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The effect of sodium bisulphite on nicotinic cholinoceptors in the frog rectus abdominis muscle

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Using electrophysiological measurements on the frog cutaneous pectoris muscle, Steinacker [1] concluded that the sulphite ion [2] is able to cleave a disulphide bond located in the vicinity of the nicotinic cholinoceptor (Fig. 1), thereby increasing the sensitivity to acetycholine. This same disulphide bond may also be modified by 1,4-dithiothreitol (Fig. 1), resulting in a decreased sensitivity to acetylcholine on a number of preparations, including the frog cutaneous pectoris muscle [3] and the frog rectus abdominis muscle [4, 5]. We report here a pharmacological investigation of the effect of sodium bisulphite on the frog rectus abdominis muscle.

Rectus abdominis muscles from *Rana temporaria* were suspended in 25 ml organ baths containing continuously aerated frog Ringer solution (composition: 115 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 4.8 mM $NaHCO_3$, 0.035 mM NaH_2PO_4, 1 mM glucose buffered with 1 mM *N*-2-hydroxyethylpiperazine-n′-2-ethanesulphonic acid (HEPES) to pH 7.4) maintained at 25° [6]. Contractions were recorded isotonically under a resting tension of 0.5 g. All contact with plastic tubing was avoided, since plasticisers have been shown to interfere with the action of dithiothreitol [7], hence all-glass apparatus was utilised.

Following the determination of an initial dose-response curve to agonist, the tissue was exposed to sodium bisulphite (0.1 to 5 mM) for up to 60 min. After washing, a second dose–response curve was recorded within 40 min of this treatment. The tissue was next exposed to 1 mM 5,5′-dithiobis(2-nitrobenzoic acid) for 60 min, in order to reform any cleaved disulphide bonds [1], and, after washing, a final dose–response curve was determined. The dose response curves resulting from a typical experiment are shown in Fig. 2.

The pA_2 value of *d*-tubocurarine against acetylcholine was also estimated on normal and bisulphite-treated tissues by the method of Arunlakshana and Schild [8] (see Fig. 3).

Sodium bisulphite, (0.1 to 5 mM) had no direct effect on the tissue and caused no significant shifts in the dose response curves of acetylcholine, either in the presence ($n = 12$) (see Fig. 2) or absence ($n = 3$) of 1 μM neostigmine. Neither did it modify the action of carbachol ($n = 6$), decamethonium ($n = 1$) or n-pentyltrimethylammonium ($n = 1$). The use of a Ringer solution containing 0.2 mM Ca^{2+} and 5.0 mM Mg^{2+} (see [1]), throughout had no effect on the inability of bisulphite to cause a shift in the dose response curve to acetylcholine ($n = 2$) nor when the second dose–response curve was determined in the presence of 1 mM sodium bisulphite ($n = 3$). Furthermore, the pA_2 value of *d*-tubocurarine against acetylcholine determined on bisulphite-treated (1 mM) muscles (6.14 ± 0.04) was not significantly different from that determined on normal tissues (6.11 ± 0.05) (see Fig. 3). The decomposition of sulphite in the presence of the tissue was insignificant as shown by an iodine–thiosulphate back titration [9].

By contrast, 1 mM dithiothreitol has been reported to produce a 3–5-fold shift in the dose–response curve of acetylcholine on the rectus abdominis muscle [4, 5], a result which we confirmed using the method described above for sodium bisulphite treatment (exposure time = 60 min, $n = 2$). The dose–response curves resulting from a typical experiment are shown in Fig. 4. Dithiothreitol (0.1 mM) has also been shown to produce a 2.4-fold increase in the affinity of *d*-tubocurarine for its binding site on the chick biventer cervicis muscle [10].

It appears therefore, that, unlike dithiothreitol, sodium bisulphite is unable to produce an effect which can be detected by the above technique. This may mean that sodium bisulphite is unable to modify the disulphide bond in frog rectus abdominis muscle, suggesting that the nicotinic cholinoceptors there are different from those in the cutaneous pectoris muscle. However, there is no evidence in literature supporting this hypothesis. Alternatively, this lack of effect could result from poor penetration of sulphite ions into the tissue, caused by their large ionic charge, and thus only the outermost disulphide bonds would be cleaved. On this basis, Steinacker's [1], finding of an increased sensitivity to acetylcholine could be explained by her experimental technique in which action potentials were presumably recorded from outermost muscle cells readily accessible to sulphite ions.

In summary, sodium bisulphite failed to alter the response of the frog rectus abdominis muscle to nicotinic agonists, thereby making doubtful an interaction between

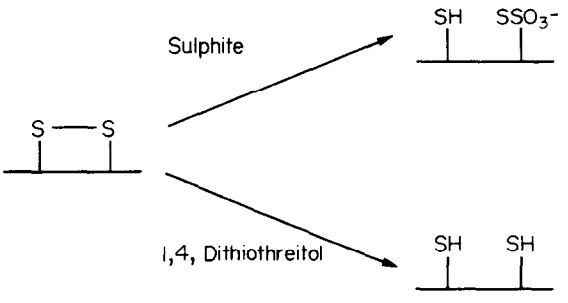

Fig 1.

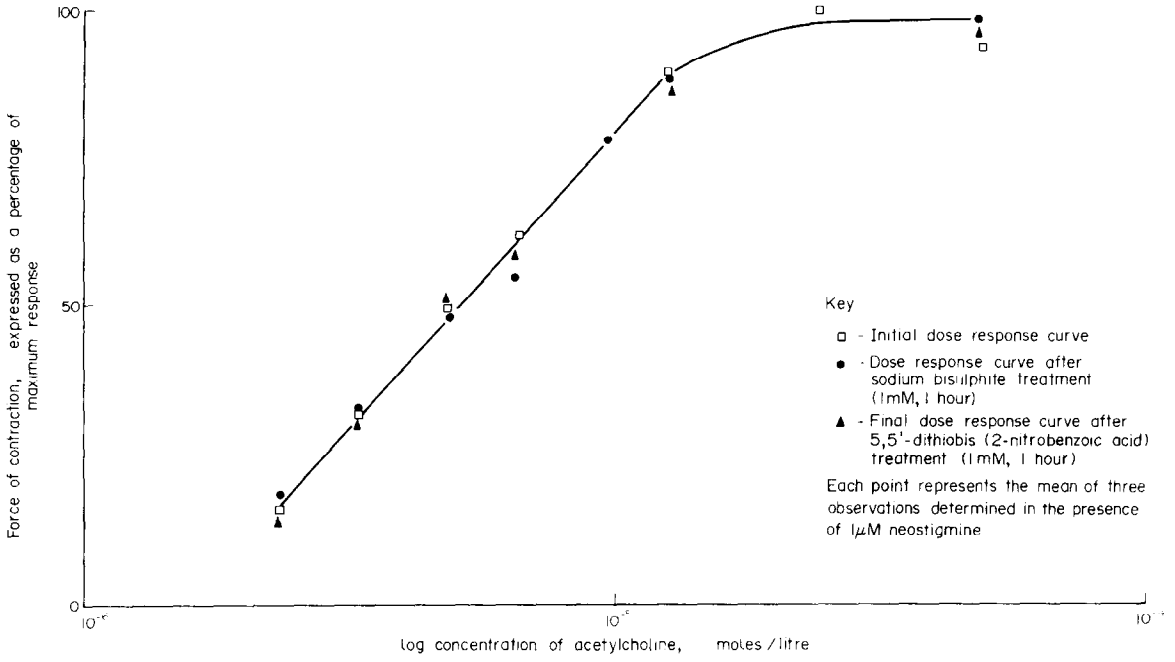
100
50
0
Force of contraction, expressed as a percentage of maximum response
Key
□ - Initial dose response curve
● - Dose response curve after sodium bisulphite treatment (1mM, 1 hour)
▲ - Final dose response curve after 5,5'-dithiobis (2-nitrobenzoic acid) treatment (1mM, 1 hour)
Each point represents the mean of three observations determined in the presence of 1μM neostigmine
log concentration of acetylcholine, moles/litre

Fig. 2.

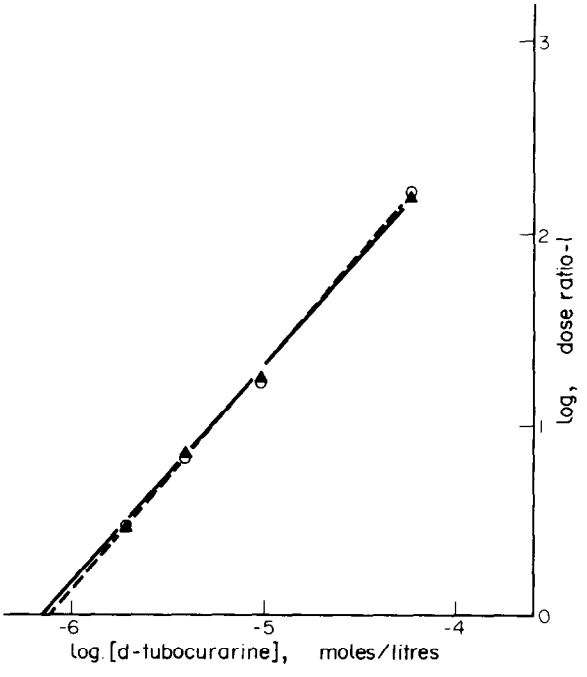
3
2
1
0
log, dose ratio-1
-6
-5
-4
log. [d-tubocurarine], moles/litres

Fig. 3.

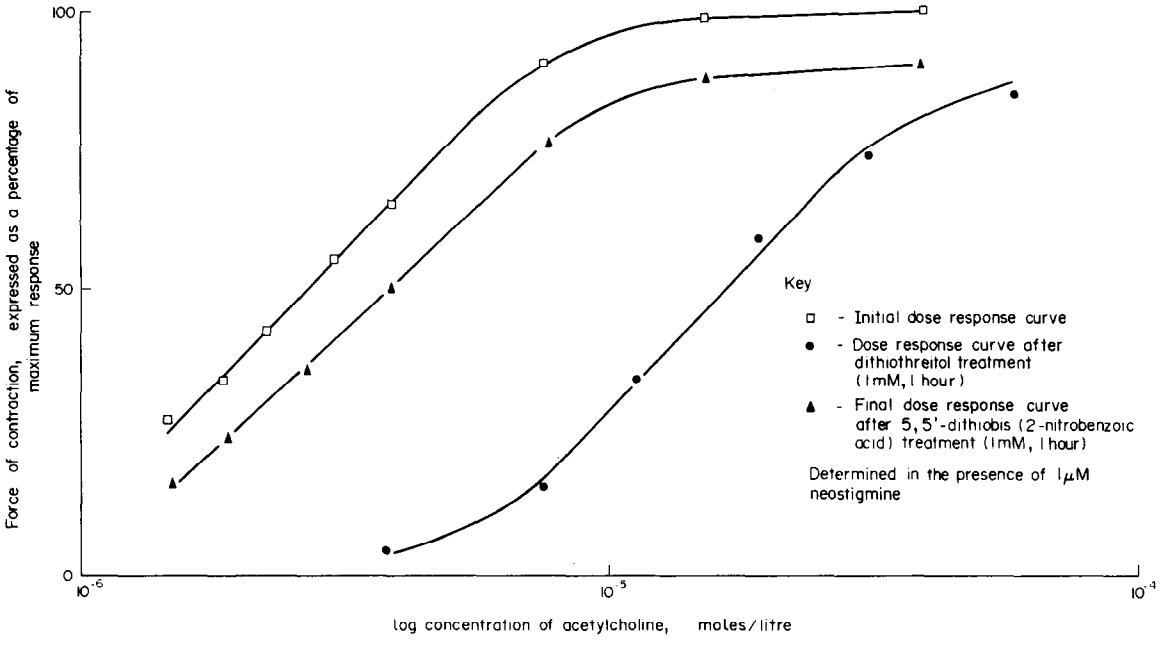

Fig. 4.

sulphite and the disulphide bond in this muscle. Electrophysiological investigations might help to clarify why the effect of bisulphite is apparently different on the two muscles.

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