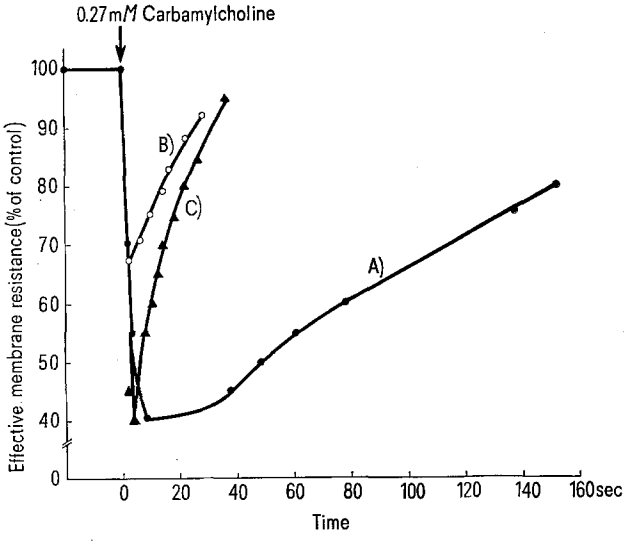
The Influence of Disulfide Bond Reduction and Lanthanum on End-Plate Receptors

Disulfide bond reduction decreases the depolarization produced by carbamylcholine (CARB) in electroplax cells[1,2], normal and denervated skeletal muscle[3–5] and the CARB contracture observed in chick biventer cervicis muscle[6], suggesting that the cholinergic receptor is a protein containing a disulfide bridge near the active site[7]. Particularly evident in the chick biventer cervicis muscle was a progressive loss of response following disulfide bond reduction and prolonged exposure to various cholinergic agonists. Since this may reflect receptor desensitization[8,9], the influence of disulfide bond reduction by dithioerythritol (DTE)[10] on the activation-desensitization sequence produced by CARB at the muscle end-plate has been investigated. The influence of lanthanum on end-plate receptors reduced by DTE was also studied because lanthanum is known to increase the CARB activation of muscle end-plates[11] and reduces the inhibition of the postjunctional membrane (PJM) produced by competitive antagonists such as curare[12].



Effect of DTE and DTE plus lanthanum on PJM activation and desensitization following continuous microperfusion with 0.27 m*M* CARB. Record A) is a control obtained in the absence of DTE and lanthanum (% change in EMR = 61.6; half-time of EMR recovery = 76 sec). Record B) was obtained from a second fibre bathed with 0.1 m*M* DTE (% change in EMR = 31.3; half-time of EMR recovery = 16 sec). The response observed in a 3rd fibre which was bathed with 0.1 m*M* DTE but perfused with 0.01 m*M* lanthanum in addition to CARB is illustrated in record C) (% change in EMR = 59.3; half-time of EMR recovery = 14 sec). For clarity, the complete recovery of the EMR is not shown in the figure. However, the half-times were calculated from the final EMR values.

All experiments were performed in vitro at room temperature (16–22 °C) on the sartorius muscle preparation of the frog (*Rana pipiens*) maintained in an isosmotic potassium propionate Ringer solution (K-propionate 122.5 m*M*; Ca-propionate 1.8 m*M*; *Tris* 1.0 m*M*). The resting potential of individual fibres ranged between 0 and −16 mV in this solution.

To estimate PJM activation[11] and desensitization[13], the effective membrane resistance (EMR) of individual fibres was measured at the end-plate region prior to, during and following microperfusion with CARB. The recording techniques employed to measure the EMR of single muscle fibres, the method of applying CARB to individual end-plates by microperfusion, the avoidance of desensitization of receptors on adjacent muscle fibres and the visualization of individual junctional regions have been previously described[11,14,15].

The maximum decrease in EMR immediately following receptor activation with CARB was calculated as a percent change as follows:

$$\left[\frac{\text{Control (preperfusion) EMR} - \text{Minimum (postperfusion) EMR}}{\text{Control (preperfusion) EMR}}\right] \times 100.$$

PJM desensitization was assessed by the half-time of EMR recovery[13]. This value is the time taken for the EMR to increase, after the initial decline, to a value equal to one-half the total change in EMR measured during desensitization.

Following DTE (0.1–0.5 m*M*) treatment, CARB activation of the end-plate membrane was decreased; the degree of PJM inhibition being dependent upon the concentration of DTE applied and the duration of exposure. KARLIN and BARTELS[1] have observed a similar effect in electroplax cells. Initial experiments revealed

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Table I. Effects of DTE treatment and La^{3+} on PJM activation and desensitization produced by 0.27 CARB

DTE (m*M*)	La^{3+} (m*M*)	Maximum change in EMR (%)	Half-time of EMR recovery (sec)	Number of fibres
–	–	60.2 ± 4.0[a]	81.1 ± 11.7[a]	7
0.1	–	32.2 ± 2.3	15.8 ± 1.1	11
0.1	0.01	55.9 ± 4.3	17.6 ± 2.6	8,7[b]

[a] Values are mean ± S.E. [b] The maximum change in EMR is from 8 fibres and the half-time of EMR recovery is from 7 fibres. The impalement was lost in 1 fibre before desensitization was complete.

Table II. Differential influence of La^{3+} on end-plate activation and desensitization produced by 0.14 m*M* CARB

La^{3+} (m*M*)	Maximum change in EMR (%)	Half-time of EMR recovery (sec)	Number of fibres
–	39.4 ± 3.0[a]	35.0 ± 5.5[a]	5
0.01	60.6 ± 2.3	75.6 ± 28.2	6,5[b]
1.0	58.0 ± 2.1	14.0 ± 1.9	6

[a] Values are mean ± S.E.; [b] The maximum change in EMR is from 6 fibres and the half-time of EMR recovery is from 5 fibres. The impalement was lost in 1 fibre before desensitization was complete. The Table shows a wide range of standard errors for the half-time of EMR recovery. However the variances were found to be equal by an F-test. With the exception that this may reflect an association of a smaller variance with a smaller mean, no clear explanation of the discrepancy between variances can be offered[19].

that the end-plate response to 0.27 m*M* CARB of muscles equilibrated in 0.1 m*M* DTE decreased with time but appeared to plateau at approximately $1^1/_2$–2 h. Therefore, all subsequent experiments were conducted on muscles kept in 0.1 m*M* DTE for approximately 2 h prior to the determination of CARB activation and desensitization. The CARB used for microperfusion was always dissolved in the same solution which bathed the muscle. Hence, DTE was present in the CARB microperfusate when DTE was present in the bathing solution.

The control or resting EMR of the DTE treated fibres (0.167 ± 0.077 megohms, mean ± S.E.) was not significantly different ($p > 0.05$, *t*-test) from the control EMR of the untreated fibres (0.170 ± 0.138 megohms, mean ± S.E.). These values are lower than those reported for polarized muscle fibres[16]. This difference is attributed to the persistent membrane depolarization produced by potassium.

The inhibition of CARB activation by DTE is evident from the Figure. Record A illustrates the time course of the change in EMR induced by continuous microperfusion of 0.27 m*M* CARB. Record B was obtained from another fibre following 0.1 m*M* DTE treatment. In both instances the EMR recovered after an initial decline indicating PJM desensitization during the sustained CARB perfusion. Although CARB activation was reduced by DTE exposure, the rate of PJM desensitization was significantly greater. The results obtained from numerous fibres are summarized in Table I.

In the presence of 0.01 m*M* La^{3+} the amount of inhibition produced by 0.1 m*M* DTE was lessened. In these experiments the La^{3+} was microperfused along with CARB (no preparations were pretreated with La^{3+} prior to CARB application). Previous studies[11] indicate that no significant change in EMR (recorded from the PJM) results from the perfusion of 0.01 m*M* La^{3+} in the absence of CARB. It was assumed that similar conditions obtained in the present study. Although La^{3+} increased the amount of PJM activation in the DTE treated muscles, the rate of PJM desensitization was not changed (Figure, Table I). These data are consistent with the view that the cholinergic receptor is a protein containing a disulfide bond[1–7] and may explain the progressive loss of response seen in earlier studies with chick muscle[6] and electroplax cells[2].

Earlier studies have shown that La^{3+} not only increases end-plate activation but also accelerates the rate of PJM desensitization produced by CARB[11,17]. However, these two effects of La^{3+} are differentially concentration dependent. In the depolarized preparation, 0.01 m*M* La^{3+} enhances receptor activation[11] without significantly increasing the rate of desensitization induced by CARB (0.054–21.6 m*M*)[18]. The desensitizing action of La^{3+} becomes apparent only at higher concentrations. For example, in the presence of 0.01 m*M* La^{3+} PJM activation with 0.14 m*M* CARB was approximately doubled and desensitization slowed, but not significantly ($p > 0.05$, *t*-test), whereas with 1.0 m*M* La^{3+} both the extent of activation and the rate of desensitization were increased (Table II). These observations suggest that receptor activation and desensitization can be separated pharmacologically and perhaps that La^{3+} has two distinct sites of action[20].

Zusammenfassung. Der Einfluss von Dithioerythritol als Reduktionsmittel und von Lanthan auf die durch Carbamylcholin induzierten Phänomene am Endplattenrezeptor in mit Kalium depolarisierten Muskelfasern wurde unteruscht. Dithioreythritol hemmte die Endplattenrezeptoraktivierung und beschleunigte die Desensibilisierungsgeschwindigkeit während 0.01 m*M* Lanthan die Endplattenaktivierung verstärkte, ohne aber einen Einfluss auf die Geschwindigkeit der Desensibilisierung mit oder ohne Dithioerythritol zu haben. 1.0 m*M* Lanthan hingegen erhöhte sowohl die Geschwindgkeit der Desensibilisierung als auch diejenige der Aktivierung bei Abwesenheit von Dithioerythritol, was den Schluss auf zwei verschiedene Wirkungsorte von Lanthan zulässt.

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The Influence of Magnesium and Calcium Ions on the Force Produced in Rigor Muscle

Removal of ATP from striated muscle fibres induces (under isometric conditions) a rigor contraction[1]. In the subsequent rigor state isometric tension is maintained and the crossbridges (which comprise part of the myosin filament) are fixed to the actin filament in the 'arrowhead' position[2]. In this position the crossbridge heads are at an angle of 45° to the actin filament (Figure 1). It is concluded from recent results that the crossbridges are elastic