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ACTIVITY OF ANALOGUES OF DECAMETHONIUM ON THE CHICK BIVENTER CERVICIS PREPARATION

BY

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The abilities of various decamethylenebisammonium compounds to cause contracture of the chick biventer cervicis preparation have been compared quantitatively with that of decamethonium. In the compounds tested the 1,10 substituents were similar and were ethyldimethylammonium, diethylmethylammonium, triethylammonium, hydroxyethyldimethylammonium and acetoxyethyldimethylammonium; decamethylenebisdimethylamine was also tested. The activity of the bisethyldimethylammonium compound is about one-third of that of decamethonium, that of the bisdiethylmethylammonium compound about one-twenty-eighth, but the other compounds are much less than one-hundredth as active. Even the bistriethylammonium compound, however, has some detectable activity although it is only a partial agonist. It is suggested that ability to depolarize the preparation depends upon the presence of methyl groups attached to the quaternary ammonium atom and also upon the presence of two such onium groups at the ends of the decamethylene chain. In these compounds the acetoxy group reduces ability to depolarize the preparation.

Decamethonium is said to block neuromuscular transmission by desensitization following an initial depolarization (Thesleff, 1955; Katz & Thesleff, 1957). The effect on blocking activity of replacement of methyl groups in the molecule by ethyl groups was studied quantitatively by Barlow, Roberts & Reid (1953) and Thesleff & Unna (1954), but it was observed by Ginzel, Klupp & Werner (1951) and by Thesleff & Unna (1954) that the mode of action of the compounds with more than one pair of methyl groups replaced by ethyl groups appeared to be different from that of decamethonium itself. The ethylated analogues were found to be much less effective than decamethonium in causing contracture of the avian muscle preparations used, but the relationship between the number of pairs of ethyl groups and the ability to produce contracture was not studied quantitatively. Ariëns & De Groot (1954) obtained some quantitative results using the frog rectus, but this preparation is not very sensitive. Although it was observed that decamethylenebis-(ethyldimethylammonium) was about half as active as decamethonium, the activity of the higher homologues could not be detected at all.

Ability to cause depolarization of multiply innervated avian muscle fibres is associated with ability to cause a sustained contracture (see, for example, Ginsborg, 1960). The relationships between the composition of the onium group and ability to cause contracture may therefore give some indication of ability to cause depolarization: in default of direct information about the effects of the ethylated compounds

at the neuromuscular junction it is therefore desirable to have quantitative information about the relationships between structure and ability to cause contracture. Analogues of decamethonium have accordingly been tested on the chick biventer-cervicis preparation (Child, 1955 ; Ginsborg & Warriner, 1960), which is about 100 times as sensitive to decamethonium as is the frog rectus.

METHODS

Compounds. Decamethonium iodide (BTM) was purchased from Burroughs Wellcome ; decamethylenebis(ethyldimethylammonium iodide) (BEDM) and decamethylenebis(diethylmethylammonium iodide) (BDEM) were prepared as described by Barlow, Roberts & Reid (1953) ; decamethylenebis(triethylammonium bromide) (BTE) was prepared as described by Barlow & Ing (1948) ; and decamethylenebis(hydroxyethyldimethylammonium bromide) (BHDM) as described by Barlow (1955). Decamethylenebis(acetoxyethyldimethylammonium bromide) (BADM) was prepared by acetylation of the hydroxy compound with acetyl bromide and acetic anhydride ; the product, recrystallized from a mixture of ethyl methyl ketone, alcohol and ether, melted at 112–13° C. Found, Br^-, 28.7 ; $C_{22}H_{46}Br_2O_4$ requires Br^-, 28.5%. This compound was previously prepared as the iodide (Barlow, 1955). Decamethylenebis-(dimethylamine hydrobromide) (BDM), recrystallized from ethyl methyl ketone and ethanol, melted at 233–4°. Found, Br^-, 41.2 ; $C_{14}H_{34}Br_2N_2$ requires Br^-, 41.0%.

Chick biventer-cervicis preparation. This was set up exactly as described by Ginsborg & Warriner (1960) in an organ bath of approximately 50 ml. capacity containing Krebs-Henseleit solution at 36.5° C. The nerve was stimulated supramaximally at a rate of 12 shocks/min and the contractions were recorded using a light isotonic lever (magnification ×7) writing on a smoked drum.

Experimental procedure. The drugs were added directly to the organ bath in a small volume (usually 0.2 ml.), producing final concentrations in the bath which ranged from about 10^{-7} M for decamethonium to about 10^{-4} M for some of the other compounds. They were left in contact with the muscle until the contracture had reached a maximum, which usually took about 10 min, then washed out and the preparation allowed to recover for 20 min. The interval between doses was thus 30 min.

Estimation of potency. Contractures were measured as the maximum deviation from the base-line. The graph of contracture against the log. of the concentration of the drug was drawn and was observed to be very steep (Fig. 1) except with decamethylenebis(triethylammonium) (BTE), for which it was much flatter. Because of the steepness of these log. dose-response curves the drugs were in some instances compared only by observing the concentrations which produced roughly comparable contractures. In most instances, however, the equipotent molar ratio relative to decamethonium was calculated from the log. dose-response curves.

Addition experiments to detect partial agonists. Ariëns & De Groot (1954) observed that decamethonium and decamethylenebis(ethyldimethylammonium) (BEDM) were only partial agonists on the frog rectus because they were incapable of producing a maximal contracture of the muscle. To see if the compounds were acting as partial agonists on the chick biventer addition experiments were performed. Concentrations of the test compound and of decamethonium were found which produced the same effects. The two substances were then added simultaneously in half these concentrations, and if the contracture produced was less than that obtained previously the test compound was concluded to be a partial agonist (Stephenson, 1956). It does not follow, however, that if the contracture was the same size the compound was not a partial agonist. It is extremely likely that in this preparation the response is obtained with only a small proportion of receptors occupied.

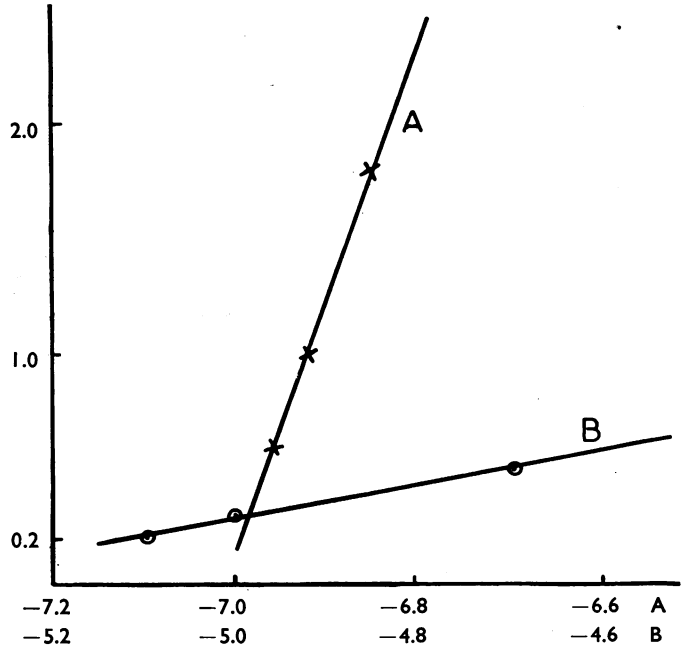

Fig. 1. Graph of size of contracture against log. drug concentration: A for decamethonium, B for decamethylenebis(triethylammonium) (BTE). Ordinate: height of the tracing (inches) observed on the drum. Abscissa: log. molar concentration.

RESULTS

Potencies relative to decamethonium. The equipotent molar ratios for the compounds relative to decamethonium are shown in Table 1, which gives the mean values and the standard error. Tella (1960) obtained a value of 210±9 for decamethylenebis(hydroxyethyldimethylammonium) (BHDM) on this preparation.

TABLE 1

ABILITY OF ANALOGUES OF DECAMETHONIUM TO CAUSE CONTRACTURE OF THE CHICK BIVENTER-CERVICIS PREPARATION

With BTE the comparison was made with small responses at the bottom of the dose-response curve

$R_3N.[CH_2]_{10}.NR_3$

R_3	Equipotent molar ratio relative to decamethonium, mean values (of n experiments)±s.e.	n
$EtMe_2$ (BEDM)	3·23±0·19	10
Et_2Me (BDEM)	27·6 ±1·4	7
Et_3 (BTE)	350 ±95	7
$M\geq_2H$ (BDM)	580 ±66	6
$(HO.CH_2.CH_2)Me_2$ (BHDM)	170 ±35	7
$(CH_3.CO.O.CH_2.CH_2)Me_2$ (BADM)	220 ±25	9

Addition experiments. The log. dose-response curve for decamethylenebis(triethylammonium) (BTE) was much flatter than that for decamethonium, and addition experiments indicated that this is a partial agonist (Fig. 2): the value in Table 1

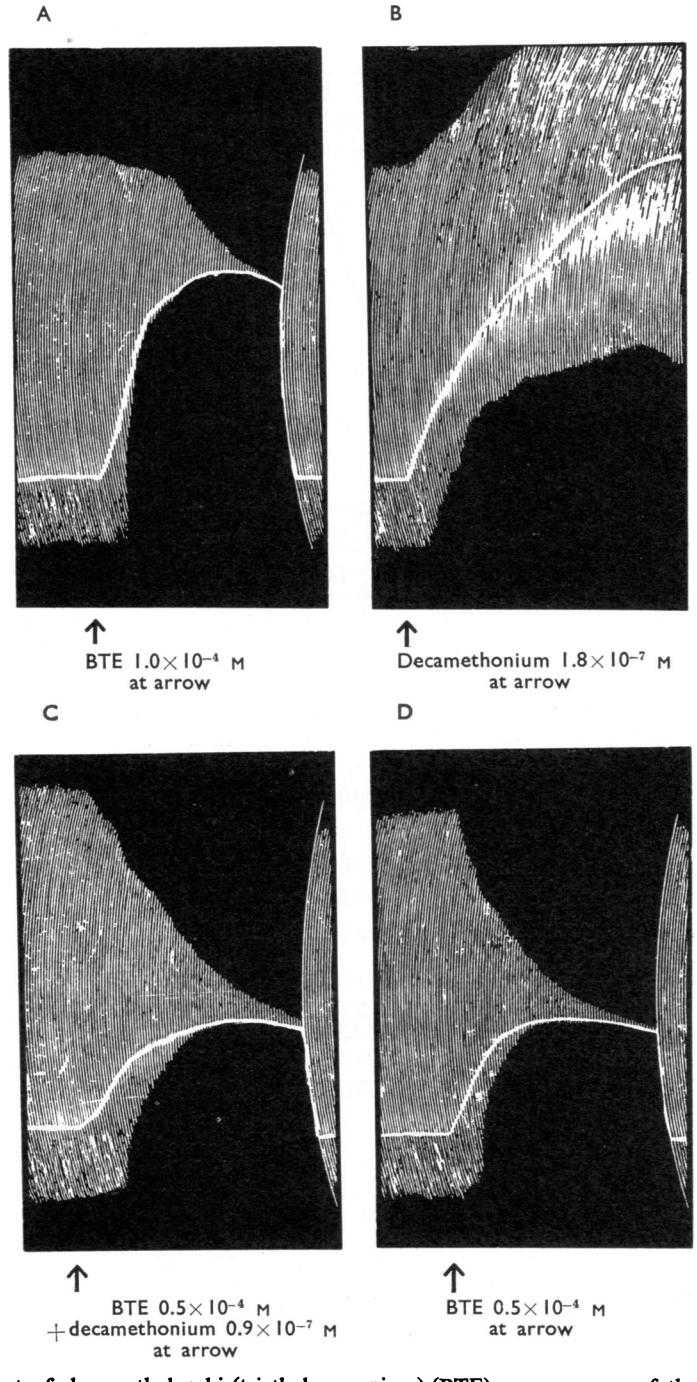

Fig. 2. Effect of decamethylenebis(triethylammonium) (BTE) on responses of the chick biventer cervicis to decamethonium. A=BTE, 1×10^{-4} M. B=decamethonium, 1.8×10^{-7} M. C=BTE, 0.5×10^{-4} M, plus decamethonium, 0.9×10^{-7} M. D=BTE, 0.5×10^{-4} M. The molarities indicate the concentration in the organ bath.

was obtained using only threshold responses. Addition was demonstrated for the bisethyldimethyl (BEDM), bisdiethylmethyl (BDEM), and bishydroxyethyldimethyl (BHDM) ammonium compounds and for the bisdimethylamine (BDM) even though the log. dose-response curve for the bisdimethylamine was not quite as steep as that for decamethonium. This compound could, therefore, have a lower efficacy than decamethonium even though this was not apparent with the relatively small contractures used in the addition experiments.

Decamethylenebis(acetoxyethyldimethylammonium) (BADM). This compound is hydrolysed by acetylcholinesterases, although only at about 30% of the rate of acetylcholine (Barlow, 1955), and accordingly some comparisons with this compound were made in the presence of physostigmine (in concentrations of from 10^{-7} to 10^{-4} M). The effects of the bisacetoxyethyldimethyl compound (BADM) were not potentiated by physostigmine (Fig. 3 ; concentrations up to 10^{-4} M were tested), so

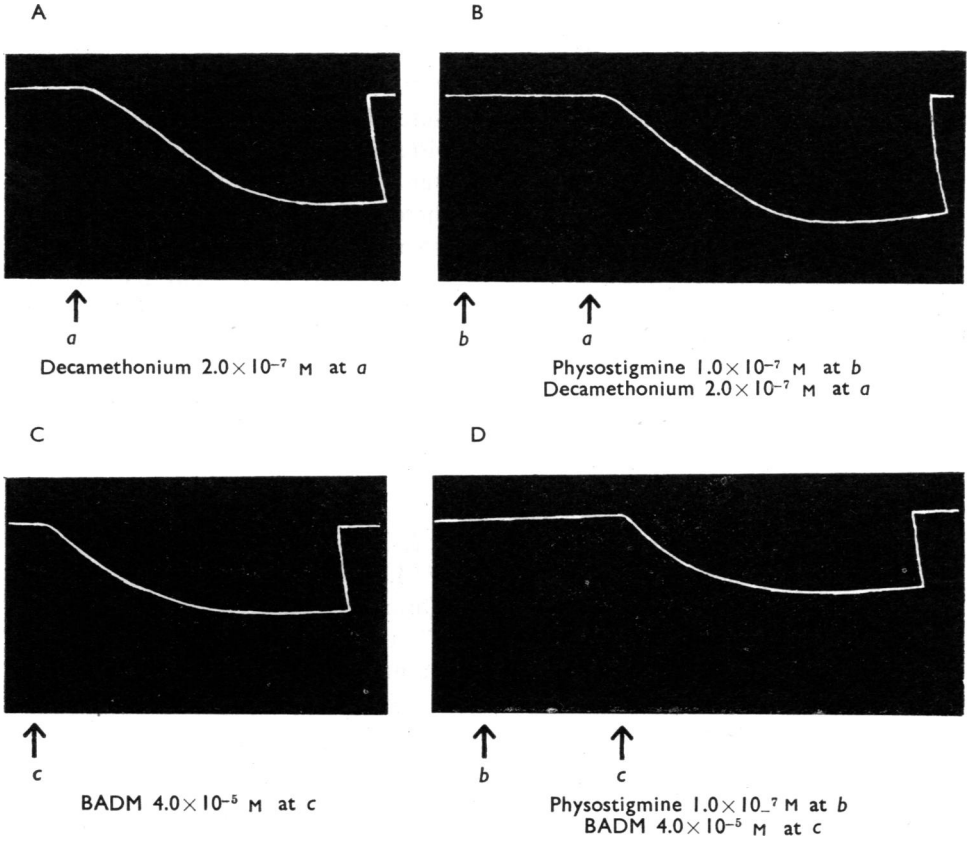

Fig. 3. Effect of physostigmine on responses of the chick biventer-cervicis to decamethonium and decamethylenebis(acetoxyethyldimethylammonium) (BADM). A=decamethonium, 2.0×10^{-7} M (*a*). B=physostigmine, 1×10^{-7} M (*b*), followed by decamethonium, 2.0×10^{-7} M. C=BADM, 4.0×10^{-5} M (*c*). D=physostigmine, 1×10^{-7} M, followed by BADM, 4.0×10^{-5} M. The molarities indicate the concentration in the organ bath. In this experiment the electrical stimulation of the preparation was omitted.

it does not appear that it is being destroyed to any extent by the cholinesterases in the preparation. This finding justifies the inclusion of the results for this compound in Table 1 which were obtained without physostigmine being present.

DISCUSSION

These results should be compared with those of Stehle, Melville & Oldham (1936), Holton & Ing (1949), and Ing (1949), who investigated analogues of acetylcholine, and by Wien & Mason (1951), who investigated analogues of hexamethonium.

The removal of a methyl group from each onium atom has a much greater effect in lowering the activity of decamethonium than it does with acetylcholine. The equipotent molar ratio for decamethylenebis(dimethylamine) (BDM) relative to decamethonium on the chick biventer is 580 compared with the ratio for acetoxyethyldimethylamine relative to acetylcholine of 50 on the cat blood pressure, 40 on rabbit intestine and 50 on the frog heart. Unfortunately the latter compound does not appear to have been tested at the sites of the nicotine-like actions of acetylcholine.

The effects of replacing one methyl group attached to each onium atom in decamethonium by an ethyl group are similar to those observed with acetylcholine. The equipotent molar ratio for decamethylenebis(ethyldimethylammonium) (BEDM) relative to decamethonium on the chick biventer was 3.2 (Ariëns & De Groot (1954) obtained a ratio of 2 on the frog rectus), whereas the equipotent molar ratio for acetoxyethylethyldimethylammonium relative to acetylcholine was 3 on the cat blood pressure before atropine, 5 after atropine, 2.5 on the guinea-pig ileum, 2 on the frog heart and 5 on the frog rectus. Further replacement of methyl groups by ethyl groups, however, reduced the activity of the analogues of decamethonium on the chick biventer much less than it reduced the activity of the analogues of acetylcholine at the sites of the muscarine-like and nicotine-like actions of acetylcholine listed above.

The low activity of the bishydroxyethyldimethylammonium compound (BHDM) compared with the bisethyldimethylammonium compound (BEDM) is particularly interesting in view of the use by Creese, Taylor & Tilton (1959) of decamethylenebis-(iodoethyldimethylammonium), labelled with ^{131}I, to study events occurring in the rat and guinea-pig diaphragm and rabbit lumbrical muscles. It was assumed that this compound was a "depolarizing agent," and although it produced effects apparently like those of decamethonium at the neuromuscular junction, in view of the present results and of the absence of direct evidence of depolarization, it may be questioned whether this assumption is strictly valid.

The results with these analogues of decamethonium on the chick biventer bear no resemblance to the effects on ganglion-blocking activity of similar changes in the structure of hexamethonium. The latter compounds, however, are only antagonists and changes in activity must be ascribed to changes in affinity, thus, hexamethylenebis(ethyldimethylammonium) must have a higher affinity for the receptors in the ganglion than hexamethonium itself. The equipotent molar ratio of the former relative to hexamethonium is 0.67 (Wien & Mason, 1951). With the analogues of decamethonium on the chick biventer, as with the analogues of acetylcholine, the

activity—agonist activity—depends upon efficacy as well as affinity. Although it is impossible as yet to measure the affinity of these agonists and hence deduce how efficacy varies with structure, it is possible to interpret the results by supposing that efficacy at the receptors of the chick biventer depends upon the length of the polymethylene chain as well as on the composition of the onium group, consequently even although the replacement of a trimethylammonium group by a triethylammonium group should lead to a great decrease in efficacy, decamethylenebis(triethylammonium) (BTE) retains some efficacy because it still possesses the decamethylene chain. In these compounds the acetoxy group does not appear to be important.

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