# The effect of disulphide bond reduction on agonist activity on the frog rectus abdominus muscle

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The cholinoceptor in the eel electroplax and at neuromuscular junctions contains a disulphide bond in close proximity to the active site. Its modification alters the response to cholinergic agonists and antagonists. In the eel electroplax, reduction of the disulphide bond by dithiothreitol (DTT) results in a 3–4 fold decrease in the effectiveness of agonists (Karlin & Bartels, 1966). A comparable result is obtained in the chick biventer muscle (Rang & Ritter, 1971). In the frog rectus abdominus muscle Mittag & Tormay (1970) found that DTT caused a 10–30 fold reduction in the effectiveness of acetylcholine and carbachol. On the other hand, Fleisch, Krzan & Titus (1974) reported in the same preparation a 5.7 fold reduction in the effectiveness of carbachol. Since frog muscle preparations are widely used for studies of individual events in the transmission process and of the actions of agonists and antagonists, we reinvestigated the action of DTT on the frog rectus abdominus muscle preparation.

The rectus abdominus muscle of *Rana temporaria* was suspended in an organ bath containing oxygenated frog Ringer, pH 8.4, at 21°C. Responses to agonists were recorded under isotonic and isometric conditions. Table 1 shows the effect of incubation of the muscle with DTT (1 mM, pH 8.4) for 60 min. In all cases, DTT caused a parallel shift of the log dose response curve for agonists. In the case of monoquaternary compounds the shift was to the right and for the bisquaternary compound, decamethonium, it was to the left. The small differences between dose-ratios obtained with isometric and isotonic recording were not significant (unpaired Student *t*-test).

The results obtained indicate that the cholinoceptor in the frog rectus muscle resembles the cholinoceptor in the eel electroplax more closely than that in the chick biventer muscle and that no generalizations are possible from observations on an individual type of cholinoceptor. Further support for this view comes from preliminary studies of the effect of DTT on the action of (+)-tubocurarine. In the frog rectus muscle, the potency of (+)-tubocurarine was unaffected by DTT contrary to the findings in the chick biventer muscle where its potency was increased 2.4 fold (Rang & Ritter, 1971).

## References

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**Table 1** Dose ratios* obtained after treatment with dithiothreitol

| *Agonist* | *Frog rectus* | | *Findings reported by other workers* | | |
|---|---|---|---|---|---|
| | *Isotonic* | *Isometric* | *Frog rectus* | *Eel electroplax* | *Chick biventer* |
| Acetylcholine | 3.6 ± 0.5 ($n = 7$) | 4.2 ± 0.5 ($n = 18$) | 10–30[1] | ≃5[2], 3–4[3] | 5[5] |
| Carbachol | 3.8 ± 0.7 ($n = 7$) | 3.9 ± 0.3 ($n = 27$) | 10–30[1] 5.7[4] | ≃5[2], 3–4[3] | 5[5] |
| Nicotine | — | 3.7 ± 0.1 ($n = 6$) | | | |
| Tetramethyl-ammonium | 4.1 ± 0.9 ($n = 4$) | — | | ≃2[2] | 2[5] |
| Decamethonium | 0.5 ± 0.1 ($n = 7$) | 0.6 ± 0.1 ($n = 18$) | | ≃0.7[2] | 1.2[5] |

*Dose ratios are based on concentrations required to produce 50% of the maximal response

(1) Mittag & Tormay (1970)
(2) Karlin (1969)
(3) Karlin & Bartels (1966)
(4) Fleisch, *et al.* (1974)
(5) Rang & Ritter (1971)