

## THE INSECTICIDE AVERMECTIN B<sub>1a</sub> ACTIVATES A CHLORIDE CHANNEL IN CRAYFISH MUSCLE MEMBRANE

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### Summary

Effects of avermectin B<sub>1a</sub> (AVM) have been tested on excised outside-out or inside-out patches of crayfish stomach muscle membrane. Continuous superfusion of AVM (0.1–1 pmol l<sup>-1</sup>) to the outside-out patches induced openings of channels (22 pS) which were similar in conductance and kinetics to the chloride channels activated by glutamate, quisqualic acid, ibotenic acid and nicotinic agonists, whereas GABA mainly activated a second, larger conductance state (44 pS). This effect was reversible. AVM did not activate the excitatory, glutamate-activated cation channel. Upon raising the AVM-concentration to 10 pmol l<sup>-1</sup> and above, an enormous increase in the rate of openings of channels (22 pS) occurred. This effect could not be washed out during the lifetime of the patch. Using inside-out patches, it was shown that the single-channel current amplitude, for both the reversible and irreversible drug actions, strongly depended on intracellular chloride concentration. Applied to the sarcoplasmic side of inside-out patches, AVM did not activate any channel. The distribution of open times for 0.1 pmol l<sup>-1</sup> AVM could be fitted by a single exponential ( $\tau = 3.3$  ms). For a higher AVM concentration (1 pmol l<sup>-1</sup>) two exponentials ( $\tau_1 = 0.5$  ms,  $\tau_2 = 2.4$  ms) were needed to fit the distribution. A similar effect was elicited by decreasing the extracellular Ca<sup>2+</sup> concentration from 13.5 to 1 mmol l<sup>-1</sup> during the application of 0.1 pmol l<sup>-1</sup> AVM. Picrotoxin blocked the activation of chloride channels for both the reversible and irreversible effects of AVM. It is suggested that AVM activates the multitransmitter-gated chloride channel in this preparation. Binding sites for the drug are discussed.

### Introduction

The avermectins are a class of polycyclic lactones from *Streptomyces avermitilis* (Burg *et al.* 1979; reviewed by Wright, 1986), which are extremely active against a broad spectrum of nematodes and arthropods (Egerton *et al.* 1979; Putter *et al.* 1981), whereas their toxicity to mammals is relatively low. The structure of avermectin B<sub>1a</sub> (AVM), one of the major components of the avermectin complex, is shown in Fig. 1.

Studies of the mode of action of avermectins in invertebrates have suggested

Key words: avermectin, crayfish muscle, chloride channels, patch-clamp.

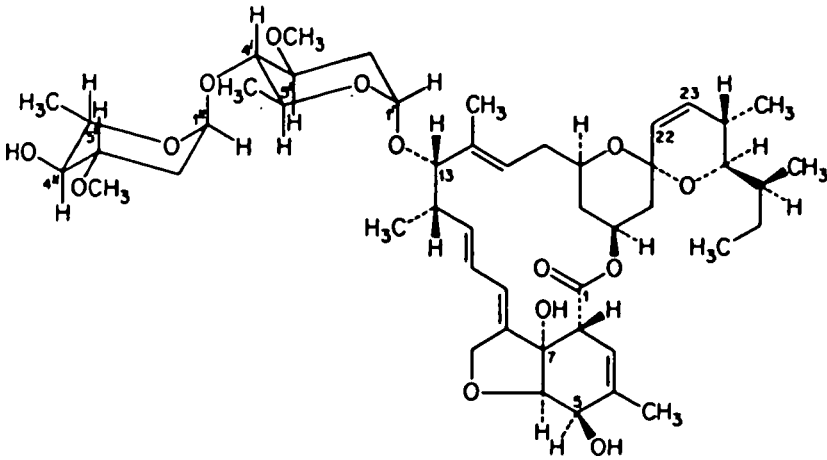


Fig. 1. Structure of the avermectin B<sub>1a</sub> molecule.

that AVM has effects on GABAergic transmission. AVM causes irreversible block of inhibitory postsynaptic potentials in the opener and stretcher muscles of Crustacea (Fritz *et al.* 1979; Mellin *et al.* 1983), which may be related to a reduction in input resistance of the muscle membrane caused by an increase of Cl<sup>-</sup> permeability (Fritz *et al.* 1979). It has also been shown that AVM blocks neurotransmission at inhibitory neuromuscular synapses, and in the ventral nerve cord, of the nematode *Ascaris suum* (Kass *et al.* 1980). Recently it has been shown that glutamate-activated channels and voltage-dependent Cl<sup>-</sup> channels may be affected by AVM. The extrajunctional glutamate H-receptor/Cl<sup>-</sup> channel complex in locust extensor tibiae muscle might be involved in the irreversible conductance increase by AVM (Duce & Scott, 1985*a,b*) and measurements of radioisotope fluxes have suggested that AVM opens a GABA<sub>A</sub> receptor Cl<sup>-</sup> channel in rat brain by acting as a partial agonist and also opens a voltage-dependent Cl<sup>-</sup> channel, which is totally insensitive to GABA (Abalis *et al.* 1986). In addition, it has recently been reported that AVM activates a chloride channel insensitive to neurotransmitters in *Ascaris* muscle (Martin & Pennington, 1988).

The aim of the present study was to look into the molecular mode of action of AVM by using the patch-clamp technique. Excised outside-out patches of crayfish stomach muscle, which are known to contain a glutamate-activated cation channel (Franke *et al.* 1986*a*) and a chloride channel which can be activated by GABA as well as by glutamate and acetylcholine (Franke *et al.* 1986*b*; Zufall *et al.* 1988*a*) seemed to be an ideal preparation for this purpose.

A preliminary report of this work has been published (Zufall *et al.* 1988*b*).

### Materials and methods

Muscles were isolated from crayfish (*Austropotamobius torrentium*), 3–5 cm long, and pinned down in a chamber described by Franke *et al.* (1986*a*). All 28

experiments were performed with the intrinsic gm6b stomach muscle (Maynard & Dando, 1974). No inhibitory innervation has been identified in this muscle (Govind *et al.* 1975). After treatment of the muscles with 1–2 mg ml<sup>-1</sup> collagenase (Sigma 1A) to remove connective tissue, excised outside-out or inside-out patches were established as described by Franke *et al.* (1986a,b) for this preparation. These excised patches were moved from the preparation to a recording chamber which could be perfused separately as described in detail by Franke *et al.* (1986a). Test solutions were superfused continuously over the excised patches. The modified van Harrevelde solution used for the preparation and the patches contained (in mmol l<sup>-1</sup>) NaCl, 220; KCl, 5.4; CaCl<sub>2</sub>, 13.5; MgCl<sub>2</sub>, 2.5; and Tris-maleate buffer, 10. The pH was adjusted to 7.6. The high-chloride intracellular solution contained (in mmol l<sup>-1</sup>) KCl, 150; NaCl, 5; MgCl<sub>2</sub>, 2; CaCl<sub>2</sub>, 1; EGTA, 10; Tris-maleate buffer, 10; and the pH was adjusted to 7.2 with KOH to give a final potassium concentration of 190 mmol l<sup>-1</sup> and a chloride concentration of 161 mmol l<sup>-1</sup>. The low-chloride intracellular solution contained 150 mmol l<sup>-1</sup> potassium propionate, 5 mmol l<sup>-1</sup> sodium propionate, 2 mmol l<sup>-1</sup> MgCl<sub>2</sub>, 1 mmol l<sup>-1</sup> CaCl<sub>2</sub>, 10 mmol l<sup>-1</sup> EGTA, 10 mmol l<sup>-1</sup> Tris-maleate buffer. The pH was adjusted to 7.2. The temperature was 18°C in all experiments. L-Glutamate was obtained from Merck; GABA, carbachol and picrotoxin from Sigma. Avermectin B<sub>1a</sub> was kindly donated by Merck, Sharp & Dohme (Rahway, NJ). A stock solution of 10<sup>-4</sup> mmol l<sup>-1</sup> AVM in dimethyl sulphoxide (DMSO) was stored frozen, and dilutions of that solution were made freshly every day with a final maximal DMSO concentration of 0.001%. After each experiment the tubing and recording chamber were washed with saline containing 10% DMSO. The patch-clamp data were recorded and evaluated as described in Franke *et al.* (1986a) and Dudel & Franke (1987). The records were filtered with an eight-pole Bessel filter at 2 kHz and sampled at 10 kHz.

## Results

### *Reversible effects of AVM*

We have recently shown that the three transmitters, GABA, glutamate and acetylcholine, activate a chloride channel in excised outside-out patches of crayfish stomach muscle (Franke *et al.* 1986b; Zufall *et al.* 1988a). There was cross-desensitization of GABA, glutamate and acetylcholine responses, suggesting that the receptors activate a common ion channel. This chloride channel has at least two conductance substates which are preferentially activated by the different agonists. Glutamate and the nicotinic agonist carbachol applied to the same outside-out patch activate the first conductance state (22 pS) whereas GABA mainly activates the second, larger conductance state (44 pS). As exemplified in Fig. 2A, the amplitude of the current steps at a membrane potential of -85 mV is about -2 pA for the first conductance state. The GABA agonist muscimol also mainly opens the 44 pS level, whereas quisqualic acid and ibotenic acid, a compound which has been described as a specific agonist of extrasynaptic

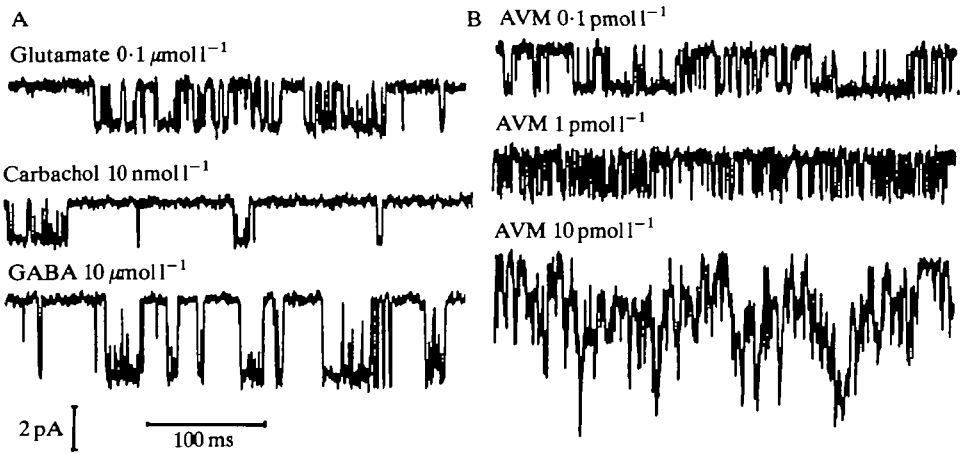


Fig. 2. Recordings from one outside-out patch superfused with (A) glutamate ( $0.1 \mu\text{mol l}^{-1}$ ), carbachol ( $10 \text{ nmol l}^{-1}$ ) and GABA ( $10 \mu\text{mol l}^{-1}$ ), which activates preferentially a substate with twice the conductance of the other agonists and (B) with AVM at three different concentrations. Patch potential  $-85 \text{ mV}$ , the single-channel currents are inward (downward excursion). Bandwidth  $0\text{--}2 \text{ kHz}$ .

glutamate-gated  $\text{Cl}^-$  channels in locust muscle (Lea & Usherwood, 1973), only open the  $22 \text{ pS}$  level in this preparation.

When outside-out patches of crayfish stomach muscle membrane were continuously superfused with AVM ( $0.1\text{--}1 \text{ pmol l}^{-1}$ ) channels opened  $1\text{--}2 \text{ min}$  after starting the superfusion (upper two traces of Fig. 2B). These openings also had a single-channel conductance of  $22 \text{ pS}$  and resembled those activated by carbachol and glutamate. AVM was never seen to activate the second conductance level like GABA agonists. This effect of AVM was reversible on washing the patch with Ringer's solution. These subpicomolar effects of AVM were observed even when the recording chamber had not previously been perfused with higher doses of AVM. AVM was not seen to activate channels on patches which did not respond to GABA, glutamate or carbachol, suggesting that AVM does not act as an ionophore. Furthermore, AVM did not activate the excitatory, glutamate-activated cation channel, which is also frequently present on these patches (Franke *et al.* 1986a).

#### *Irreversible effects of AVM*

Upon raising the AVM-concentration to  $10 \text{ pmol l}^{-1}$  and above, an enormous increase in the rate of opening of channels occurred within several seconds (see bottom trace of Fig. 2B). This effect could not be washed out during the lifetime of the patch ( $30\text{--}60 \text{ min}$ ) and is therefore referred to as irreversible.

Single openings at this high rate were not easy to analyse. Many of the openings in  $10 \text{ pmol l}^{-1}$  AVM were so brief that they were not recorded at full amplitude because of the limited frequency response of the recording system. Nevertheless,

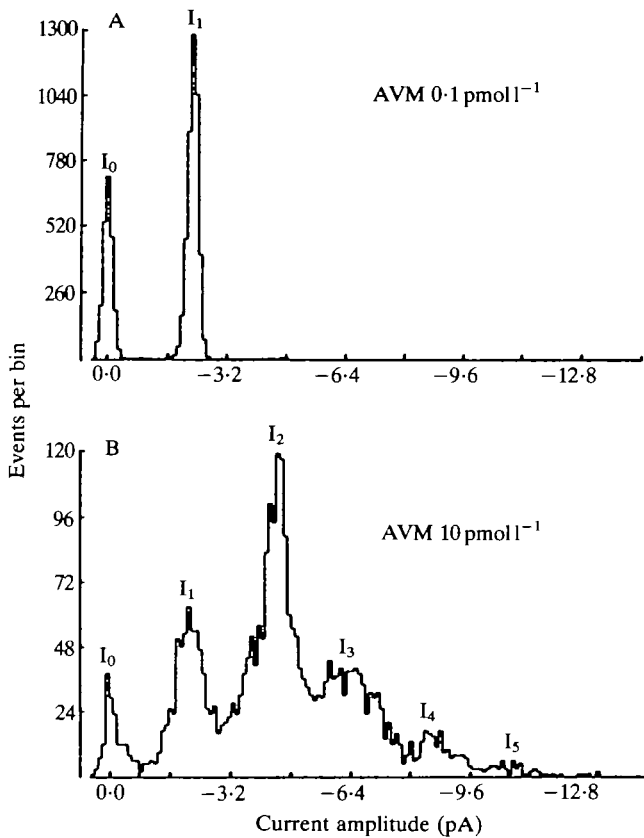


Fig. 3. Amplitude distributions of single-channel currents longer than 0.5 ms for one outside-out patch activated by AVM  $0.1 \text{ pmol l}^{-1}$  (A) and  $10 \text{ pmol l}^{-1}$  (B).  $I_0$  represents the closed state,  $I_1$  the first open state.  $I_2$ – $I_5$  represent the current level at simultaneous openings. Binwidth  $0.1 \text{ pA}$ .

the amplitude histogram of openings longer than 0.5 ms (Fig. 3B) showed distinct peaks, which represent the superposition of single channel openings with the amplitude  $I_1$  elicited by  $0.1 \text{ pmol l}^{-1}$  AVM (Fig. 3A). Therefore, the irreversible effect of AVM is probably due to channel openings with the same conductance as those involved in the reversible drug action.

#### *AVM activates a chloride channel*

To find out whether the AVM-induced currents were dependent on  $\text{Cl}^-$  concentration, inside-out patches were established as described by Franke *et al.* (1986b) and the intracellular  $\text{Cl}^-$  concentration was varied during recording of single channels. Single-channel amplitudes of the currents induced by  $0.1 \text{ pmol l}^{-1}$  AVM were dependent on intracellular chloride concentration (Fig. 4) in a similar fashion to the  $\text{Cl}^-$  channels activated by carbachol (Zufall *et al.* 1988a). Between 20 and  $100 \text{ mmol l}^{-1}$  intracellular  $\text{Cl}^-$  concentration, the single-channel current

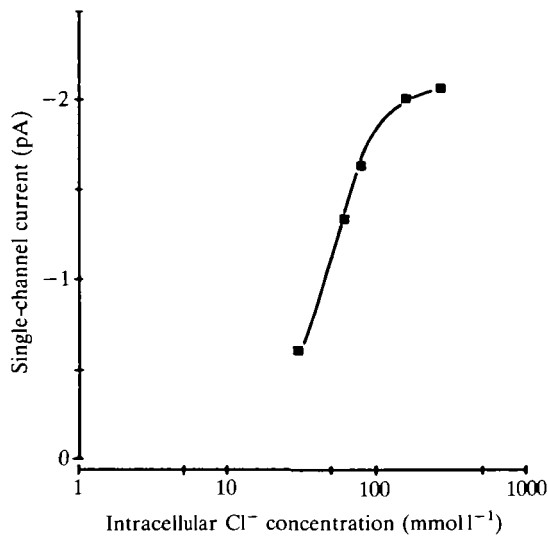


Fig. 4. Evaluation of amplitudes of single-channel currents triggered by  $0.1 \text{ pmol l}^{-1}$  AVM at different intracellular  $\text{Cl}^{-}$  concentrations recorded at an inside-out patch. The abscissa is on a logarithmic scale; membrane potential  $-85 \text{ mV}$ . The curve connecting the current values is fitted by eye.

amplitude rose from  $-0.6$  to  $-1.6 \text{ pA}$ . Above  $100 \text{ mmol l}^{-1} \text{ Cl}^{-}$ , the current approached a saturation level. Each point of the curve in Fig. 4 represented at least 200 single-channel current amplitudes which were evaluated and plotted in amplitude histograms as shown for Fig. 3.

A similar relationship between single-channel amplitude and intracellular chloride concentration was also found for channels that had been irreversibly activated by AVM (data not shown).

#### *AVM does not act from the inside of the membrane*

To examine whether channels possessed binding sites for AVM on the inside of the membrane, AVM was applied to the cytoplasmic side of five inside-out patches. A low dose of AVM ( $0.1 \text{ pmol l}^{-1}$ ) inside the patch pipette activated some chloride channels, showing that they were present in these patches. Application of a high concentration of AVM ( $100 \text{ pmol l}^{-1}$ ) to the inside of the membrane did not enhance the rate of channel openings. Thus, a direct action of AVM ( $100 \text{ pmol l}^{-1}$ ) on the cytoplasmic side of these channels seems to be ruled out under the conditions employed. A possible internal action in the intact cell with cytoplasmic contents present cannot be eliminated by these experiments. The relatively poor membrane permeability of AVM argues against, but does not eliminate, this possibility.

#### *Kinetic properties*

The open-time and closed-time distributions for the AVM-induced  $\text{Cl}^{-}$  channel

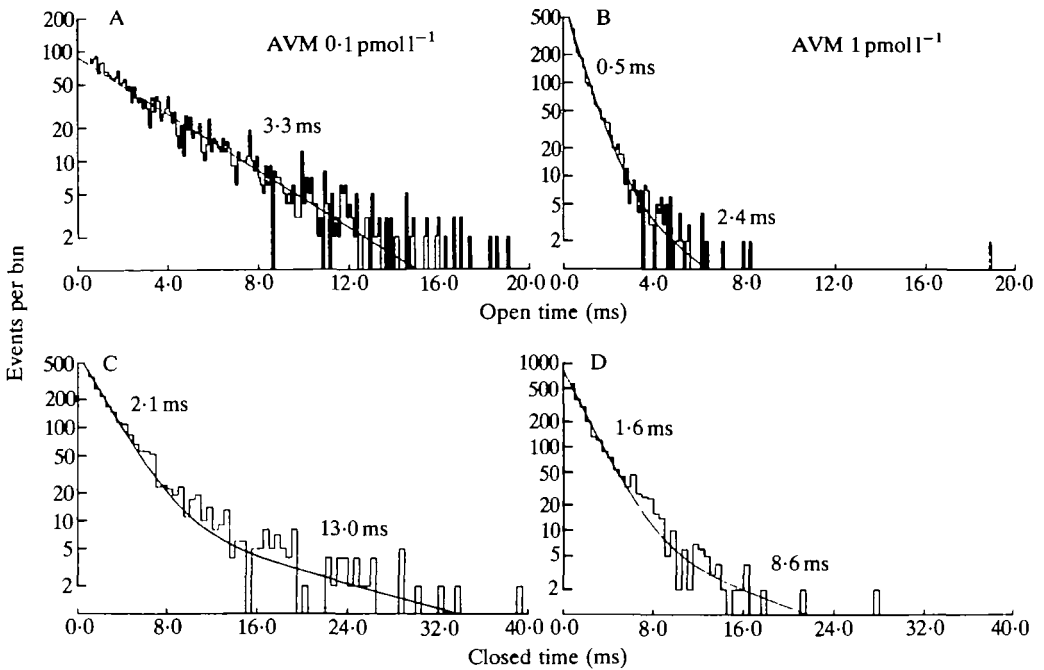


Fig. 5. Distributions of open times (A,B) and closed times (C,D) of single-channel currents elicited by  $0.1 \text{ pmol l}^{-1}$  (A,C) and  $1 \text{ pmol l}^{-1}$  AVM (B,D). The ordinates are on logarithmic scales. The distributions of open times in A is fitted by a single exponential, in B by two exponentials. (C,D) Distributions of closed times, fitted by two exponentials. Binwidth  $100 \mu\text{s}$  (A,B) and  $400 \mu\text{s}$  (C,D).

openings of the experiment shown in Fig. 2 were evaluated in time histograms shown in Fig. 5. For  $0.1 \text{ pmol l}^{-1}$  AVM the open-time distribution could be fitted by a single exponential with a time constant of 3.3 ms (Fig. 5A), representing the mean open time. This value is in the range found for the glutamate- and carbachol-activated  $\text{Cl}^-$  channels, which lies near 2.5 ms (Zufall *et al.* 1988a). As AVM concentration was increased, the mean open time decreased. For  $1 \text{ pmol l}^{-1}$  AVM, two exponential components were needed to fit the open-time distribution. One fast component with a time constant of 0.5 ms and one slower component with a time constant of 2.4 ms were detectable. In contrast the  $\text{Cl}^-$  channels activated by glutamate, carbachol or GABA show mean open times that are apparently concentration-independent (F. Zufall, unpublished observations). The mean current, which was calculated by integrating the digitized traces and dividing the sum by the respective recording time, decreased from  $-1.39$  to  $-0.52 \text{ pA}$  when the AVM concentration was raised from  $0.1$  to  $1 \text{ pmol l}^{-1}$  according to the reduced mean open time. The distribution of closed times (Fig. 5C,D) could be fitted by the sum of two exponentials with time constants of 2.1 ms and 13.0 ms (AVM  $0.1 \text{ pmol l}^{-1}$ ) or 1.6 ms and 8.6 ms (AVM  $1 \text{ pmol l}^{-1}$ ). The short time constants represent the short closings separating individual openings in a burst, whereas

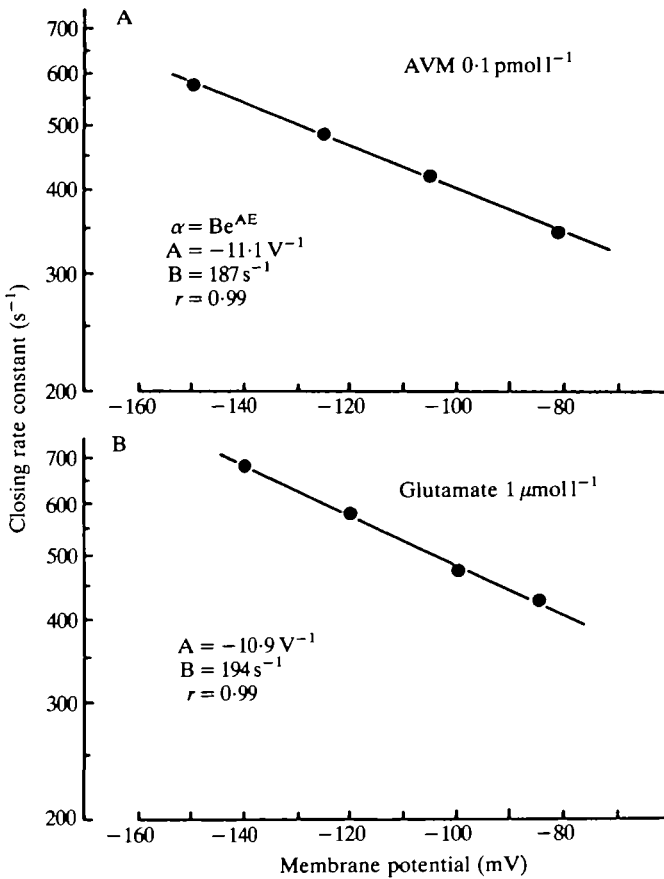


Fig. 6. Voltage-dependence of  $\alpha$ , the closing rate constant calculated as the reciprocal of the mean open time, on the membrane potential. The channels were activated by  $0.1 \text{ pmol l}^{-1}$  AVM (A) and  $1 \text{ } \mu\text{mol l}^{-1}$  glutamate (B). The regression lines represent exponential curves with the parameters indicated on the respective graph.

closed times between the bursts are represented by the longer time constant. The irreversible activation of  $\text{Cl}^-$  channels by higher concentrations of AVM (lower trace of Fig. 2A) shows many superpositions of channel openings and cannot be evaluated kinetically with our computer program. Nevertheless, the openings seem to be very short because a lot of them failed to reach their full amplitude having been attenuated by the filtering.

In a further series of measurements, the dependence of the mean open time of the AVM-induced channels on membrane potential was determined and compared to the response of glutamate-activated openings (Fig. 6). The current records were obtained after the change in membrane potential. The closing rate constant  $\alpha$  is calculated as the reciprocal of the mean open time (the distribution of open time contained only one exponential for this low AVM concentration). Fig. 6A shows that hyperpolarization up to  $-150 \text{ mV}$  shortened the mean open



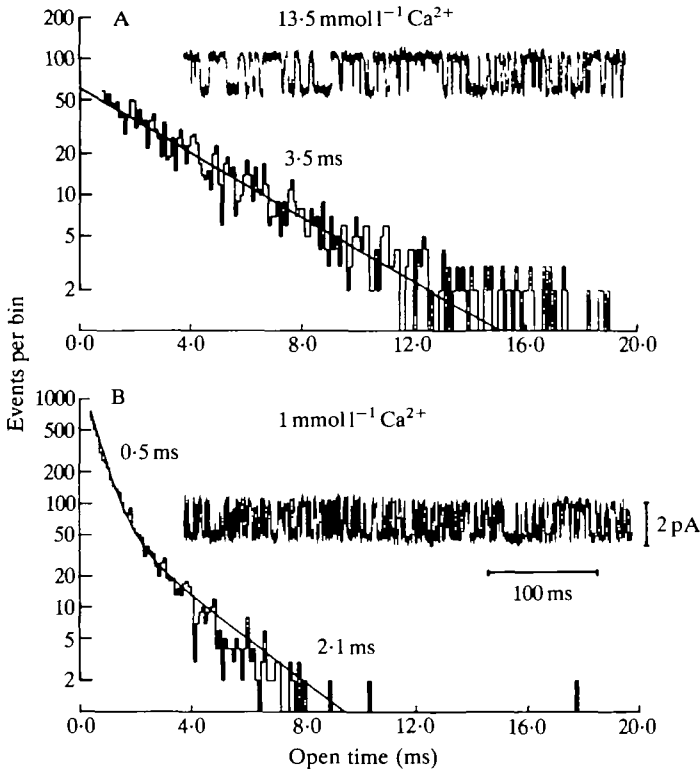


Fig. 7. Evaluations of durations of openings elicited by  $0.1 \text{ pmol l}^{-1}$  AVM in  $13.5 \text{ mmol l}^{-1} \text{ Ca}^{2+}$  (A) and in  $1 \text{ mmol l}^{-1} \text{ Ca}^{2+}$  (B) on the same outside-out patch. The distributions were described by one or two time constants which are indicated on the different curves. The abscissae are on a logarithmic scale. The insets are original recordings.

time of the AVM-activated channels. Compared to the value at  $-85 \text{ mV}$ , the reciprocal of the mean open time,  $\alpha$ , fell with an exponent of  $A = -11.1 \text{ V}^{-1}$ . A similar dependence of the mean open time on membrane potential was found for the glutamate-activated  $\text{Cl}^-$  channels (Fig. 6B). Although their mean open time was somewhat shorter, the closing rate constant gave nearly the same exponent of  $A = -10.9 \text{ V}^{-1}$ . A similar exponent of  $-7.7 \text{ V}^{-1}$  has been found for the voltage-dependence of the rate constant  $\alpha$  of decay of inhibitory postsynaptic currents in crayfish muscle (Dudel, 1977).

The mean open time of AVM-activated  $\text{Cl}^-$  channels was reduced as extracellular  $\text{Ca}^{2+}$  concentration was lowered from  $13.5$  to  $1 \text{ mmol l}^{-1}$  (Fig. 7). The mean open time for this patch at  $13.5 \text{ mmol l}^{-1} \text{ Ca}^{2+}$  was  $3.5 \text{ ms}$  (Fig. 7A). The open-time distribution at  $1 \text{ mmol l}^{-1} \text{ Ca}^{2+}$  (Fig. 7B) could only be fitted by two exponentials with time constants of  $0.5$  and  $2.1 \text{ ms}$ . This effect was reversible (not shown). Thus it appears that a reduction of the extracellular  $\text{Ca}^{2+}$  concentration has the same effect as raising the concentration of AVM. A strong inhibitory effect

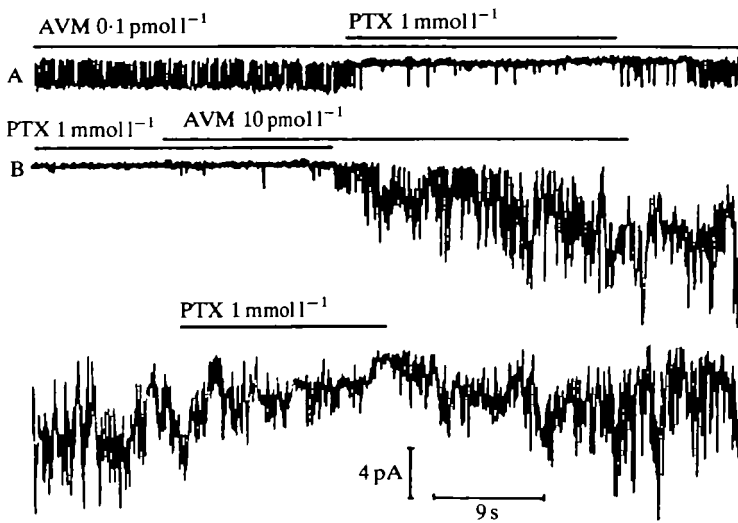


Fig. 8. Recordings from an outside-out patch at low time resolutions. In A the response to  $0.1 \text{ pmol l}^{-1}$  AVM is continued and  $1 \text{ mmol l}^{-1}$  picrotoxin (PTX) is added for 22.5 s. Note that during PTX application the channel activity is almost completely blocked. (B) PTX ( $1 \text{ mmol l}^{-1}$ ) was added first, followed by  $10 \text{ pmol l}^{-1}$  AVM. As long as PTX is present no channel opening appears. After the end of PTX application, AVM elicits superimposed channel activity with no obvious desensitization. Even after the end of AVM application, the channel activation continues. If PTX is added again for 18 s, the channel activity decreases, but then increases again without any further adding of AVM. Bandwidth 0–500 Hz.

of extracellular  $\text{Ca}^{2+}$  upon the channels activated by ACh and GABA has also been found (Zufall *et al.* 1988a).

#### *The effect of picrotoxin*

To further characterize the AVM-gated  $\text{Cl}^-$  channels we tested the effect of picrotoxin, a well-known antagonist of inhibitory currents (Takeuchi & Takeuchi, 1969; Smart & Constanti, 1986) (see Fig. 8). For this muscle, picrotoxin has been shown to block activation of chloride channels by GABA and, to a lesser degree, by glutamate or ACh (Franke *et al.* 1986b; F. Zufall, unpublished observation). The recordings of Fig. 8 are from one outside-out patch. In Fig. 8A  $0.1 \text{ pmol l}^{-1}$  AVM elicited some channel activation. No superpositions of openings were seen. When picrotoxin ( $1 \text{ mmol l}^{-1}$ ) was added, channel activation was almost completely blocked. After removal of picrotoxin from the bathing solution, AVM, which was permanently present in the superfusing solution, again activated more channels. In Fig. 8B (continuous recording) picrotoxin was applied first and then a higher concentration of AVM ( $10 \text{ pmol l}^{-1}$ ) was added which normally led to an irreversible effect. No channel activation was seen. However, when the picrotoxin superfusion was stopped, the typical irreversible effect of  $10 \text{ pmol l}^{-1}$  AVM with a high rate of channel openings was found. Even after the end of the AVM

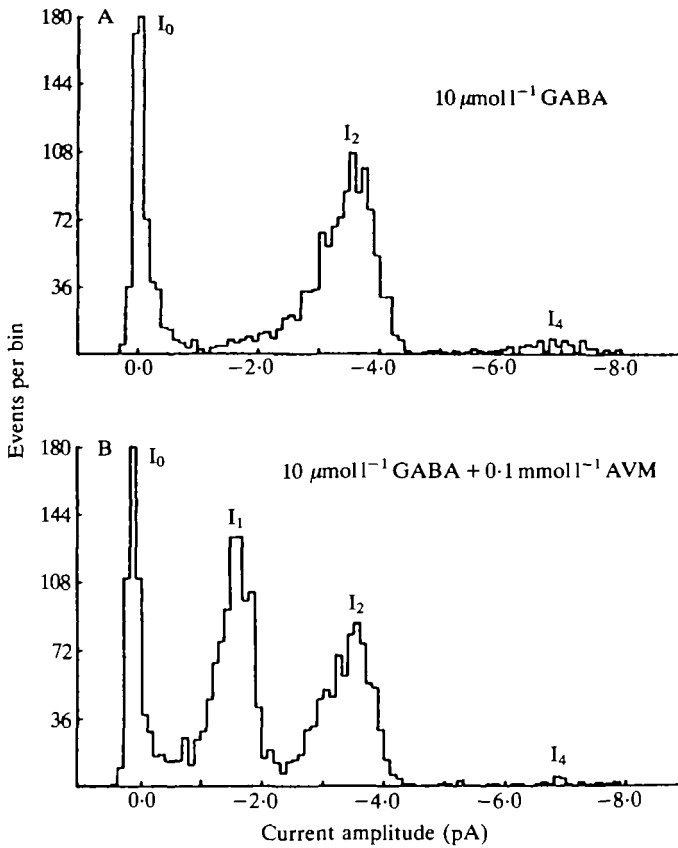


Fig. 9. Amplitude distributions of single-channel currents from one outside-out patch. (A) Channels opened by  $10 \mu\text{mol l}^{-1}$  GABA; (B) channels opened by  $10 \mu\text{mol l}^{-1}$  GABA in the presence of  $0.1 \text{ pmol l}^{-1}$  AVM. The recording time in A and B was 25 s.

application the channel activation continued. When picrotoxin ( $1 \text{ mmol l}^{-1}$ ) was then added again, the rate of channel activation was reduced while the drug was present. After termination of the application of picrotoxin the number of channel openings increased again without any additional application of AVM.

#### *The addition of GABA and AVM*

It was mentioned above that in this preparation GABA and muscimol mainly activate a higher conductance state ( $44 \text{ pS}$ ) of the chloride channel. However, AVM only opens channels with a conductance of  $22 \text{ pS}$ . We tried to take advantage of these properties to examine the possible interaction between AVM- and GABA-activated  $\text{Cl}^-$  channels. Such recordings can only be evaluated for low doses of AVM when there is no superposition of openings. Fig. 9A shows the amplitude distribution of chloride channels activated by GABA ( $10 \mu\text{mol l}^{-1}$ ). GABA almost exclusively triggered the open state  $I_2$ . Some superpositions ( $I_4$ ) occurred. When AVM at the low concentration of  $0.1 \text{ pmol l}^{-1}$  was added

(Fig. 9B) there was a big peak in the open state of  $I_1$ , representing the AVM-induced openings. The two GABA peaks are not significantly changed during the AVM application, suggesting that AVM at this concentration may not affect the activation of chloride channels by GABA.

### Discussion

A major result of this study was the finding that the insecticide AVM opens chloride channels. The activation of such channels may be responsible for the increase of chloride permeability leading to a reduction in input resistance found in measurements with intracellular microelectrodes in various invertebrate preparations (Fritz *et al.* 1979; Mellin *et al.* 1983; Duce & Scott, 1985*a,b*; Beadle & Lees, 1985; Albert *et al.* 1986). AVM opens chloride channels in outside-out patches in the absence of intracellular messengers such as cyclic AMP and ATP. This suggests that AVM activates the channel directly and not *via* a second messenger pathway.

The chloride channels activated by AVM have the same conductance as, and, at low concentrations, similar kinetic parameters to (open time, closed time), those activated by glutamate and carbachol (Franke *et al.* 1986*b*; Zufall *et al.* 1988*a*). In addition, they are sensitive to picrotoxin, show a  $Ca^{2+}$ -dependent gating and a similar voltage-dependence of the mean open time. No other chloride channel has yet been identified in these membranes. It therefore seems reasonable to suggest that AVM activates the multitransmitter-gated chloride channel in this preparation. Does AVM activate the channel at a transmitter binding site or somewhere else on the channel protein? AVM is highly lipid-soluble and did not activate chloride channels if applied to the sarcoplasmic side of an inside-out patch. The activation therefore seems to occur at the part of the receptor-channel complex facing the extracellular space. In fibres of locust leg muscle which are insensitive to GABA and have no inhibitory innervation, AVM caused an irreversible increase in  $Cl^-$  permeability (Duce & Scott, 1985*a,b*). In the same type of fibres, a high-density of extrasynaptic  $Cl^-$  channels (H-receptor) activated by glutamate and ibotenate have been found (Dudel *et al.* 1988). These  $Cl^-$  channels are similar in some respects (conductance and kinetics) to the multitransmitter-activated channels described here, although they have some pharmacological differences. It seems likely that in the studies of Duce & Scott (1985*a,b*) these channels were involved in the effect of AVM. They suggested a site of action independent of the GABA-receptor complex. Their results are supported by the finding of Tanaka & Matsumura (1985) that AVM selectively increases  $Cl^-$  permeability, but does not alter the binding of GABA, benzodiazepam and picrotoxin to muscle membranes of the cockroach. In the system investigated here, AVM activated the same conductance state of a  $Cl^-$  channel as glutamate, ibotenic acid and nicotinic agonists. GABA and muscimol preferentially activated a second, higher-conductance state. Therefore, an interaction with the GABA binding site seems unlikely. However, it cannot be completely excluded because

GABA, too, can activate the first subconductance state to a small extent. We found that picrotoxin reduced the effect of AVM on single-channel activation. In a previous study, we demonstrated that picrotoxin is not specific for GABA; it can also block the activation of  $\text{Cl}^-$  channels by glutamate (Franke *et al.* 1986b). Therefore the picrotoxin effect gives no further information on the binding site of AVM.

The threshold concentration for the activation of the AVM binding site of  $<0.1 \text{ pmol l}^{-1}$  is the lowest value for an effect of AVM described until now. In insects a threshold of  $100 \text{ } \mu\text{mol l}^{-1}$  to  $10 \text{ mmol l}^{-1}$  has been found; in crustaceans and molluscs,  $10 \text{ nmol l}^{-1}$  to  $10 \text{ pmol l}^{-1}$ . The reversible action of AVM at low concentrations confirms the results of Duce & Scott (1985a). Higher concentrations induced an irreversible effect in all preparations examined. This dose-dependent behaviour has also been found in our studies on single-channel currents. It remains unclear whether AVM has more than one site of action, as proposed by Wright (1986): a high-affinity binding site responsible for the reversible action at low concentrations and a nonspecific binding site involved in the irreversible effect at high concentrations. For our experiments this interpretation seems rather unlikely because we showed that channels with the same characteristics (conductance, ion specificity, sensitivity to picrotoxin) are activated by low and high concentrations of AVM.

It seems possible that AVM at higher concentrations, in addition to the agonistic effect, blocks the open chloride channel because the mean open time and the open probability decrease with increasing AVM concentrations. Ogden & Colquhoun (1985) reported similar results for the nicotinic endplate channel of frog skeletal muscle and suggested an open-channel block by the agonist itself.

When the concentration of AVM was further increased, irreversible activation of chloride channel openings was induced, probably due to irreversible binding of AVM to the receptor-channel complex. The suggested irreversible binding may be responsible for the extreme toxicity of this compound.

In conclusion, it was demonstrated in the present study that AVM acts as a powerful agonist of transmitter-activated chloride channels. To further characterize the binding site(s) of AVM, a preparation with chloride channels activated only by either glutamate or GABA should be investigated. This is the case in the locust extensor tibiae muscle, which was recently reported by Dudel *et al.* (1988) to contain a glutamate-activated chloride channel insensitive to carbachol and GABA.

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