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TETRAMETHYLENEDISULPHOTETRAMINE: AN INHIBITOR OF γ-AMINOBUTYRIC ACID INDUCED DEPOLARIZATION OF THE ISOLATED SUPERIOR CERVICAL GANGLION OF THE RAT

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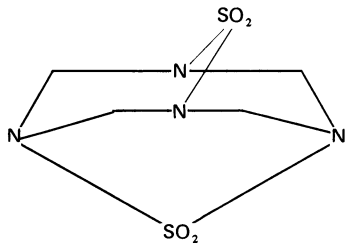
Tetramethylenedisulphotetramine (TETS), a potent convulsant, antagonized the depolarizing action of γ-aminobutyric acid (GABA) in the isolated superior cervical ganglion of the rat. No antagonism of responses to the cholinomimetic agent carbachol was observed. TETS appeared to act in a non-competitive manner and was reversible. Its activity profile was comparable to that of bicuculline in the same tissue except that the latter appears to act in a competitive manner.

Introduction It has recently been suggested that 2,6-dithia-1,3,5,7-tetraza-adamantane-2,2,6,6-tetraoxide [tetramethylenedisulphotetramine] (TETS) might be a γ-aminobutyric acid (GABA) antagonist (Smythies, 1974). In accordance with this speculation, TETS has convulsant activity (Hagen, 1950; Haskell & Voss, 1957; Voss, Haskell & Gartenberg, 1961) and has been stated to reduce possible GABA-mediated inhibition in the mammalian central nervous system (Curtis & Johnston, 1974).



Formula of tetramethylenedisulphotetramine (TETS)

In the present experiments we have assessed the antagonistic activity of TETS towards the depolarizing action of GABA on isolated sympathetic ganglia of the rat. This depolarization is mediated through receptors analogous to those in the central nervous system (Bowery & Brown, 1974) and the ganglion preparation is more suited for quantitative measurements since drugs can be applied in known concentrations.

Methods The procedure was essentially that described previously (Bowery & Brown, 1974) except that the moving-fluid electrode system was replaced by a vertical superfusion technique. Two Ag/AgCl electrodes were placed in electrical contact with the tissue, one via the thread attached to the post-ganglionic trunk and the other directly on the ganglion body close to the preganglionic trunk. The potential difference between these electrodes was monitored continuously on a Bryans 28000 potentiometric recorder. The tissue was superfused with Krebs solution at a rate of 1 ml min^{-1} and drugs were applied by addition to this solution.

Ganglionic uptake of tritiated GABA was determined as described previously (Bowery & Brown, 1972). Comparison was made between contralateral ganglia obtained from the same rats.

Synaptic transmission was monitored by suspension of the ganglion in a three-compartment bath (pre-, soma and post-, described in detail elsewhere) each chamber being perfused separately with Krebs solution at 0.6 ml min^{-1}. Stimulation (1 Hz, 1 ms) was applied to the preganglionic trunk through bipolar electrodes whilst recording from platinum electrodes placed in the soma and post-ganglionic trunk compartments.

TETS was prepared according to the method of Hecht & Heneka (1949) and its convulsant activity checked by intraperitoneal injection into mice: convulsions occurred at <0.25 mg kg^{-1}. Solutions containing TETS were obtained by dilution in Krebs solution from a freshly prepared stock of TETS dissolved in acetone (10 mg ml^{-1}). The apparent maximum concentration attainable was $<200\ \mu M$. In excess of this a precipitate rapidly formed. However, in one experiment a solution of $340\ \mu M$ was used on the assumption that a maximum concentration was in solution. Acetone up to 0.5% in Krebs solution had no direct effect on the preparation or on the responses to GABA or carbachol.

(+)-Bicuculline methochloride (methyl bicucul-

line chloride) was prepared by a modification of the method of Johnston, Beart, Curtis, Game, McCulloch & McLachlan (1972).

Results TETS antagonized the depolarizing action of GABA at concentrations of 10 μM upwards. Antagonism was fully reversible on washing. The effect appeared to be non-competitive; the log dose-response curve was shifted to the right and the maximum depolarization was diminished (Figure 1a). In contrast, methylbicuculline and bicuculline produced a parallel shift without depression, as previously noted for bicuculline alone (Figure 1b; see also Bowery & Brown, 1974).

TETS did not significantly inhibit the depolarization produced by carbachol at concentrations in excess of 130 μM (Figure 1c) and did not affect transmission of orthodromic stimuli. GABA depresses orthodromic transmission (de Groat, 1970; Adams & Brown, 1973). This depression appeared resistant to both TETS and bicuculline. The lack of effect by bicuculline confirms results previously obtained by de Groat, Lalley & Block (1971).

Glial cells in sympathetic ganglia accumulate GABA by a high-affinity carrier-mediated process (Bowery & Brown, 1972; Young, Brown, Kelly & Schon, 1973). Accumulation of GABA following incubation for 30 min in 0.1 μM [^{3}H]-GABA was not reduced by 130 μM TETS (added 30 min before GABA). Ganglionic bath fluid concentration ratios were: minus TETS (control) 9.82 ± 0.69; plus TETS (test) 11.61 ± 1.10 (mean ± s.e.mean, n = 4 in each case).

Discussion The results of these experiments would accord with Smythies' (1974) prediction that TETS blocks GABA receptors, in that it preferentially antagonized GABA-depolarization as against carbachol depolarization. Further, a preliminary test (Adams & Brown, unpublished) has indicated that TETS does not itself alter the resting potential or membrane conductance of ganglion cells.

The potency of TETS as a GABA-antagonist is not dissimilar to that of bicuculline or methyl bicuculline. However, the nature of the antagonism appears to be rather different, in that TETS reduced the maximum depolarization. This was not indicative of irreversible inhibition: effects of both TETS and bicuculline were rapidly reversed on washing. One possibility is that TETS occludes the GABA-ionophore in addition to, or instead of blocking receptors.

Previous experiments (de Groat, 1970; Bowery & Brown, 1974) have demonstrated an essential identity between GABA-receptors at the ganglion

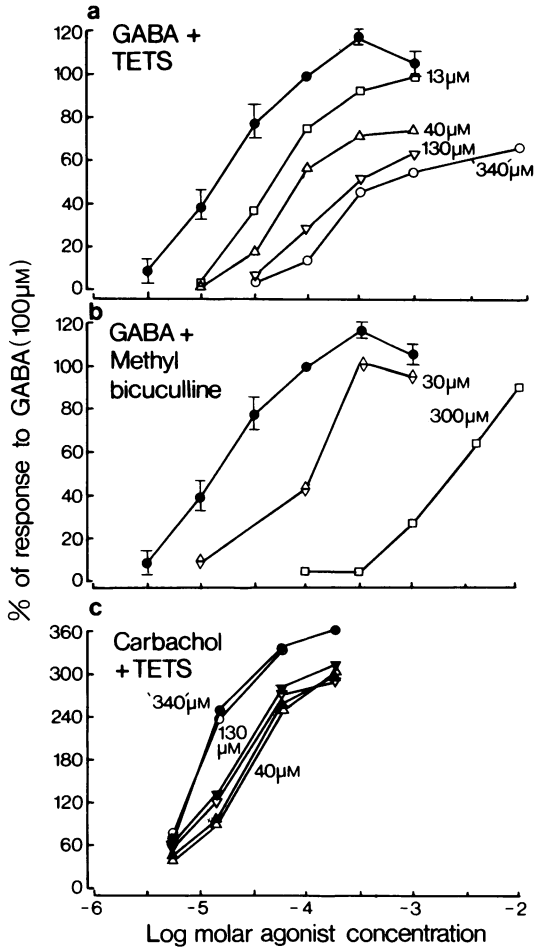


Figure 1 The effects of tetramethylenedisulphotetramine (TETS) (a & c) and methyl bicuculline (b) on ganglionic depolarization produced by γ-aminobutyric acid (GABA) (a & b) and carbachol (c). Ordinates in each case: peak depolarizations produced during 1 min applications of either GABA or carbachol, plotted as a percentage of the response to 100 μM GABA in that preparation (100 μM GABA = 100%). Abscissae: log molar concentrations of GABA (a & b) or carbachol (c). Solid symbols represent the control responses to either GABA or carbachol; open symbols the responses obtained in the presence of different concentrations (μM) of TETS or methyl bicuculline as indicated on the figure. The mean control log-dose response curve to GABA (a & b) was calculated from results obtained in 6 separate ganglia. Vertical bars represent s.e.mean. GABA or carbachol was applied at not less than 15 min intervals. TETS or methyl bicuculline was in contact with the tissue at least 20 min before application of the agonists. One concentration of TETS or methyl bicuculline was examined on a single ganglion. Note that '340' μM refers to an incomplete solution of TETS (see methods section).

and in the mammalian central nervous system with respect to ligand specificities. Consequently, it seems highly probable that TETS is capable of antagonizing actions of GABA elsewhere in the mammalian nervous system. However, it should be emphasized that ganglia are not sensitive to glycine or glutamic acid so we cannot determine in this preparation whether the effects of amino acids acting on other receptors are sensitive to TETS. Preliminary observations by Dray (1975) suggest that intravenous TETS may block the effects of iontophoretic glycine and GABA in the rat mid brain.

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