Disulphide bond reduction in nicotinic receptors

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Dithiothreitol (DTT) reduces disulphide bonds (Cleland, 1964). Karlin & Winnik (1968) have shown that electroplaques exposed to DTT became less sensitive to carbachol, but were depolarized by hexamethonium. 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman, 1959) restored the tissue to its original state. We have tested these reagents on the contractile responses of chick biventer cervicis muscle. Fig. 1 shows responses to hexamethonium appearing in the presence of DTT, and responses to carbachol diminishing. Contractions produced by KCl or caffeine were unaffected by DTT. The effect was not prevented by concurrent application of (+)-tubocurarine, showing that the disulphide bonds involved are not rendered inaccessible by receptor occlusion.

The effect of DTT on the potency of various mono and bis-quaternary agonists was studied. Among the *n*-alkyltrimethylammonium (alkyl-TMA) series, butyl, pentyl and hexyl-TMA became about 50% as active, while the potency of shorter and longer chain compounds (up to decyl-TMA) was little affected. Among the symmetrical bis-trimethylammonium series, $(CH_3)_3N^+$-(CH_2)n-$N^+(CH_3)_3$, C_2 (n=2) became much less active; C_3, C_4, and C_5 were inactive both before and after DTT treatment; C_7 and C_8 became more active, while the long chain compounds (C_9–C_{15}) were unchanged in potency. All of these effects of DTT were fully reversed by reoxidation with DTNB (Fig. 1).

The potency of (+)-tubocurarine was roughly doubled by DTT treatment, and the effectiveness of the irreversible antagonist diphenyldecamethonium mustard ($DPC_{10}M$) (Rang & Ritter, 1969a, b) was also much increased by prior application of DTT. A concentration of $DPC_{10}M$ that produced a dose ratio of 1·25 in a normal preparation produced one of 9·0 when applied after the tissue had been exposed to DTT.

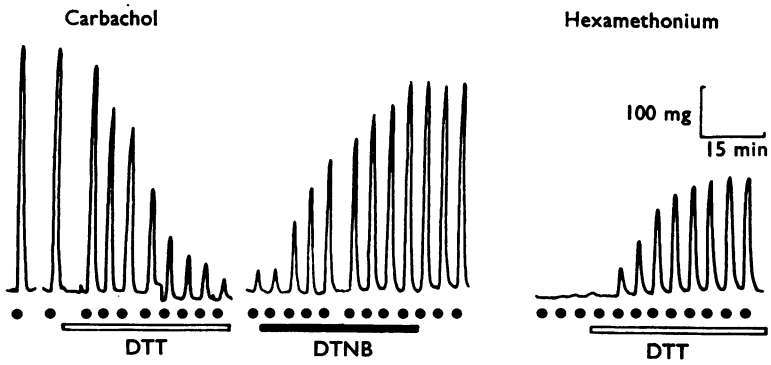

FIG. 1. Contractions of chick biventer muscle strips in response to carbachol, $1{\cdot}6\times10^{-5}$M (left hand panel), and to hexamethonium, $8{\cdot}0\times10^{-5}$M (right hand panel). DTT, 10^{-4}M, was present for the duration of the open rectangles, and DTNB, 4×10^{-4}M, for the duration of the solid one. The agonists were applied for 90 sec as indicated by the solid circles.

These results suggest that the receptors are proteins containing disulphide bonds, disruption of which alters their properties without rendering them ineffective.

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The effect of theophylline on sodium transport across frog-skin in the absence of chloride

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It is widely accepted (see Orloff & Handler, 1967) that neurohypophysial vasopressin stimulates the transport of water and sodium across various epithelia via increased intracellular production of cyclic 3′,5′-AMP, and that theophylline-induced stimulation of active sodium transport results from decreased degradation of endogenous 3′,5′-AMP, caused by inhibition of phosphodiesterase. Recently, this view has been challenged by the suggestions that the action of theophylline on sodium transport across frog-skin is secondary to an increased chloride permeability, and that phosphodiesterase inhibition may be unimportant (Cuthbert & Painter, 1968b; Cuthbert, Painter & Prince, 1969).

In the present experiments frog skins (*Rana temporaria*) were mounted between pools of Ringer solutions for measurement of short-circuit current (I_{SC}), open-circuit potential (E) and for calculation of total resistance (R), using standard techniques (Ussing & Zerahn, 1951).

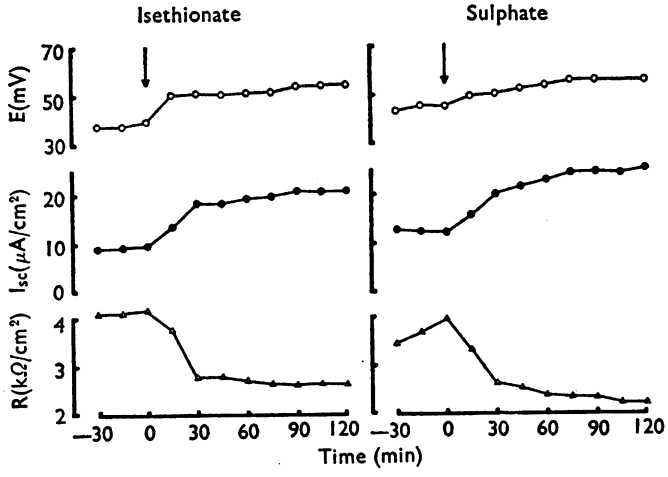

FIG. 1. Open-circuit potential difference (E), short-circuit current (I_{sc}) and resistance (R) in *Rana temporaria* skins mounted in, left, sodium isethionate Ringer, and, right, sodium sulphate Ringer, solutions. The arrows indicate the time of addition of theophylline ($2{\cdot}5 \times 10^{-3}$M) to the inner solution.