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Effect of Avermectin B_{1a} on Chick Neuronal γ-Aminobutyrate Receptor Channels Expressed in *Xenopus* Oocytes

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SUMMARY

Chick brain mRNA was isolated and injected into *Xenopus* oocytes. This led to the expression of γ-aminobutyrate (GABA) channels easily accessible for current measurements using the voltage clamp technique. The effect of the anthelmitic natural product avermectin B_{1a} on the GABA current was studied quantitatively. In the presence of the drug, GABA-induced chloride currents were strongly enhanced in a dose-dependent manner. Half-maximal stimulation of the current evoked by 5 μM GABA was found with about 0.1 μM avermectin B_{1a}. Avermectin B_{1a} did not affect the reversal potential of the current or the maximal response elicited by GABA, and did not alter the membrane permeability in the absence of GABA. The major effects of avermectin B_{1a} were a shift of the K_a for GABA from 21 μM to 2 μM, and a decrease of the apparent Hill coefficient for GABA from 1.7 to 1.1. Furthermore, in the presence of avermectin B_{1a}, desensitization of the GABA current was strongly inhibited. The benzodiazepine-binding site ligand Ro 15-1788 did not affect the action of avermectin B_{1a} if present at concentrations up to 1 μM. The stimulatory effects of the drug were additive to the ones by the barbiturate pentobarbital, if both agents were added at low concentrations. At higher concentrations each of these agents inhibited the stimulatory effects of the other.

Avermectin B_{1a} is a macrocyclic lactone from *Streptomyces avermitilis* with powerful antiparasitic efficacy. An extensive review of its chemical structure, antiparasitic properties, and the putative mode of action has recently been published by Campbell *et al.* (1). Ivermectin, the 22,23-dihydro derivative of avermectin B_{1a}, is presently being evaluated for its activity against microfilariae in man (2). Data obtained from experiments carried out with *Ascaris suum* (3) and on the stretcher muscle of the walking leg of lobster (4) indicated an involvement of GABA neurotransmission, because all effects of avermectin B_{1a} could be blocked by the noncompetitive GABA antagonist picrotoxin. The low density of GABA synapses in helminths and crustaceans hindered efforts to establish whether avermectin B_{1a} acts as a GABA agonist, an allosteric modulator of GABA action, or at a presynaptic site. Further studies have indicated the presence in rat brain of binding sites for avermectin B_{1a} with nM affinity (5). Subsequent studies have shown that there is allosteric interaction between the binding sites for GABA, benzodiazepines, barbiturates, picrotoxin, and avermectin B_{1a} (6–10), indicating a site of action at the $GABA_A$ receptor channel complex (11, 12). These findings prompted us to investigate quantitatively the action of avermectin B_{1a} on the GABA-induced chloride current. The current measurements were carried out on GABA channels expressed in *Xenopus* oocytes (13–17). This preparation allows accurate current measurements and precise drug concentration control with the exclusion of presynaptic influences. It is therefore ideally suited for the study of drug action (18–20).

Materials and Methods

Ivermectin and the avermectin preparation, which contained 92% avermectin B_{1a} and 6% avermectin B_{1b}, were a gift from Merck, Sharp and Dohme (Rahway, NJ). Ro 15-1788 was used as the preparation available for intravenous application (Anexate, Hoffmann-La Roche).

Expression of the GABA channel in *Xenopus* oocytes. Total mRNA was prepared from forebrain of 2-day-old chicks following the procedures of Cathala *et al.* (21) with the modifications described earlier (22). Injection of mRNA into the oocytes and the removal of the follicular cell layers have been described elsewhere (22). The denuded oocytes were placed on a nylon grid and impaled with two microelectrodes.

Current measurements. Membrane currents were measured using the two-electrode voltage clamp technique. The bath volume was 0.4 ml and the medium contained 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM Hepes-NaOH (pH 7.4). The chamber was constantly perfused at a rate of 6 ml/min. Avermectin B_{1a} and ivermectin were dissolved in ethanol/dimethyl sulfoxide (8:2) at a concentration of 8 mg/ml. Stock solutions of GABA and/or the drugs were diluted into the medium mentioned above and applied by bath perfusion. All experiments were carried out at 20°. The membrane potential was always kept at −100 mV. Where curves were fitted, a nonlinear least square fit (Gauss-Newton-Marquardt) was used with the equations given in the text.

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ABBREVIATIONS: GABA, γ-aminobutyrate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Results

After injection with chick brain mRNA, *Xenopus* oocytes expressed in the plasmalemma several functional voltage- and ligand-gated ion channels (16, 17). Specifically, they showed GABA responses that could be allosterically modified in the presence of benzodiazepine-binding site ligands (15, 18, 20). Fig. 1 shows the inward current response of such an oocyte to the application by bath perfusion of 5 μM GABA. The oocyte was kept at −100 mV under voltage clamp. Subsequently, the same oocyte was exposed to 1 μM avermectin B_{1a} and, 3 min later, to 5 μM GABA in combination with avermectin B_{1a} (Fig. 1). This GABA response was stimulated severalfold in comparison to that under control conditions. Picrotoxin (3 μM) strongly inhibited the reponse elicited by the combined drug application of GABA and avermectin B_{1a}. This inhibition amounted to 84% of the current amplitude. A similar inhibition of the GABA current has been observed in the absence of avermectin B_{1a} (20), thus indicating independent sites of action for picrotoxin and avermectin B_{1a}. If the oocyte was not preequilibrated with avermectin B_{1a} before the combined application of drugs, the rise time to peak of the current resonse was markedly increased, indicating a slow onset of the stimulation by avermectin B_{1a}. The drug, applied at 1 μM in the absence of GABA, in some cases elicited small membrane currents amounting to up to 50 namp in oocytes displaying maximal GABA responses of 1.5–3 μamp. Fig. 2 shows the avermectin B_{1a} concentration dependence of the stimulation of the control current elicited by 5 μM GABA. Best fit of the data by the equation $I(c)-1 = (I_{max}-1)c^n/(c^n+K_a^n)$ was obtained with a Hill coefficient of $n = 0.88$, and with half-maximal stimulation K_a = 77 nM. I_{max} was preset to 83% of the theoretical maximal GABA response as estimated from the control GABA application. The reason for this decrease in the maximal GABA response in the presence of avermectin B_{1a} is explained below.

The shape of the current-voltage relationship and the reversal potential of the GABA response were not affected by the presence of 1 μM avermectin B_{1a} (Fig. 3). The reversal potential of the current elicited by GABA was always in the range between −22 and −30 mV, which is in good agreement with the

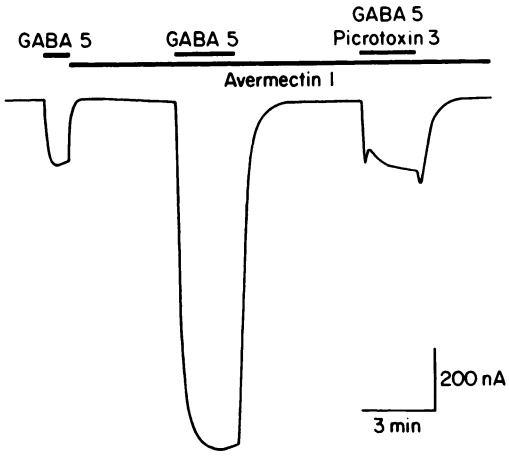

Fig. 1. Effect of avermectin B_{1a} on the GABA-induced current response. The membrane of the oocyte was kept at −100 mV under voltage clamp. The temperature was 20°. Continuous perfusion was switched for the periods indicated by the *bars* to the same medium containing either GABA alone or combined with the drugs indicated. The *numbers* indicate the respective drug concentrations in μM.

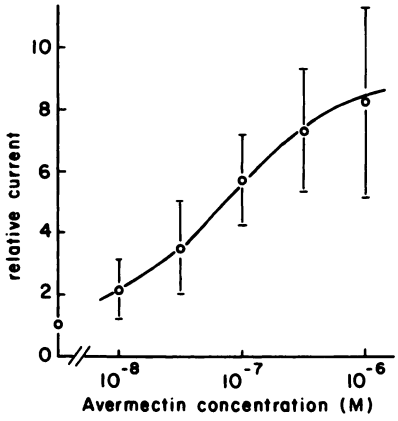

Fig. 2. Concentration dependence of the stimulatory effect by avermectin B_{1a}. The measurements were standardized by assigning the value of 1 to the current amplitude elicited by a control application of 5 μM GABA. Different concentrations of avermectin B_{1a} were applied for 3 min alone and then in combination with 5 μM GABA. For each *point* the mean ± standard deviation is given for at least three determinations performed on different oocytes. The *solid line* shows the fit obtained as indicated in the text.

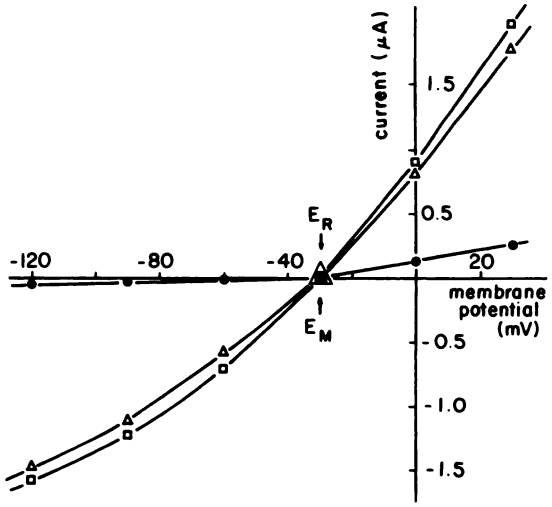

Fig. 3. Current-voltage relationship of the modified GABA current. Data were obtained from an experiment of the type shown in Fig. 5. Before application of 100 μM GABA alone and in conjunction with 1 μM avermectin B_{1a}, and during peak current amplitude, increasing voltage steps of 100 msec duration were applied from a holding potential of −100 mV. The current amplitude at the end of a step was plotted against membrane potential. ●, resting conditions; △, 100 μM GABA; □, 100 μM GABA and 1 μM avermectin B_{1a}.

reversal potential of −24 mV for chloride ions in the *Xenopus* oocyte (23). This indicates that the stimulation of the GABA response by avermectin B_{1a} is not due to a change in the ion selectivity of the channel.

The GABA concentration dependence of the avermectin B_{1a} (1 μM)-stimulated current is shown in Fig. 4. The GABA concentration response curve was fitted with the equation $I(c) = I_{max}(c^n/(c^n+K_a^n))$. Best fit was obtained with a Hill coefficient of 1.07, a K_a for GABA of 2.1 μM, and a relative maximal current, I_{max}, of 9.5. Due to the slow rundown of the GABA current in the presence of avermectin B_{1a}, every oocyte was only used for a single determination. The varying levels of channel expression in different oocytes made it necessary to standardize the current responses. The values characterizing the GABA concentration-response curve in the presence of

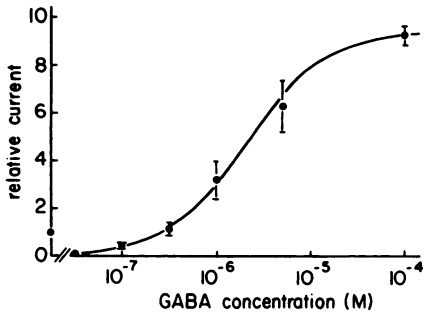

Fig. 4. Effect of avermectin B_{1a} on the GABA concentration-response curve. All current measurements were standardized as indicated in Fig. 2. Avermectin B_{1a} (1 μM) was applied first alone for 4 min and then together with different concentrations of GABA. Each concentration of GABA was applied to at least three different oocytes. Mean ± standard deviation is given. Each oocyte was only used for one GABA concentration. The *points* were fitted with the equation given in the text.

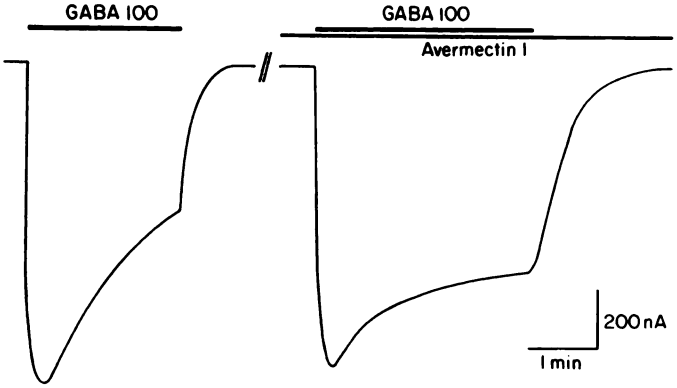

Fig. 5. Effect of avermectin B_{1a} on the desensitization and the maximal size of the GABA current. GABA (100 μM) was applied first alone and subsequently in combination with 1 μM avermectin B_{1a}. The same concentration of Avermectin B_{1a} was applied during the last 3 min of the 10-min wash-out interval.

avermectin B_{1a} should be compared to the respective values obtained in the absence of the drug (20) in the same preparation, namely $n = 1.7$ for the Hill coefficient and $K_d = 19$ μM for GABA, and a relative maximal current $I_{max} = 11.5$. The maximal response that could be achieved in the presence of avermectin B_{1a} was smaller than in the absence of this drug, and amounted to 83% as derived from the above fits. It should be stated that this value was obtained after 4 min exposure to the drug. Longer exposure progressively decreased I_{max} further. A similar decrease can also be seen in the experiment shown in Fig. 5. At a lower drug concentration (0.1 μM), the run-down of the response was also evident, and amounted to about 20% within 30 min. The reason for this rundown is not known at present.

Avermectin B_{1a} also drastically slowed desensitization of the GABA current (Fig. 5). The inactivation of the GABA current in the prolonged presence of GABA may be described by an exponential decay plus a time-independent fraction (20, 24). In the present experiments carried out using 100 μM GABA, this constant fraction amounted to 13 ± 2% ($n = 3$) in the absence and 59 ± 2% ($n = 3$) in the presence of 1 μM avermectin B_{1a}. If all the receptor channel entities occupied by avermectin B_{1a} were not available for densensitization at all, this fraction would be expected to be considerably higher (>90%), as judged from the above avermectin B_{1a} concentration dependence.

It was interesting to test whether avermectin B_{1a} acts on one of the binding sites for other allosteric modulators of the GABA channel. The benzodiazepine compound Ro 15-1788, known as "benzodiazepine antagonist" (25), did not affect the extent of current stimulation by avermectin B_{1a}. In these measurements the current amplitude elicited by the application of 5 μM GABA together with drugs was expressed relative to the current response to the application of 5 μM GABA alone. The relative current elicited in the presence of 1 μM avermectin B_{1a} was 628 ± 107% ($n = 6$) as compared to 614 ± 107% ($n = 3$) in the presence of both 1 μM avermectin B_{1a} and 1 μM Ro 15-1788. At a concentration of 1 μM, the latter compound did not affect currents elicited by GABA in the oocytes but abolished the action of drugs acting at the benzodiazepine-binding site (20). Pentobarbital and avermectin B_{1a}, if added at relatively low concentrations (100 and 0.1 μM, respectively), showed a synergistic stimulatory effect, when applied together (Fig. 6, c and d). In contrast to this finding, when applied together at relatively high concentrations (300 and 1 μM, respectively), each of these drugs antagonized the stimulatory effect of the other (Fig. 6, a and b).

In separate experiments the effect of ivermectin, a dihydro derivative of avermectin B_{1a}, on the GABA concentration-response curve was investigated. In these experiments no difference as compared to the action of avermectin B_{1a} could be detected (results not shown).

Discussion

The results described here confirm that the anthelmintic avermectin B_{1a} can bind to the $GABA_A$ receptor-activated chlo-

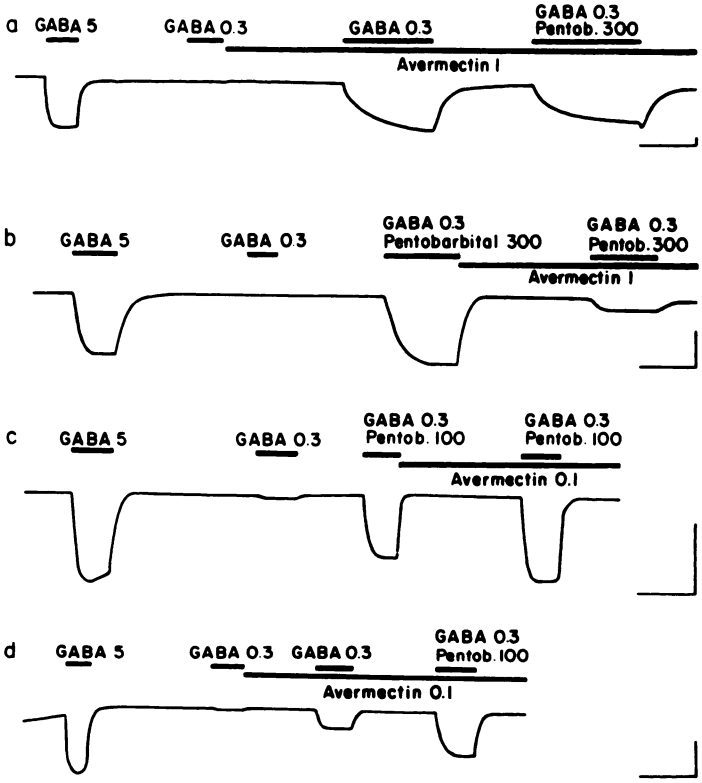

Fig. 6. Interaction of avermectin B_{1a} with pentobarbital. Each *trace* shows control applications of 5 μM GABA and 0.3 μM GABA. Subsequently, 0.3 μM GABA was applied together with pentobarbital or avermectin B_{1a} alone or in combination. Whereas the two drugs counteract each other at higher concentrations (a and b), synergism is observed at small concentrations (c and d). The *calibration bars* indicate 2 min (*horizontal*) and 50 namp (*vertical*).

ride channel. Under voltage clamp conditions it could be shown that the functional properties of this channel are profoundly altered as a result of this interaction. Four major effects on the chloride currents elicited by GABA could be seen: 1) a decrease of the apparent K_a for GABA from 19 to 2 μM, 2) a decrease in the Hill coefficient from 1.7 to 1.1, 3) a drastic increase in the nondesensitizing current, and 4) a slow run-down of I_{max}. Whereas the effects on the GABA concentration-response curve and the inhibition of desensitization of the GABA current both act together to increase the chloride current, the slow run-down observed at very high concentrations of avermectin B_{1a} opposes this action. Avermectin B_{1a} itself did not activate the chloride channel or alter its ion selectivity. Avermectin B_{1a} did not seem to act at any of the binding sites of either picrotoxin, benzodiazepines, or β-carbolines. The interaction with pentobarbital is more complex. Whereas the synergistic effect at low concentrations of both avermectin B_{1a} and barbiturate are compatible with an action at a common site, the cross-inhibitory effect of the two drugs at higher concentration seems to argue for a different mode of action. Evidence for a new and different site of action from other modulatory compounds has been provided by binding studies of avermectin B_{1a} to rat brain membranes. Drexler and Sieghart (26) found little if any displacement of avermectin B_{1a} from its binding site by 0.5 mM pentobarbital. Picrotoxin (0.1 mM) even had a stimulatory effect.

It is interesting to compare the effect of avermectin B_{1a} on the GABA-controlled chloride channel with the recently described effects of the steroid general anesthetic alphaxolone in bovine chromaffin cells (27) and in spinal cord neurons (28), both maintained in culture. Alphaxolone has also been reported to stimulate GABA currents without effect on the reversal potential. In contrast to our finding with avermectin B_{1a}, high concentrations of pentobarbital enhanced currents stimulated by high concentrations of alphaxolone (27, 28).

Ivermectin is presently used as a potent anthelmintic in animals and its use in the management of human oncocerchiasis is presently being evaluated (2). In spite of the apparently poor penetration of the blood-brain barrier by ivermectin (2), possible central side effects can be inferred from our findings. The enhancement of the $GABA_A$ receptor-mediated inhibition could lead to a central effect similar to the action of relaxant benzodiazepines. This effect could possibly be potentiated by the inhibitory effect on the desensitization of the GABA current. This expected enhancement of the GABA-mediated inhibition is counteracted, however, by the slow run-down of the GABA current described above. The latter effect requires more detailed analysis at lower avermectin B_{1a} concentrations to enable a prediction of the overall effect of avermectin B_{1a} on central inhibition.

In conclusion, we describe here the effects of avermectin B_{1a} on chick neuronal GABA-gated chloride channels expressed in *Xenopus* oocytes after injection with mRNA. This preparation enables quantitative measurement of drug effects using bath application of drugs and under voltage clamp conditions.

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