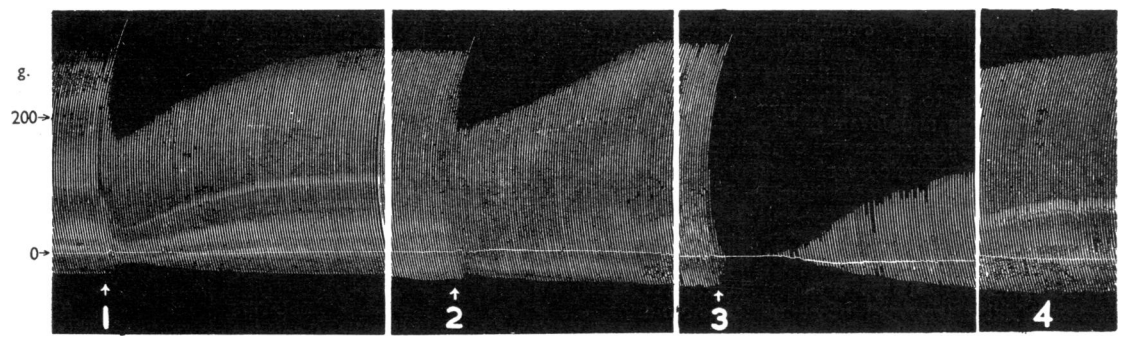

FIG. 2.—Cat, chloralose, 3.4 kg. Record of isometric contractions of tibialis anticus stimulated indirectly with supramaximal break shocks at the rate of 10/min. At 1, C10 3μg./kg., at 2, T7 1 mg./kg., and at 3 both substances given together. Injections were by the close-arterial route. Panel 4 shows recovery in 24 min. Recovery time 11 min. with C10 and 7 min. with T7 given singly.

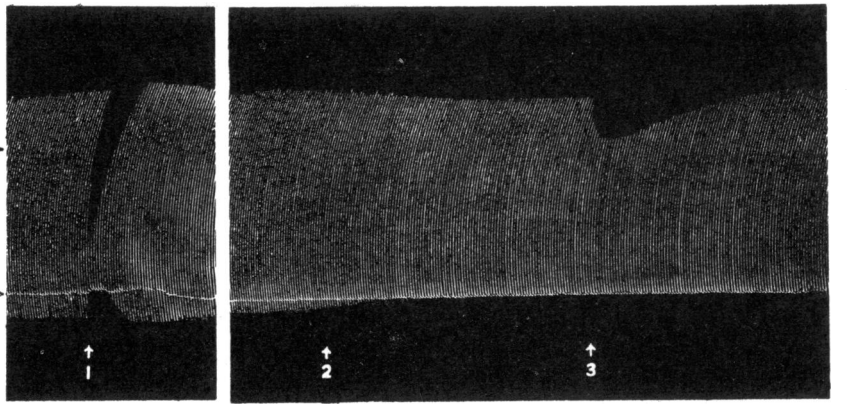

FIG. 3.—Cat, 3.1 kg., chloralose. Tibialis anticus stimulated as in Fig. 2. At 1 and 3, T7 0.8 mg./kg., and at 2, gallamine 0.08 mg./kg. by the close-arterial route. Note less depression of twitch by T7 after gallamine.

prevent the depression which followed on rapid stimulation.

The response to a close-arterial injection of ACh was also antagonized by the ethylenediamines (Fig. 5), and they enhanced the depression produced by larger doses of ACh following neostigmine.

If adrenaline (1.5 μg./kg.) is injected intra-arterially after neostigmine the twitch tension is further increased. The ethylenediamines caused a sharp inhibition of this second response (Fig. 6).

Avian Striated Muscle

Intravenous injection of gallamine produced flaccid paralysis in the chick (Fig. 7, No. 1) and the ethylenediamine caused a spastic paralysis. The posture assumed was not identical with the characteristic opisthotonos resulting from C10. (Compare Fig. 7, Nos. 2 and 3.)

Since the ethylenediamines are central stimulants, it was decided to test whether central stimulation interfered with the production of opisthotonos by C10. Leptazol gave rise to clonic convulsions terminating in a flaccid paralysis; leptazol with C10 produced a spastic paralysis (Fig. 7, No. 4) in which the characteristic manifestations were modified so that the final appearance more closely resembled that given by T3 or T7.

Amphibian Striated Muscle

The ethylenediamines, like C10, produced contracture in the normal frog rectus and in the muscle soaked in a solution of DFP 10^{-6} for 30 min. Their potency was one-tenth that of C10. Monoiodoacetic acid in an amount ten times greater than T3 or T7 (i.e., 1.25×10^{-3}) produced a contracture, although very slowly.

The contractures produced by the ethylenediamines and C10 had a slower onset than those caused by ACh. They took longer to reach a plateau and, on washing out, relaxation was

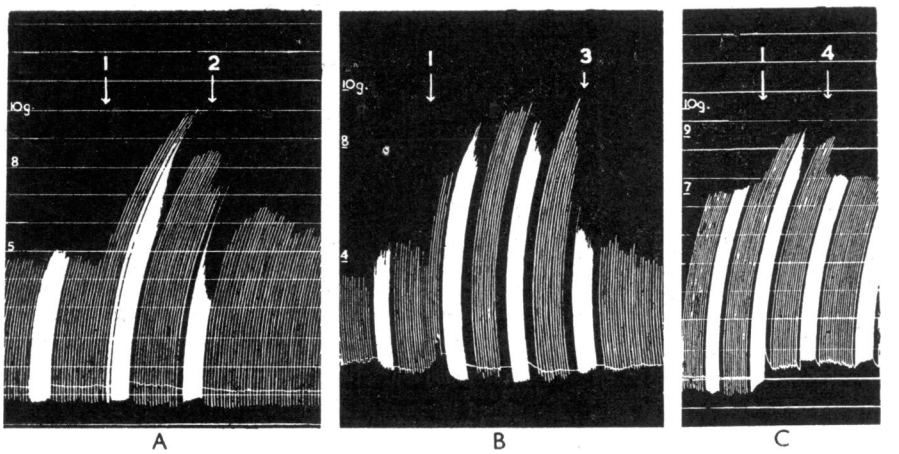

FIG. 4.—Rats, 300 g., 280 g., and 330 g. in A, B, and C respectively. Urethane 400 mg./kg. i.p. Isometric contractions of gastrocnemius stimulated indirectly with supramaximal shocks at 8/min. and 120/min. Close-arterial injections of 1.5 μg. neostigmine at 1 in A, B, and C. In A the stimulant effect of neostigmine is antagonized by T7 1 mg./kg. at 2. In B 20 μg./kg. of C10 at 3 exerts a similar effect. In C procaine 5 mg./kg., at 4, also antagonizes the increase in tension following neostigmine, but in addition prevents the characteristic decrease of tension during rapid stimulation.

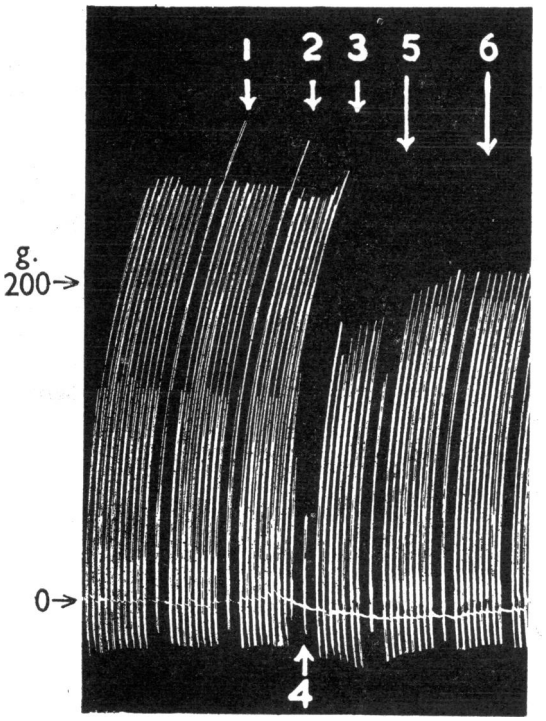

FIG. 5.—Cat, chloralose, 2.9 kg. Record of indirect stimulation of tibialis anticus at 8/min. ACh, 10 μg., by close-arterial injection at 1, 2, 4, 5, and 6 causes a twitch. 3 mg./kg. T7 at 3 inhibits the responses to both stimuli.

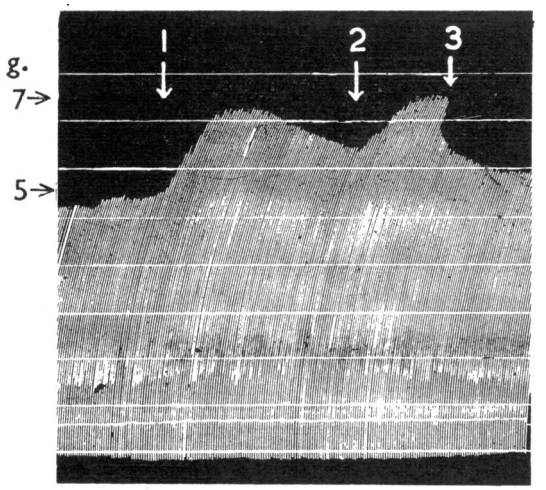

FIG. 6.—Gastrocnemius of rat, pentobarbitone sodium 50 mg./kg. i.p. Increase in tension caused by neostigmine 1.5 μg. at 1 during indirect stimulation at 20/min. is enhanced by 0.5 μg. adrenaline at 2. This effect is inhibited at 3 by T7, 3 mg./kg.

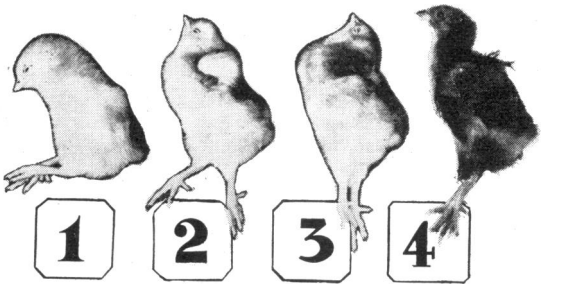

FIG. 7.—Post-mortem photographs of week-old chicks injected i.v. with (1) gallamine 5 mg./kg., (2) T7 100 mg./kg., (3) C10 1.0 mg./kg., (4) C10 1.0 mg./kg. with leptazol 10 mg./kg.

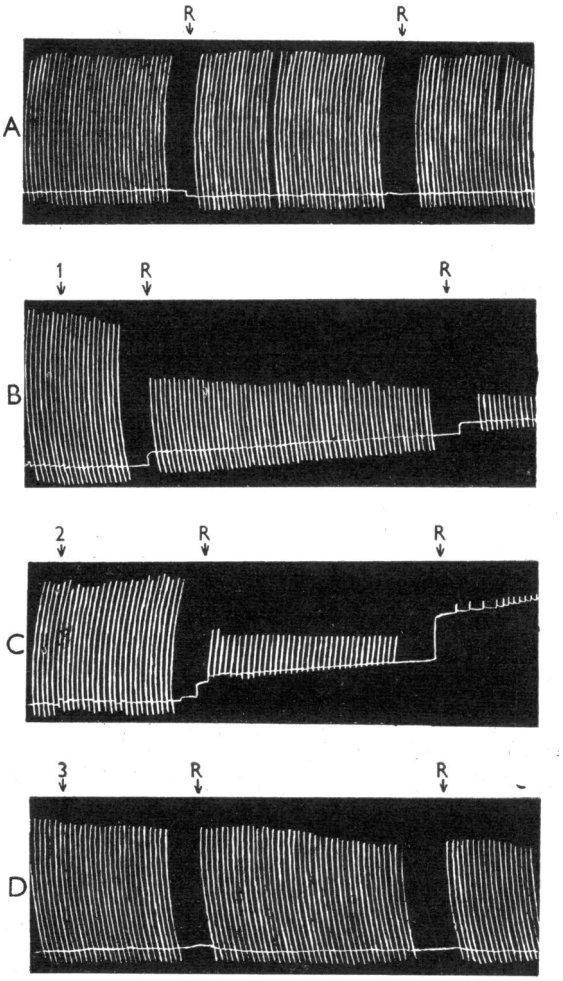

FIG. 8.—Isolated frog gastrocnemius muscles in Ringer's solution, directly stimulated at 10/min. with maximal break shocks from an induction coil. R=rest period, A is a control, B and C show the resemblance between the effects of 7.5 mg. of T7 given at B1 and 10 mg. of monoiodoacetic acid at C2 by contrast with C10 7 mg. at D3.

slower. They resembled the responses to ACh in being antagonized by gallamine (2×10^{-3}), and by a high concentration of atropine (10^{-3}). The response to repeated additions of ACh was reduced by the ethylenediamines but potentiated by C10. This potentiation was attributed by Paton and Zaimis (1949) to the weak anticholinesterase activity shown by C10. Ethylenediamines also antagonized the responses to potassium chloride administered at regular intervals. C10 did not produce the same result as the ethylenediamines when added to the bath fluid of the isolated and directly stimulated frog gastrocnemius. This difference was most clearly observed if, following a brief period of stimulation, the muscle was rested for 10 min. (compare Fig. 8b and d). At the end of this time the tone of the muscle in contact with the ethylenediamines was increased and remained so on further stimulation, when the magnitude of the muscle twitch was diminished. A similar though more pronounced result was obtained with monoiodoacetic acid (Fig. 8c). C10 was without this effect.

Monoiodoacetic acid (1 mg./10 g.) produced its typical spastic paralysis when injected into the ventral lymph sacs of intact frogs, but the ethylenediamines (1 mg./10 g.) and C10 (0.1 mg./10 g.) were without effect.

Action on Cholinesterase

The action of the ethylenediamines on true and pseudo-cholinesterase was investigated. Concentrations of the ethylenediamines from 10^{-6} to 10^{-3} were used, and within this range they had little activity against either enzyme.

After completing the investigation it was clear that T3 and T7 had comparable activities. It was therefore decided to include only figures obtained with T7.

Discussion

The brief stimulant action of the ethylenediamines on the muscle twitch in the cat and rat and the production of contracture in the frog rectus suggest a depolarizing action at the motor end-plate similar to that of decamethonium (Paton, 1951). The sustained tension during a tetanus and the stability of the block, as well as the failure of adrenaline and neostigmine to relieve it, are compatible with this view. In addition the ethylenediamines produce a twitch in the resting tibialis anticus muscle of the cat and sum with C10 in paralysing the indirectly stimulated muscle. The cat tibialis muscle appears more sensitive to the ethylenediamines than does the rat gastrocnemius. It is known that the activity of a substance producing neuromuscular block by long lasting depolarization is diminished by a substance raising the end-plate threshold to ACh. This is observed in the cat tibialis anticus with the ethylenediamines when gallamine is interposed between the injections.

The ethylenediamines and C10 did not prevent the falling off in muscle tension during rapid stimulation of the rat gastrocnemius and cat tibialis anticus following neostigmine. Thus, unlike procaine and botulinum toxin, they do not appear to inhibit the release of ACh or reduce its depolarizing action like curare. Furthermore, the effect of ACh by close-arterial injection is antagonized as much as, if not more than, the response to a nerve volley. In the slowly stimulated preparation an increase in muscle tension follows the injection of adrenaline. This is antagonized by the ethylenediamines.

The characteristic effect of C10 in the chick was not observed with the ethylenediamines. The latter are central stimulants, and it was interesting to observe that C10 failed to produce a complete opisthotonos when administered with leptazol. Other anomalies appear on comparing C10 with the ethylenediamines. Small doses of the latter produce a reversible block, but with larger doses (3 mg./kg.) only partial recovery occurs during which the muscle tension again decreases. Control experiments have shown that this secondary decrease in tension is not the result of normal fatigue. Whereas C6 antagonizes the action of C10 (Paton and Zaimis, 1949) it does not relieve the paralysis caused by large doses of the ethylenediamines, and during such paralysis the response to direct stimulation of the muscle is diminished. Lastly, there is the deepening paralysis following a sustained tetanus. This would suggest anticholinesterase activity (Bacq and Brown, 1937), but the ethylenediamines depress, and do not potentiate, the response to ACh in the frog rectus. At this concentration they also reduce the contracture due to KCl, and they can still elicit a contracture following the prolonged contact of the muscle with DFP. Manometric tests have confirmed the absence of anticholinesterase activity in the ethylenediamines.

These differences can be reconciled if account is taken of the action of the ethylenediamines on the isolated frog gastrocnemius. C10 appears to be without effect on the directly stimulated muscle, while the ethylenediamines exhibit an action similar to that of the thiolprive group of substances. Guttman, Horton, and Wilbur (1937) have shown that interference with lactic acid formation by monoiodoacetic acid abolishes post-

tetanic potentiation in indirectly stimulated muscle. This would account for the anomaly noted with the ethylenediamines on stimulating the muscle indirectly after a tetanus. It is reasonable to assume that the reduction in the response to direct stimulation of the muscle, the inability of the muscle to recover completely from large doses of the ethylenediamine, and the failure of C6 to relieve the paralysis caused by these compounds, are also the result of this interference with muscle metabolism. The absence of the spastic paralysis typical of monoiodoacetic acid on injecting the ethylenediamines into the ventral lymph sac of the frog would indicate that they are less potent than monoiodoacetic acid itself. This is confirmed on comparing the results of equimolar concentrations of these substances on the frog gastrocnemius.

In conclusion, it appears that these ethylenediamines exert a specific depolarizing action on the motor end-plate in addition to a direct action on the muscle itself.

Summary

1. *N'* - benzyl - *N'* - 2 - naphthyl - *N* - dimethylethylenediamine (T3) and its *N'*-ethyl analogue (T7) are neuromuscular blocking agents of the decamethonium type which also directly depress the muscle.

2. On close-arterial injection they produce a twitch in the resting tibialis muscle of the cat; they increase and then decrease the twitch response to indirect stimulation in the cat and the rat; they give additive effects with C10. They produce contracture in the frog rectus before and after DFP, and partial opisthotonos in chicks. They have no anticholinesterase activity.

3. Larger amounts reduce the response of mammalian muscle to direct stimulation *in vivo* and produce contracture in amphibian muscle *in vitro*.

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