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EFFECT OF TETRAMETHYLENEDISULPHOTETRAMINE ON THE MEMBRANE CONDUCTANCE INCREASE PRODUCED BY γ-AMINO-BUTYRIC ACID AT THE CRAB NEUROMUSCULAR JUNCTION

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The effect of tetramethylenedisulphotetramine (TETS) on the increase in membrane conductance produced by γ-aminobutyric acid (GABA) at the hermit crab neuromuscular junction was investigated. TETS produced a dose-dependent antagonism of GABA, which was characterized by a non-parallel shift of the log dose-conductance curve for GABA with a reduced maximal conductance change.

Introduction In the preceding paper Bowery, Brown & Collins (1975) showed that tetramethylenedisulphotetramine (TETS) antagonized the depolarizing action of γ-aminobutyric acid (GABA) on rat sympathetic ganglia, in an apparently non-competitive manner. The present paper describes the action of TETS on the responses of crustacean muscle to GABA. GABA is probably the inhibitory transmitter at crustacean neuromuscular junctions and produces an increased membrane conductance.

Methods Conductance changes produced by GABA in the abductor muscle of large claws of the hermit crab (*Eupagurus bernhardus*) were measured as described previously (Earl & Large, 1974). Conductance measurements were made 1 min after the addition of each concentration of GABA. TETS was dissolved in acetone at 10 mg ml^{-1} and an appropriate dilution in Ringer solution added 2 min before GABA. Control solutions contained an equivalent amount of acetone. The composition of the Ringer solution was (mM): NaCl, 445; KCl, 12.2; $CaCl_2$, 29.6; $MgCl_2$, 5.75 and $NaHCO_3$, 1.79. TETS was synthesized by J.F. Collins.

Results At concentrations of 20 μM upwards TETS consistently reduced the conductance change produced by GABA (9 experiments). The log dose-conductance curve for GABA showed a parallel shift to the right at low TETS concentration and a reduced maximum response at high concentrations (Figure 1). TETS was rapidly washed out, usually within the time required to wash out the GABA (10-20 minutes).

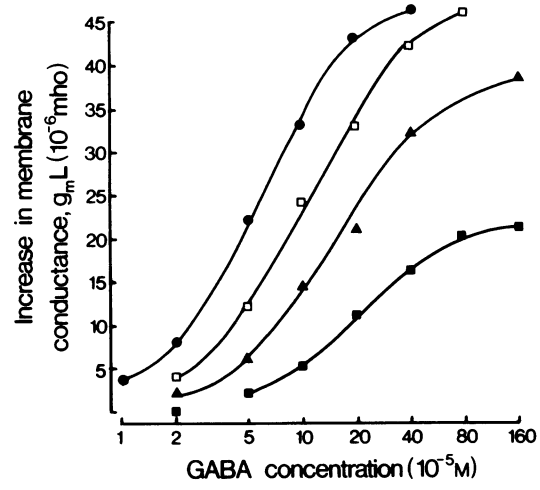

Figure 1 Effect of tetramethylenedisulphotetramine (TETS) on the relation between membrane conductance $g_m L$ (where g_m is the membrane conductance of 1 cm of muscle fibre and L is the half-length of the fibre) and the concentration of γ-aminobutyric acid (GABA). (•) Control; (□), 2×10^{-5} M TETS; (▲), 5×10^{-5} M TETS; (■), 1×10^{-4} M TETS (these results obtained from a single cell).

TETS alone did not alter membrane conductance and did not reduce the amplitude of excitatory junction potentials beyond that produced by the equivalent amount of acetone (6 experiments).

Discussion The present experiments confirm the antagonism of TETS to GABA previously observed in ganglia (Bowery *et al.*, 1975). Antagonism does not appear to derive from a non-specific effect on membrane conductance and probably does not extend to glutamate since the excitatory junction potentials were not depressed (glutamate is probably the excitatory transmitter at the crustacean neuromuscular junction, see Gerschenfeld, 1973).

The antagonism of GABA by TETS appears to have the same characteristics already observed

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with picrotoxin and bicuculline at this synapse (Earl & Large, 1974); namely, the antagonists reduce the maximal response of GABA and hence suggest a non-competitive type of antagonism. However it is interesting to note that, in low concentrations, TETS produces a parallel shift of the GABA dose-response curve, as is found with picrotoxin (unpublished observation, Earl & Large).

As judged from the depression in maximal conductance change, TETS appears to be about 10 times more potent than bicuculline but 10 times less potent than picrotoxin (i.e. the order of potency is bicuculline < TETS < picrotoxin). In the ganglion the order of increasing potency is approximately picrotoxin < bicuculline ≈ TETS (Bowery *et al.*, 1975). This may reflect chemical differences between crustacean and mammalian GABA receptors (cf. Bowery & Brown, 1974).

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