MYASTHENIC-LIKE FEATURES OF THE NEUROMUSCULAR TRANSMISSION FAILURE PRODUCED BY TRIETHYLCHOLINE

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Received May 22, 1962

THE triethyl analogue of choline (triethyl-2-hydroxyethylammonium) has been shown to produce a slowly-developing failure of transmission in frequently excited mammalian nerve-muscle preparations. The cause of this, it has been suggested, is an action on the nerve endings through which the amount of acetylcholine released by a nerve impulse is reduced (Bowman and Rand, 1961a,b,c). Among a series of choline-analogues studied, optimal activity in this respect was found with the triethyl analogue (Bowman and Rand, 1962). In conscious animals, injection of triethylcholine causes a slowly developing muscular weakness which is accentuated by exercise and which closely resembles the symptoms of myasthenia gravis (Bowman and Rand, 1961a,b). However, preliminary observations appeared to suggest a difference between the defect in myasthenia gravis and that produced by triethylcholine. In the myasthenic patient, edrophonium and neostigmine cause a striking improvement in muscular power, but even when doses twice as great as the usual anti-curare doses were employed, these agents possessed only an insignificant ability to restore maximal twitches depressed by triethylcholine (Bowman and Rand, 1961b). The effects of larger doses of edrophonium and neostigmine on the muscular weakness produced by triethylcholine have now been examined both in conscious rabbits and in nerve-muscle preparations of anaesthetised cats.

Triethylcholine chloride (15 mg./kg.) was injected intravenously into each of 5 conscious rabbits. Every 5 min. after injection the righting-reflex was tested up to 20 times in rapid succession and the trial at which each rabbit failed to right itself was noted. The rabbits were left undisturbed between each test. Between 25 and 40 min. after injection, the rabbits lost the strength to right themselves after 2–10 trials and this degree of weakness lasted for about 20 min. A gradual recovery then occurred so that by 100 min. after injection they again responded to all 20 trials. Control tests previously carried out on the same rabbits showed that in the absence of drug treatment all of them continued to right themselves to all 20 trials applied at 5 min. intervals for up to 150 min., although their movements became less vigorous towards the end of this period.

On subsequent different days, the same doses of triethylcholine were administered and at the height of the ensuing muscular weakness, edrophonium (1 mg./kg.), neostigmine (0·25 mg./kg.) or choline (5 mg./kg.) was injected intravenously: the rabbits were atropinised (3 mg./kg. i.v.) before neostigmine or choline was administered. Edrophonium caused

a striking but temporary improvement in muscular strength, and neostigmine caused a slightly greater and longer lasting improvement which merged with the spontaneous recovery. However, neither of these was as effective as choline which caused a return almost to normal activity within 5–10 min. These results are expressed graphically in Fig. 1.

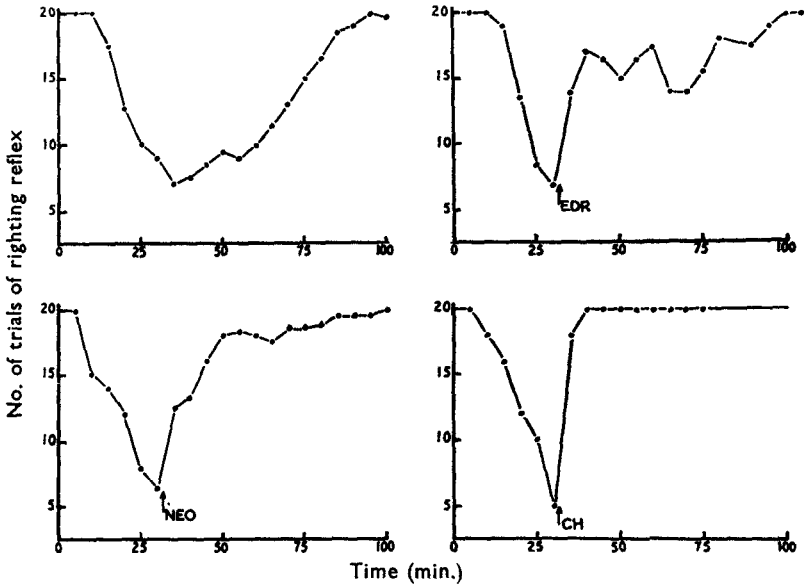

FIG. 1. The effects of edrophonium (EDR), neostigmine (NEO) and choline (CH) on the muscular weakness produced by triethylcholine in conscious rabbits. Each point represents the mean results of trials in 5 rabbits. Triethylcholine (15 mg./kg.) was injected intravenously at time 0 in each case. No antagonist was administered in the first experiment. For further details, see text.

In experiments on cats under chloralose anaesthesia the tension of the tibialis anterior muscle was recorded isometrically on a cathode ray oscilloscope by means of a RCA 5734 transducer valve; gross muscle action potentials were simultaneously led off by means of belly-tendon electrodes. Clonic contractions of the muscle were elicited by stimulation of the motor nerve with supramaximal shocks of 100 μsec. duration at a frequency of 10/sec. for 1 sec. every 10 sec. Fig. 2a is one of a series of identical clonic contractions recorded before triethylcholine. Between *a* and *b* a large dose of triethylcholine (100 mg./kg.) was injected intravenously. In order to hasten the onset of transmission failure, 12 tetani, each of 1 sec. duration and 100/sec. frequency, were delivered within the space of 1 min. after 10 min. had elapsed since the injection. In the normal muscle such high-frequency stimulation did not cause appreciable fatigue but after triethylcholine, the subsequent test responses were markedly depressed (Fig. 2b). This degree of transmission failure was maintained at a constant level for about 90 min. providing the stimulation was delivered at regular intervals throughout. After this period a gradual

recovery of the contractions occurred. Recovery could be hastened by allowing the preparation short periods of rest. Intravenous injections of edrophonium (0·5 mg./kg.), neostigmine (0·25 mg./kg.) or choline (5 mg./kg.) reversed the transmission failure. Fig. 2c illustrates the partial improvement in contractions produced by edrophonium. Twenty min. later the effect of edrophonium had worn off and the contractions had again become constant at a depressed level (Fig. 2d). Neostigmine produced about the same degree of antagonism as edrophonium but its duration of action was considerably longer. An injection of choline caused complete restoration of contractions (Fig. 2e) and this effect was persistent.

These results therefore show that, as in myasthenia gravis, edrophonium and neostigmine cause a marked improvement in the strength of muscles depressed by triethylcholine. Comparison of Fig. 2b and c with Fig. 6 of a paper by Desmedt (1961) illustrates the striking resemblance to the picture obtained with neostigmine in a similar experiment on a myasthenic patient.

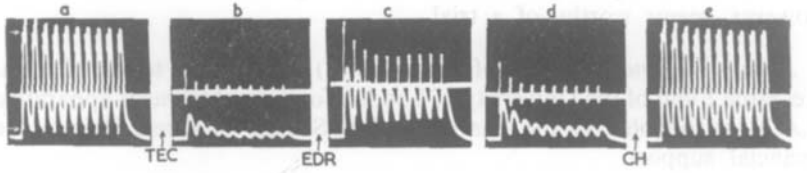

FIG. 2. Cat, chloralose anaesthesia. Isometric myogram (lower trace) and gross electromyogram (upper trace) recorded simultaneously on a cathode ray oscilloscope. The small horizontal arrows in 'a' indicate the levels of the peaks of the negative and positive deflections of the muscle action potentials which can be distinguished as small bulges in the tension trace. The gain of the action potentials was doubled in b, c and d. Between a and b, triethylcholine, between b and c, edrophonium and between d and e, choline was injected intravenously. c was recorded 5 min. after edrophonium and e was recorded 10 min. after choline. For further details see text.

A study of the literature makes it clear that both pre-junctional (Desmedt, 1960; Dahlbäck and others, 1961) and post-junctional defects (Churchill-Davidson and Richardson, 1953) may exist in generalised myasthenia gravis. There is also evidence (Stricker and others, 1960) that the defect is caused by a substance present in the blood stream and many extensive searches have been made for such an agent (e.g. Wilson, Obrist and Wilson, 1953; Wilson and Wilson, 1955; Nowell and Wilson, 1961; Nastuk and Strauss, 1961). If a circulating agent does exist, it might well possess properties like those of triethylcholine and there is evidence that an ethonium compound is normally present in nervous tissue. Thus Lorenté de No (1949), while working on the effects of quaternary ammonium ions on conduction in frog nerve fibres, extracted a quaternary compound with the properties of an ethonium ion from ox brain. More recently, Calvey, Nowell and Wilson (personal communication) found in chromatographic studies that a substance with an R_F value like that of the ethylcholines was present in the thymus glands of myasthenic patients and of foetal whales. An abnormal excess of

such a substance, possibly arising through some disfunction in choline metabolism, might therefore be the agent responsible for the defect in myasthenia gravis. Triethylcholine is not highly active in producing transmission failure in the experimental animal but under these circumstances it has to compete with the large amounts of choline normally present. Many choline analogues are acetylated by choline acetylase (Burgen and others, 1956), and it is possible that in exercise, when the traffic of nerve impulses is high, the motor nerve endings accept some choline analogue in place of choline and so release an inactive transmitter. The continual bombardment of the motor end-plates by such a false transmitter might then induce the post-junctional changes described by Churchill-Davidson and Richardson (1953).

Our experiments with triethylcholine have shown that choline is the best antagonist to its blocking action, but as yet there is no evidence that choline has any beneficial effects in myasthenia gravis. Acute treatment with choline might not produce a striking improvement because there is no reason to suppose that the post-junctional defect in myasthenics would immediately revert to normal. Prolonged treatment with choline, however, seems worthy of a trial.

Acknowledgements. One of us (W.C.B.) is grateful to the Central Research Fund of the University of London for a grant towards apparatus. B.A.H. is indebted to the Pharmaceutical Society of Great Britain for financial support.

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Discussion

The paper was presented by DR. BOWMAN. The following points were made in the discussion.

Twenty-five simple analogues of choline were examined, of which the best was triethylcholine. Some bis-quaternary compounds had also been

studied. Those with methyl groups attached to the quaternary nitrogen possessed a depolarising action, while those with large groups on the quaternary nitrogen had a curare-like action; compounds with groups intermediate in size behaved like triethylcholine. Chronic toxicity studies were made on triethylcholine in dogs, cats and rabbits, and no cumulative paralysing action was produced. The animals were not tested to find if the muscles had become truly myasthenic.