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Actions of dihydroavermectin B_{1a} on insect muscle

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1 Muscle bundle 33 of the locust (*Schistocerca gregaria*) extensor tibiae muscle, which is sensitive to γ-aminobutyric acid (GABA) and receives inhibitory innervation, exhibited both reversible and irreversible responses to dihydroavermectin B_{1a} (DHAVM). These responses involved increases in Cl^- permeability.

2 DHAVM (0.000075–0.0075 μg ml^{-1}) induced reversible dose-dependent increases in Cl^- permeability and partially blocked GABA-induced Cl^- conductance. These effects appear to be due to an interaction of DHAVM with the GABA receptor-Cl^- ion channel complex.

3 DHAVM (0.01–1.0 μg ml^{-1}) induced an irreversible increase in Cl^- conductance which continued to rise after DHAVM application was stopped. At these concentrations DHAVM potentiated GABA-induced Cl^- conductances which were in turn reduced by microperfusion of DHAVM (0.01–1.0 μg ml^{-1}) during bath application of GABA.

4 DHAVM (0.0001–1.0 μg ml^{-1}) induced only irreversible increases in Cl^- conductance when applied to fast muscle bundles (21–26) of the locust extensor tibiae muscle, which are GABA-insensitive and have no inhibitory innervation.

5 The actions of DHAVM on locust muscle appear to involve more than one site. Reversible actions of DHAVM appear to be related to GABA sensitivity and may involve the GABA receptor-ionophore complex. This is unlikely to be the site of action for the irreversible increases in Cl^- conductance caused by DHAVM.

Introduction

Avermectins (AVM) are a family of polycyclic lactones from *Streptomyces avermitilis* (Burg *et al.*, 1979). One of the major components of the AVM complex is AVM B_{1a} which has been found to have broad spectrum anthelminthic and insecticidal properties (Egerton *et al.*, 1979; Ostlind *et al.*, 1979).

AVM B_{1a} has been shown to block neurotransmission at inhibitory neuromuscular synapses and in the ventral nerve cord of the nematode *Ascaris suum* (Kass *et al.*, 1980). Investigations of AVM at the excitatory neuromuscular junction of insects have been restricted to the demonstration that AVM does not affect excitatory postsynaptic potentials in cockroach (*Periplaneta americana*) muscle (Mellin *et al.*, 1983). However, AVM B_{1a} (1–10 μg ml^{-1}) has also been shown to block inhibitory postsynaptic potentials in an irreversible manner in the opener and stretcher muscles of crustacea (Fritz *et al.*, 1979; Pong *et al.*, 1980; Mellin *et al.*, 1983). The actions of AVM in these preparations involve a reduction in input resistance due to an increase in Cl^- permeability (Fritz *et al.*, 1979) and a number of investigators have implicated the involvement of the γ-aminobutyric acid (GABA) receptor-Cl^- ion channel complex in this mode of action.

GABA has been shown to mediate inhibitory neurotransmission on insect muscle fibres (Usherwood & Grundfest, 1965). We have recently demonstrated that distal muscle bundles (32,33,34) of the locust (*Schistocerca gregaria*) extensor tibiae show varying sensitivity to GABA (Duce & Scott 1983a). The extensor tibiae also has so-called 'fast' muscle bundles which receive fast excitatory but not inhibitory innervation and do not show a change in input resistance in response to bath application of GABA (10^{-3} M). Therefore, we have taken advantage of this difference in GABA sensitivity between muscle bundles of the locust extensor tibiae to investigate the role of the GABA receptor-ionophore complex in the action of AVM on insect muscle.

Throughout this study the AVM used was 22,23-

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dihydroavermectin B_{1a} (DHAVM). This compound has been found to be about 30% as active as avermectin B_{1a} in studies on parasite toxicity and avermectin-induced GABA release from synaptosomes (Pong *et al.*, 1980). A preliminary account of this work has been presented (Duce & Scott, 1983b).

Methods

The metathoracic leg of *Scistocerca gregaria* was dissected in locust saline (composition, mM: NaCl 170, KCl 10, $CaCl_2$ 2, $MgCl_2$10 and HEPES 10; pH 6.8), to display the extensor tibiae muscle. The preparation was equilibrated for one hour in a constant perfusion (3 ml min^{-1}) of locust saline containing 2% DMSO. Recording and current-passing electrodes were inserted into the centre of the muscle fibres. The intracellular current-passing electrode containing 1 M potassium citrate was used to inject hyperpolarizing current pulses (6–50 nA, 200–400 ms, 0.1 Hz) within 50 μm of the intracellular recording electrode (also contained 1 M potassium citrate) and input resistance was recorded. GABA was applied to the bath via the perfusion system and DHAVM was microperfused onto the muscle surface by gravity feed from a micropipette. In experiments using chloride-free solution the composition of this medium was (mM): Na isethionate 170, $MgSO_4$ 10, Ca proprionate 2, K_2SO_4 5, HEPES 10; pH 6.8.

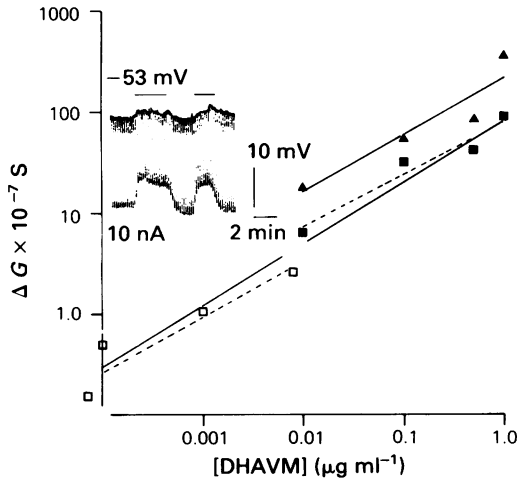

Figure 1 The dose-dependence of the mean change in input conductance induced by various doses of dihydroavermectin B_{1a} (DHAVM) in muscle bundle 33. Ordinate scale: mean change in input conductance $\Delta G \times 10^{-7}$ s (log scale); abscissa scale: log dose of DHAVM (μg ml^{-1}). (■- - - -■) The irreversible change in conductance 3–5 min after DHAVM application (correlation coefficient (r) = 0.971, by linear regression). (▲———▲) The irreversible change in conductance 60 min after DHAVM application. (r = 0.932). (□- - - -□) Reversible changes in conductance induced by low doses of DHAVM (r = 0.938). (□———■) Pooled data from reversible responses with low doses, and irreversible responses with higher doses measured 3–5 min after DHAVM application. (r = 0.985). Inset: trace showing the increase in input conductance induced by 0.0075 μg ml^{-1} DHAVM. Downward deflections represent transient changes in membrane potential caused by injection of constant hyperpolarizing current pulses.

Results

Reversible effects of dihydroavermectin B_{1a}

Microperfusion of DHAVM (0.000075–0.0075 μg ml^{-1} i.e. 9×10^{-11} – 9×10^{-9} M), onto muscle bundle 33 induced reversible dose-dependent increases in input conductance (Figure 1, Table 1). Doses of DHAVM are expressed in μg ml^{-1} to facilitate comparison with concentrations used in other studies. Changes in the duration of application from 2–10 min did not alter reversibility. These reversible changes in input conductance were abolished when DHAVM was applied in Cl^--free medium. Applications of 10^{-3} M GABA induced an increase in input conductance of $17.7 \pm 2.0 \times 10^{-7}$ s (mean ± s.e.mean, n = 35). When DHAVM (0.000075–0.0075 μg ml^{-1}) was applied during a GABA-induced conductance change the GABA response was reversibly reduced in a dose-dependent manner (Figure 2a,b; Table 1). Low doses of DHAVM never enhanced GABA-induced changes in input conductance.

Table 1 Reversible conductance increases and inhibition of GABA-induced conductance changes by microperfusion of dihydroavermectin B_{1a} (DHAVM) onto muscle bundle 33

Dose DHAVM (μg ml^{-1})	*Reversible conductance increase* ($\Delta G \times 10^{-7}$ s)	*Reversible inhibition of GABA (10^{-3} M) conductance (% control response to GABA)*
0.000075	0.16 ± 0.06* (5)	82.7 ± 5.1 (3)
0.0001	0.5 ± 0.07 (6)	36.5 ± 3.3 (7)
0.001	1.05 ± 0.09 (5)	31.7 ± 1.5 (5)
0.0075	2.58 ± 0.35 (6)	18.0 ± 3.7 (5)

*In 2 out of 5 preparations 0.0000.75 ± μg ml^{-1} induced no change in input conductance. All values represent the mean ± s.e.mean of n (number in parentheses) observations.

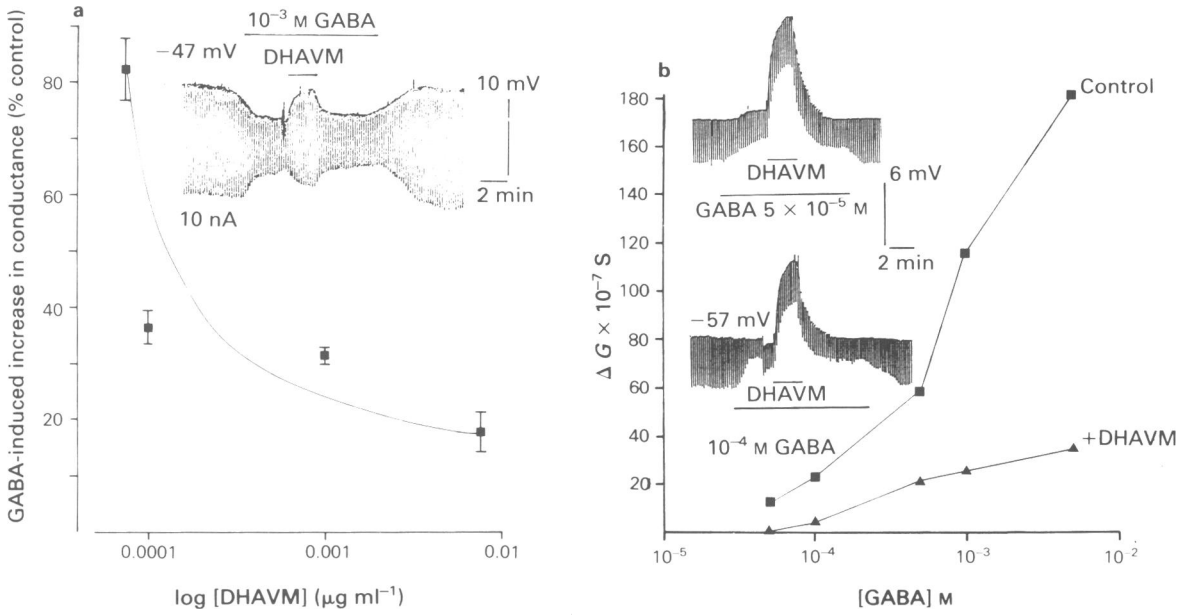

Figure 2(a) Effect of dihydroavermectin B_{1a} (DHAVM) on the response to GABA of muscle bundle 33. The line was fitted by eye. Inset: trace showing that the increase in input conductance induced by GABA (10^{-3} M) is reduced on addition of DHAVM (0.0075 μm ml^{-1}). **(b)** Dose-response relationship of GABA-induced changes in input conductance, in the presence (▲———▲), and in the absence; (■———■) of DHAVM 0.001 μg ml^{-1}. The inset trace illustrates a marked increase in input conductance with very little change in membrane potential in response to application of GABA, presumably because the membrane potential was close to the Cl^- equilibrium potential. During DHAVM microperfusion a marked depolarization occurred in this experiment. This may be interpreted as a reduction in GABA-induced conductance caused by simultaneous application of DHAVM which acts as a blocker of Cl^- channels gated by GABA. Application of DHAVM alone caused little change in membrane potential (see Figure 1).

Irreversible effects of dihydroavermectin B_{1a}

Doses of DHAVM (0.01 – 1.0 μg ml^{-1} i.e. 1.21×10^{-8} – 1.21×10^{-6} M) induced irreversible changes in input conductance (Figures 1 and 3, Table 2). Input conductance continued to rise for more than one hour after DHAVM microperfusion had stopped (Table 2). The involvement of Cl^- in this response was demonstrated in two ways. First, when DHAVM was microperfused in the presence of Cl^--free medium little effect was seen; however, after DHAVM microperfusion was stopped a characteristic increase in input conductance was observed on return to normal medium (Figure 4). Second, after applying DHAVM in normal medium and inducing an increase in input conductance the preparation was perfused with Cl^--free medium, this resulted in the input conductance returning to control levels (Figure 5).

Cl^- conductance increases induced by DHAVM were accompanied by changes in membrane potential of up to 10 mV. Both depolarizing and hyperpolarizing changes occurred, presumably dependent on the

Figure 3 Irreversible increase in input conductance induced by dihydroavermectin B_{1a} (DHAVM; 0.01 μg ml^{-1}). Note the conductance continues to increase after the addition of DHAVM is stopped.

Table 2 Irreversible conductance increases and potentiation of GABA-induced conductance changes induced by dihydroavermectin B_{1a} (DHAVM) microperfusion onto muscle bundle 33

Dose DHAVM ($\mu g\,ml^{-1}$)	Irreversible conductance increase ($\Delta G \times 10^{-7}$ s)		*GABA response after DHAVM (% of control GABA response)*
	3–5 min after application DHAVM	*60 min after application DHAVM*	
0.01	6.3 ± 1.37	17.9 ± 4.5 (6)	160.8 ± 8.1 (25)
0.1	33.3 ± 6.9	56.2 ± 9.7 (7)	270.4 ± 24 (13)
0.5	42.0 ± 5.8	82.5 ± 6.5 (3)	———
1.0	91.1 ± 7.8	360.0 ± 59.8 (8)	434.1 ± 79.7 (16)

All values represent the mean ± s.e. mean of *n* (number in parentheses) observations.

relationship between the membrane potential and the Cl-equilibrium potential. The Cl^- equilibrium potentials for individual preparations varied widely (unpublished data). GABA ionophoretic potentials gave a mean reversal potential of -61.2 ± 7 mV (mean ± s.e.mean). However, changes in membrane potential associated with increases in input conductance induced by DHAVM and GABA were in the same direction for any individual muscle fibre.

The interactions of GABA 10^{-3} M with these higher irreversible doses of DHAVM were investigated as follows. GABA (10^{-3} M) was applied to the bath and the conductance increase was measured; the preparation was then washed and DHAVM (0.01 – 1 $\mu g\,ml^{-1}$) microperfused, which resulted in an irreversible increase in input conductance. GABA (10^{-3} M) was reapplied and the response was found to be potentiated (Figure 6, inset). A further application of DHAVM (0.01 $\mu g\,ml^{-1}$) during this potentiated GABA-induced conductance produced a reversible reduction in the GABA response.

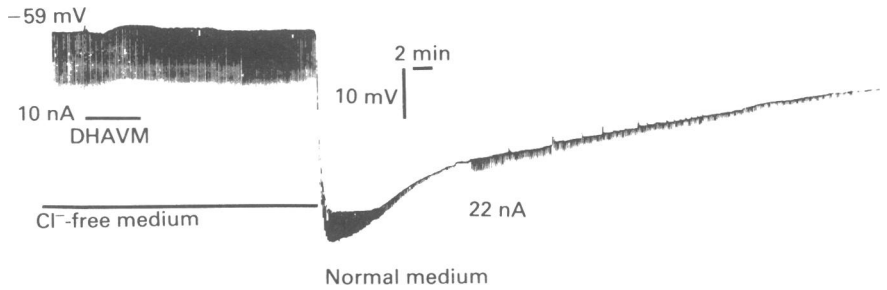

Figure 4 The result of the addition of dihydroavermectin B_{1a} (DHAVM; 0.1 mg ml^{-1}) in chloride-free medium (muscle bundle 33) on the input conductance. On returning to normal medium a transient hyperpolarization was initiated followed by a large increase in input conductance.

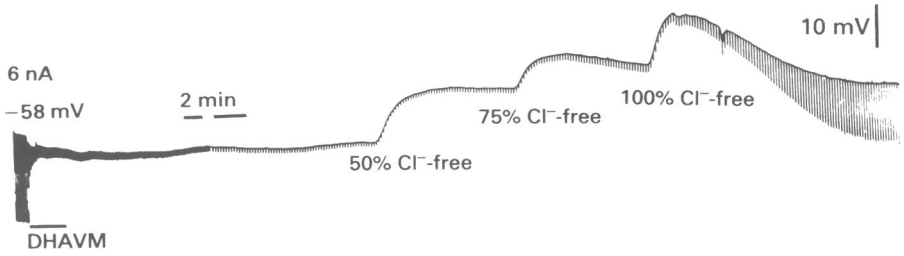

Figure 5 The effect of replacing normal medium, in a step-wise manner, with reduced-chloride medium (until zero chloride was reached) on the induction of an increase in input conductance in muscle bundle 33 with dihydroavermectin B_{1a} (DHAVM; 1.0 $\mu g\,ml^{-1}$). Note transient depolarizations and a gradual decrease in input conductance.

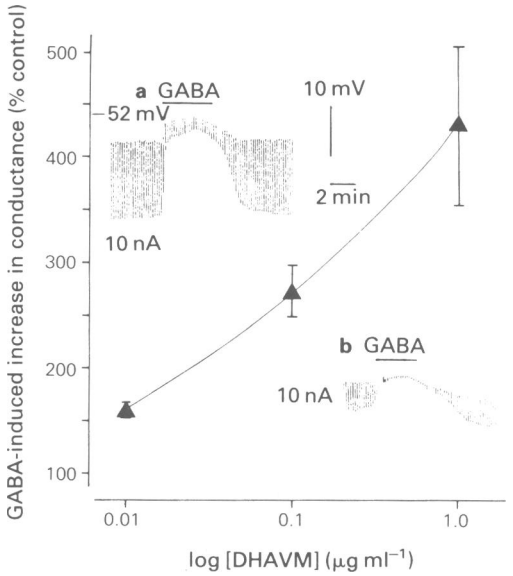

Figure 6 The effect of dihydroavermectin B_{1a} (DHAVM; 0.1 μg ml^{-1}) on the GABA response of muscle bundle 33. Insets: Records illustrating the potentiation of GABA (10^{-3} M) – induced conductance increase by DHAVM; (a) before and (b) after application of DHAVM (0.1 mg ml^{-1}). The potentiation of GABA-induced conductance after membrane resistance was reduced by DHAVM was measured directly on the oscilloscope screen. In cases when the resistance was very low the injected current was increased from 6–50 nA to facilitate the measurement of the hyperpolarizing electrotonic potentials.

Table 3 Irreversible conductance increases induced by dihydroavermectin B_{1a} (DHAVM) microperfusion on to GABA insensitive fast muscle fibres

Dose DHAVM (μg ml^{-1})	Irreversible conductance increase ($\Delta G \times 10^{-7}$ s)	
	3–5 min after application DHAVM	*60 min after application DHAVM*
0.001	1.8 ± 0.5*	6.5 ± 1.5 (5)
0.0075	4.7 ± 1.3	13.1 ± 0.4 (3)
0.01	13.6 ± 3.3	47.4 ± 9.9 (5)
0.1	28.1 ± 3.4	76.6 ± 2.5 (3)
1.0	111.1 ± 14.9	474.0 ± 84.4 (3)

*In 2 out of 5 preparations 0.001 μg ml^{-1} induced no change in input conductance. All values represent the mean ± s.e.mean of *n* (number in parentheses) observations.

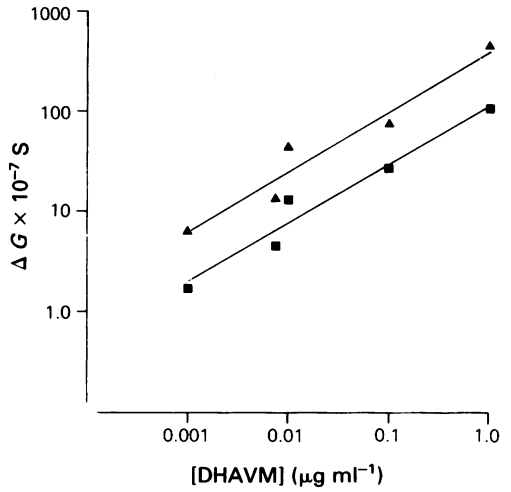

Figure 7 The dose-dependence of the change in input conductance caused by microperfusion of dihydroavermectin B_{1a} (DHAVM) onto muscle bundles 21–26 (insensitive to GABA). The change in conductance was measured 3–5 min (■) (correlation coefficient = 0.966) and 60 min (▲) (correlation coefficient = 0.978), after DHAVM application.

Effects of dihydroavermectin B_{1a} on muscle bundles insensitive to GABA

Bath application of GABA (10^{-3} M) had no effect on input conductance of fibres in muscle bundles 21,22,23,24,25, and 26 (n = 18). GABA (10^{-3} M) only caused small increases (0.56 ± 0.015 × 10^{-7} s) in input conductance in a few (n = 4) experiments. Microperfusion of DHAVM (0.001–1.0 μg ml^{-1}) onto fibres of muscle bundles 21–26 induced irreversible increases in Cl^- permeability (Figure 7 and Table 3). These irreversible increases in input conductance continued to rise for at least 60 min after the DHAVM microperfusion had been stopped. This phenomenon appears to be similar in magnitude and time course to the irreversible change induced by high doses of DHAVM in muscle bundle 33.

Discussion

The extensor tibiae preparation of the locust leg used in this study has proved particularly useful for elaborating some of the mechanisms involved in the action of DHAVM in insect muscle. In particular it has been possible to discriminate between DHAVM responses involving the GABA receptor-Cl^- ion channel complex and those in which the GABA receptor is not implicated.

The fast muscle bundles 21–26 were predominantly

(18 out of 22) without sensitivity to bath application of GABA (10^{-3} M). In four cases low GABA sensitivity was found and this may be attributed to a detectable level of activation of extrajunctional GABA receptors, previously described by Cull-Candy & Miledi (1981). Muscle bundle 33 on the other hand receives inhibitory innervation and is sensitive to GABA (Duce & Scott 1983a,b). Low doses (0.000075–0.0075 $\mu g\ ml^{-1}$) of DHAVM induced reversible dose-dependent increases in Cl^- permeability, when applied to muscle bundle 33, and reversibly reduced GABA-induced Cl^{-1} conductance. The GABA dose-response curve (Figure 2b) indicates that DHAVM depressed the GABA response in a non-competitive fashion. The reversible effects of DHAVM on both GABA responses and the increase in input conductance appear to be due to the interaction of DHAVM at sites on the GABA receptor-Cl^- ion channel complex.

DHAVM (0.01–1.0 $\mu g\ ml^{-1}$) induced irreversible increases in Cl^- conductance in both GABA-sensitive (muscle bundle 33) and GABA-insensitive (bundles 21–26) fibres. This effect is consistent with the data from work on crustacean muscle fibres (Fritz *et al.*, 1979; Mellin *et al.*, 1983), where blockade of neurotransmission was shown to be associated with reduction of input resistance of the muscle fibres and was not reversible. The concentrations of AVM used by these authors were an order of magnitude higher than the highest dose used in this present study.

The irreversible increases in Cl^- permeability seen in muscle bundles 21–26 and muscle bundle 33 continued to rise gradually for at least 60 min after DHAVM microperfusion was stopped. The mechanism of this long time course is unknown, but possibly the DHAVM enters the lipid phase of the membrane and either alters the properties of the lipids or some other membrane components. Such mechanisms have been implicated in the actions of some anaesthetics (Haydon *et al.*, 1977; Gage & Hamil, 1981). The irreversible increase in input conductance induced by DHAVM was found to be due to an increase in Cl^- permeability. This is consistent with the effects seen in other systems: increases in conductance in crustacean muscle (Fritz *et al.*, 1979; Mellin *et al.*, 1983) and AVM stimulated GABA binding to rat brain synaptic membranes (Pong & Wang, 1982) were also Cl^--dependent. In no other study have DHAVM-induced conductance changes been found to be reversible, although reversibility of AVM-induced GABA release from mammalian synaptosomes has been described (Pong *et al.*, 1980).

GABA-induced conductance increases are potentiated following an irreversible increase in Cl^- permeability produced by microperfusion of DHAVM. However, re-application of this same dose of DHAVM during the potentiated GABA response results in a reduction of the GABA-induced conductance. These interactions support the contention that DHAVM has more than one site of action. It appears that once DHAVM has acted to produce irreversible increases in conductance, although it may remain present in the membrane and produce potentiation of GABA responses, it no longer has access to the site on the GABA receptor-ion channel complex responsible for inhibition of GABA responses. However, when reapplied and present in solution DHAVM is able to reduce GABA-induced conductance.

Potentiation of GABA-mediated transmission in rats has been found to be elicited by AVM and it was suggested that this effect may involve an increase in GABA release and/or an increase in GABA binding sites (Williams & Yarborough, 1979), and evidence for AVM-induced increases in GABA binding has been presented (Pong & Wang, 1982).

The finding that DHAVM induced an irreversible increase in Cl^- permeability in fibres that were insensitive to GABA leads us to suggest a site of action independent of the GABA receptor-Cl^- channel complex in locust muscle. The observed potentiation of GABA (10^{-3} M)-induced conductance changes produced by DHAVM is consistent with this proposition.

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