Short communications

The effect of α-bungarotoxin on acetylcholine receptors

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Fluctuations of membrane potential ('membrane noise') during acetylcholine-induced depolarization have been studied by intracellular recording from endplates of frog muscle fibres. When acetylcholine sensitivity had been greatly reduced by an irreversible inhibitor (α-bungarotoxin), analysis of the membrane noise showed that neither the amplitude nor the time course of the 'elementary' potential change (due to the molecular depolarizing action of acetylcholine) were altered.

α-Bungarotoxin, a small protein (mol. wt. 8000) isolated from the venom of *Bungarus multicinctus* (Chang & Lee, 1963) inhibits the action of acetylcholine on muscle in a practically irreversible manner (see Lee, 1970; Miledi & Potter, 1971). The toxin might block by reducing the size of the elementary potential change evoked by acetylcholine molecules (Katz & Miledi, 1970); alternatively, the toxin might interfere with the access of acetylcholine to the receptors. Even in the latter case, it is conceivable that the attachment of a toxin molecule to one receptor might alter the response to acetylcholine at adjacent receptor sites. The aim of the present experiments was to study the effect of α-bungarotoxin on the elementary 'ion gates' opened by acetylcholine molecules in the endplate membrane.

Methods.—The method used for this purpose was to record and analyse the characteristic 'membrane noise' which accompanies a local depolarization induced by acetylcholine (Katz & Miledi, 1972, 1973a). It has previously been shown that a statistical analysis of the acetylcholine noise enables one to determine the approximate value of the elementary depolarization (a fraction of a microvolt) produced by the transient opening of each individual ion gate. Furthermore, frequency analysis of the noise variance gives one an indication of the average duration, or 'life-span', of these ionic channels (Katz & Miledi, 1971).

Results and Discussion.—In 12 experiments, at 22·5° C, the mean life-span of the acetylcholine-induced ion channels was $1{\cdot}14 \pm 0{\cdot}062$ ms, while at 1·2° C it was increased to $5{\cdot}38 \pm 0{\cdot}44$ ms. It has previously been shown (Katz & Miledi, 1972) that curare, while reducing the statistical probability of interaction between acetylcholine and receptor molecules, does not modify the properties (conductance and life-time) of the ion gate opened by a successful molecular action. A similar result was obtained in 5 experiments on frog endplates, at 4° C, in which acetylcholine potentials and acetylcholine noise were recorded intracellularly, before and after application of α-bungarotoxin (1 μg/ml for more than 30 min, sufficient to reduce the size of miniature endplate potentials to less than one tenth).

Acetylcholine was applied iontophoretically; from the relation between mean depolarization and its variance, the elementary voltage amplitude a was determined as described in detail elsewhere (Katz & Miledi, 1972). The mean value of a (5 experiments at 4° C in the presence of neostigmine, 1 μg/ml) before addition of α-bungarotoxin was 0·623 μV; after exposure of the muscles to α-bungarotoxin (1 μg/ml for more than 30 min) the mean value of a was 0·595 μV. The reduction of α, to $92 \pm 6{\cdot}3\%$ of the value for the unblocked preparation, was not statistically significant, even though the miniature endplate potentials had been reduced to undetectably small size.

Figure 1 shows the results of a frequency analysis in one of these experiments. There was no significant change in the spectral distribution after α-bungarotoxin. The 'time constant' of the elementary voltage change, derived from the 1/2 power frequencies in 5 experiments similar to the one illustrated in Fig. 1, was $15 \pm 1{\cdot}17$ ms in the control preparations compared with $13{\cdot}7 \pm 0{\cdot}42$ ms after the application of α-bungarotoxin. A more important point is that there was no difference in the higher frequency (10–100 Hz) attenuation ($78 \pm 8{\cdot}2$ in the 5 muscles before toxin, 80 ± 4 after toxin) which provides a sensitive index of any change in the life-span of the ion channel (Katz & Miledi, 1972, 1973a).

One may conclude from these results that α-bungarotoxin, like curare, reduces

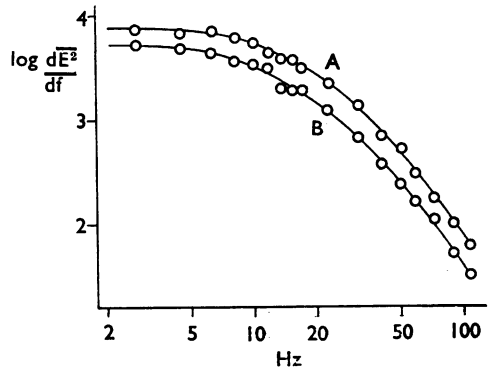

FIG. 1. Spectral distribution of acetylcholine noise recorded intracellularly. (A) before, and (B) after α-toxin. Logarithmic scales. Ordinates: variance density ($d\overline{E^2}/df$) in log units; abscissae: frequency (Hz).

the number of ion gates opened by a given concentration of acetylcholine, but does not alter the size and time-course of the elementary conductance change associated with each successful molecular activation. Evidence has been presented (Katz & Miledi, 1973b) that α-bungarotoxin, like curare, reduces the binding capacity of the endplate receptors for the quanta of acetylcholine released from nerve endings into the synaptic cleft. It appears, therefore, that both substances act by rendering receptor molecules unable to combine with acetylcholine, though the kinetics differ in the two cases and the precise molecular mechanism may not be the same.

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