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Activation of rat recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptor by the insecticide ivermectin

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Abstract

In the present study, the activation of rat recombinant $\alpha_1\beta_2\gamma_{2S}$ γ-aminobutyric acid (GABA)-ergic Cl^- channel expressed in human embryonic kidney (HEK) 293 cells by ivermectin was investigated. Maximal activation of the channel occurred with GABA concentrations of 10 mM or 20 μM ivermectin both achieving about the same current amplitudes. With those saturating concentrations, the currents rose with GABA within 1 ms to the maximal values, whereas the rise time for ivermectin was about 500 times longer. In contrast to activation with GABA, no desensitisation in the presence of the agonist was observed with ivermectin. With both agonists, two different open states were detected. On simultaneous application of GABA and ivermectin the current amplitudes and the kinetics were determined by the agonist applied in the concentration eliciting the higher open probability. It is concluded that GABA and ivermectin activated the channel independently resulting in different kinetic properties.

Keywords: Ivermectin; $GABA_A$ receptors; Subunit composition; Patch clamp

1. Introduction

Avermectins, a class of macrolidic substances widely used as insecticides and anthelmintics, were first isolated as fermentation products of the bacterium *Streptomyces avermitilis*. Beside the natural occurring avermectins, there are a lot of semi-synthetic derivatives including ivermectin, a mixture of ivermectin B_{1a} and ivermectin B_{1b}, generated by hydroxylation of the respective avermectin derivates. The anthelmintic and insecticidal action of these substances seems to be caused by their potentiating and/or agonist action on glutamate-gated Cl^- channels (Arena, 1994; Adelsberger et al., 1997; Martin et al., 1997). This group of inhibitory ligand-gated ion channels use the transmitter glutamate, normally acting as an excitatory transmitter via cation channels, as the ligand and has been found exclusively in invertebrates so far (Cleland, 1996). Homology analysis of cloned subunits, for example, from *C. elegans* (Cully et al., 1994) and *Drosophila melanogaster* (Cully et al., 1996) suggest that glutamatergic chloride channels belong to the superfamily of ligand-gated ion channels, which also comprises γ-aminobutyric acid (GABA)-ergic and glycinergic Cl^- channels, as well as nicotinic acetylcholine receptor subunits (Vassilatis et al., 1997). Therefore, several studies on the action of ivermectin on members of this channel family have been performed. Prolonged pre-application of ivermectin to the neuronal nicotinic acetylcholine receptor α7 from human and chick resulted in potentiation of the acetylcholine-elicited current (Krause et al., 1998). The mode of action on GABAergic Cl^- channels is controversial and probably depends on the subunit composition of the receptor. On GABAergic receptors expressed in *Xenopus* oocytes after injection of chick brain mRNA, pre-application of avermectin B_{1a} potentiated the GABA-elicited currents (Sigel and Baur 1987). On cultured chick spinal cord neurons, it seemed to increase membrane conductance through sites different from GABA recognition sites (Matsumoto et al., 1986). An agonistic action of avermectin was described for Cl^- channels on rat cultured hippocampal neurons (Schönrock and Bormann, 1983). Binding studies on rat cultured cerebellar granule neurons led to the conclusion

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that it binds to two sites on the GABAergic receptor resulting in activation of the receptor after binding to the high-affinity site and blocking it on further binding to the low-affinity site (Huang and Casida, 1997).

The large number of different subunits forming GABAergic Cl^- channels results in receptors with different kinetic and pharmacological properties depending on the subunit composition (Sieghart, 1995). This could be an explanation for the different modes of action of ivermectin on GABAergic receptors observed in different preparations. In most of the preparations, the receptor composition was unknown or the substances could have acted even on more than one receptor at the same time. Therefore, in the present study, a molecularly defined GABAergic receptor consisting of rat $\alpha_1\beta_2\gamma_{2S}$-subunits was chosen to investigate the action of ivermectin. The kinetic properties were compared to those elicited by GABA.

2. Materials and methods

2.1. Cell culture and electroporation

cDNAs for the α_1-, β_2- and γ_{2S}-subunits were cloned into the vector pCDM8 (Invitrogen) and the S65T-green fluorescent protein (GFP)-gene (Heim et al., 1995) into the vector pcDNA3 (Invitrogen) for mammalian expression. Plasmid DNA was purified from 25 to 500 ml bacteria cultures using plasmid kits for various purification scales (Qiagen). For improved transfection results, purified DNA was subjected to a second round of ethanol precipitation. Pellets were dissolved in sterile buffer (10 mM Tris–HCl, pH 8.0) to approximately 1 μg plasmid/μl. Remaining particles were removed by centrifugation and the DNA was stored at −20°C in 5 or 10 μg aliquots.

Human embryonic kidney (HEK) 293 cells (ATCC CRL-1573) were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37°C and 5% CO_2 in a humidified atmosphere. For transfection, cells were harvested at approx. 90% confluence by treatment with trypsin/EDTA and resuspended in electroporation buffer (50 mM K_2HPO_4, 20 mM K-acetate, pH 7.35) to a density of approx. 8×10^5 cells/ml. $MgSO_4$ was added to the cell suspension to a final concentration of 25 mM. The cell suspension (0.4 ml) was then added to purified plasmid DNA (5 μg for each GABA receptor subunit and 10 μg for S65T-GFP) and mixed. Electroporation at room temperature was performed instantly after addition of cells to DNA in 0.4 cm cuvettes at 225 V, 750 μF and 329 Ω, resulting in a mean pulse time of 25 ms. After transfection, cells were immediately seeded onto round, untreated 12 mm glass coverslips in 15 mm wells filled with 0.5 ml medium (20–30 μl cell suspension per well) and incubated at 37°C overnight.

2.2. Electrophysiological recordings

Cell were superfused with an extracellular solution containing (in mM): 162 NaCl, 5.3 KCl, 0.67 Na_2HPO_4, 0.22 KH_2PO_4, 15 HEPES and 5.6 glucose, pH 7.4 adjusted with NaOH. Successfully electroporated cells were detected by their green fluorescence using a fluoresceine filter set. More than 90% of the cells expressing green fluorescent protein were also positive for the GABAergic Cl^- channel. The patch-pipettes were pulled form borosilicate-glass with an outer diameter of 1.5 mm (Clark Electromedical Instruments, Pangbourne, England) to a resistance of 8–12 MΩ. For recordings in the outside-out configuration, the pipettes were filled with a solution containing (in mM): 140 KCl, 2 $MgCl_2$, 10 EGTA and 10 HEPES, pH 7.4 adjusted with NaOH. For on-cell measurements, pipettes were filled with extracellular solution containing the agonist. Ivermectin (containing 92.8% ivermectin B_{1a} and 7.2% ivermectin B_{1b}; Sigma, Deisenhofen, Germany) was pre-dissolved in dimethyl sulfoxide (DMSO) in a concentration of 100 mM, resulting in DMSO concentrations of maximally 0.05% in the final solution. GABA was dissolved in the extracellular solution in a concentration of 100 mM. All agonist stock solutions were prepared every day. Application of the agonists to outside-out patches was performed using a piezo-driven liquid filament switch for fast application allowing application and deapplication times of well-defined agonist concentrations in less than 0.3 ms (Franke et al., 1987). Outside-out patches were polarized to values of −30 to −50 mV, whereas for single channel recordings in the on-cell configuration, polarizations of +150 mV were necessary in order to obtain a sufficient signal-to-noise ratio. All measurements were performed at room temperature.

2.3. Data analysis and computer simulations

Currents were recorded with an EPC-9 (HEKA, Lamprecht, Germany) patch clamp amplifier at a sampling frequency of 20 kHz and stored on hard disk. Macroscopic currents were filtered with 1 kHz and single channel currents with 2 kHz. Off-line analysis of the data was performed on Pentium microcomputers. The rise time of averaged current traces was defined as the time from 10% to 90% of the peak current. To determine the optimal number of time constants, recordings were fitted alternatively with three and two time constants for higher GABA concentrations and two and one for lower GABA concentrations. Then, the deviation of the fits from the recordings was determined using the 'Minimum-χ^2 Method' (Colquhoun and Sigworth, 1995) and the fits with the lower deviations were used to define the number of time constants at the different GABA concentrations. The decay time constants of the averaged currents after rapid washout of the agonists were determined using the same fit proce-

dure as described above for the determination of the desensitisation time constants. For evaluation of single channel recordings, only measurements in which no simultaneous openings occurred were used. Currents reaching more than 66% of the maximal channel amplitude were classified as openings, whereas declines to less than 50% of the currents were classified as closures. Open and closed times were fitted using the Marquard–Levenberg Routine. All data are given as means ± s.d.

3. Results

3.1. Ivermectin activated the rat recombinant $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptor

Activation of the channel with 10 mM GABA resulted in currents rising within 1 ms to their maximal amplitudes (Fig. 1A). With this agonist concentration, the maximal open probability was achieved. Towards lower GABA concentrations, the relative current amplitudes declined in a dose-dependent manner and reached a value of 0.074 ± 0.023 with 1 μM GABA (Figs. 1B and 5B). For half-maximal activation of the receptor, an EC_{50} value for GABA of 7.05 μM was determined. The slope of the steepest part of the dose–response relationship in a double-logarithmic plot, termed Hill coefficient, gives information about the minimal number of agonist-binding sites at the receptor. For this receptor, the value was 2.2, indicating at least three binding sites for GABA (Jahn et al., 1997). The rise time of the currents increased towards lower agonist concentrations and was about 280 ms with 1 μM GABA (Fig. 1C). After reaching the maximal amplitude with 10 mM GABA, desensitisation of the channel in the presence of the agonist was observed. The time course of the desensitisation could be well fitted with three time constants of about 25, 209, and 1650 ms (Krampfl et al., 2000). Desensitisation reached a steady state at about 20–50% of the maximal current. Towards lower GABA concentrations, the extent of desensitisation and the number of time constants declined. At GABA concentrations < 1 mM, desensitisation was fitted with two and < 10 μM with one time constant. After eliciting currents with GABA concentrations ≤ 3 μM, no more desensitisation was detected.

Activation of the channel with ivermectin did not result in desensitisation even at prolonged application times of the agonist for 3 s (Fig. 1A). The maximal current amplitude, achieved with 20 μM ivermectin, reached a value of 0.96 ± 0.37 of that observed with 10 mM GABA. The decline of the current amplitude towards lower ivermectin concentrations was dose-dependent and with 0.1 μM ivermectin, a relative current amplitude of 0.09 ± 0.03 was found (Fig. 1B). The EC_{50} value of 2.3 μM for half-maximal activation with ivermectin was lower than the one

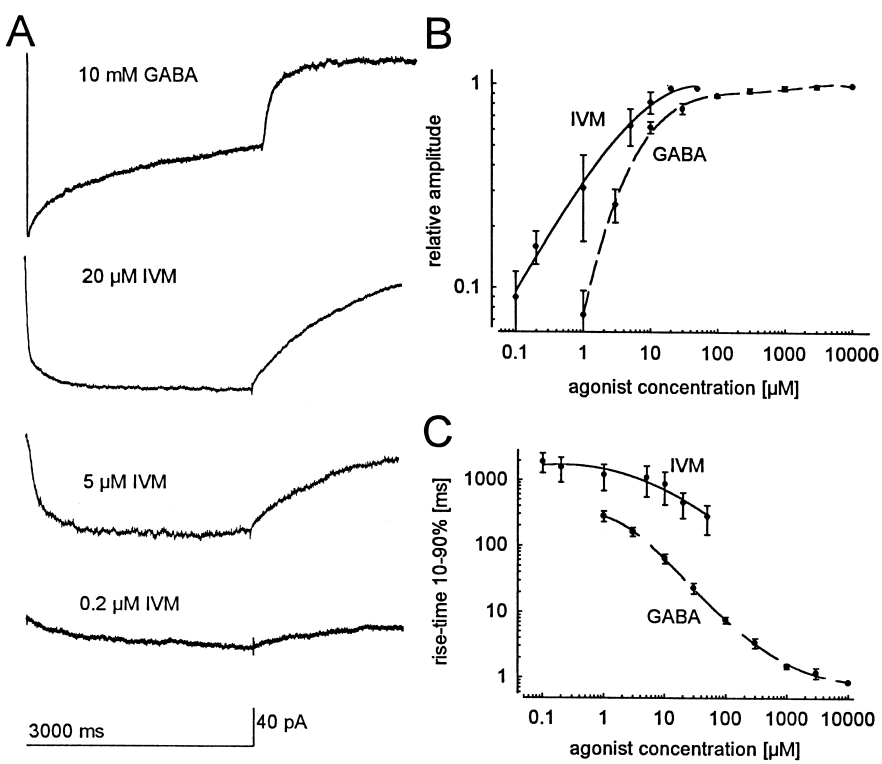

Fig. 1. Ivermectin activated the $\alpha_1\beta_2\gamma_{2S}$ GABAergic receptor. (A) Averaged current traces (10–30 each) of an outside-out patch activated with 10 mM GABA, 20, 5, and 0.2 μM ivermectin. (B) Relative current amplitudes after activation of the channels with different GABA (dashed) and ivermectin (solid) concentrations (Double-logarithmic plot). (C) Current rise times after activation of the channels with different GABA (dashed) and ivermectin (solid) concentrations (Double-logarithmic plot). IVM = ivermectin.

determined with GABA as the agonist. When the channel was activated with ivermectin, the slope of the steepest part of the dose–response relationship was 1.7 indicating two agonist-binding sites, one less than for GABA. The rise times of ivermectin-activated currents were much longer than those of GABA-activated currents and increased from 450 ± 190 ms with 20 μM to 1920 ± 642 ms with 0.1 μM ivermectin (Fig. 1C).

The decline of the current after rapid washout of GABA was biexponential with mean time constants of $\tau_{decay1} = 174 \pm 38$ ms and $\tau_{decay2} = 515 \pm 90$ ms, respectively, using 10 mM GABA (Fig. 2). At lower GABA concentrations, the values of both time constants declined. The short time constant reflects the burst length of the channel determined by the relation of the dissociation rates of the agonist to the rates into the openings. After washout of the agonist when the receptor recovers from the desensitised states, openings occur before dissociation of GABA (Haas and Macdonald, 1999). The decay time of the current elicited by these openings is determined by the rates out of the desensitised states generating the longer time constant. When switching off 10 μM ivermectin, the decay of the current was monoexponential with a time constant of $\tau_{decay} = 1019 \pm 374$ ms ($n = 38$). With ivermectin, no concentration-dependent shift of the decay time constant was seen.

3.2. The single channel kinetics after activation with GABA or ivermectin showed differences

To avoid superimposed openings, single channel measurements in the on-cell configuration were performed at low agonist concentrations. The channel exhibited at least three subconductance states with a main conductance state of 25 ± 2.1 pS ($n = 5$). The two smaller subconductance states showed conductances of 33% and 66% of the main conductance states. No differences with respect to the distributions or the conductances of the states were obvious after activation with GABA or ivermectin. With either agonist, the openings occurred in groups divided by longer closed periods (Fig. 3). After activation of the channel with 0.1 μM GABA, the groups of openings lasted from about 50 to several hundred milliseconds (Fig. 3A). At a higher time resolution, it was obvious that the openings are arranged in subgroups containing one to several openings divided by closures of a few milliseconds. The closed periods between these subgroups had lengths in the range of 10 to a few hundred milliseconds.

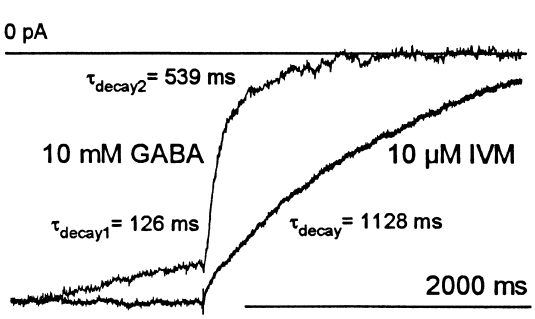

Fig. 2. Decay of the currents after rapid washout of 10 mM GABA and 10 μM ivermectin. IVM = ivermectin.

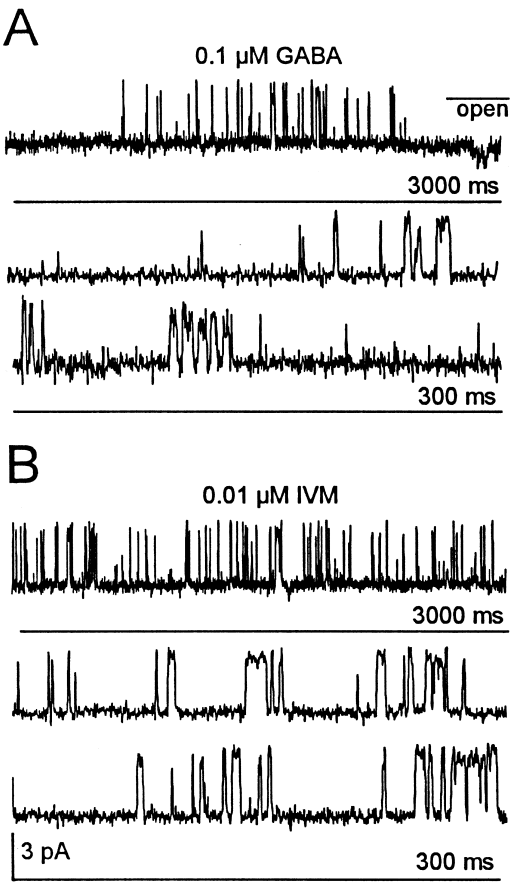

Fig. 3. Current traces of single channel openings recorded on-cell at a polarization value of +150 mV and a filter cut-off frequency of 2 kHz using (A) 0.1 μM GABA and (B) 0.01 μM ivermectin as the agonist. The upper traces show channel activity over a time of 3000 ms whereas in the lower traces the time scale is enlarged to 300 ms. IVM = ivermectin.

Activation of the channel with 0.01 μM ivermectin also resulted in groups of openings but with lengths up to several seconds being remarkably longer than the groups elicited by GABA (Fig. 3B). At a higher time resolution, again, subgroups containing one to several openings divided by closed periods of different lengths could be observed. Open time histograms of single channel openings could both be well fitted by two time constants with values of $\tau_{O1} = 0.29 \pm 0.11$ ms and $\tau_{O2} = 2.72 \pm 0.74$ ms ($n = 5$) after activation with 0.1 μM GABA and $\tau_{O1} = 0.39 \pm 0.14$ ms and $\tau_{O2} = 3.73 \pm 2.48$ ms ($n = 5$) after activation with 0.01 μM ivermectin (Fig. 4A). No statistically significant differences (Student's *t*-test, *p*-value ≤ 0.01) with respect to the values of the two time constants and the relative proportions τ_{O1}/τ_{O2} could be detected after activation with the two different agonists.

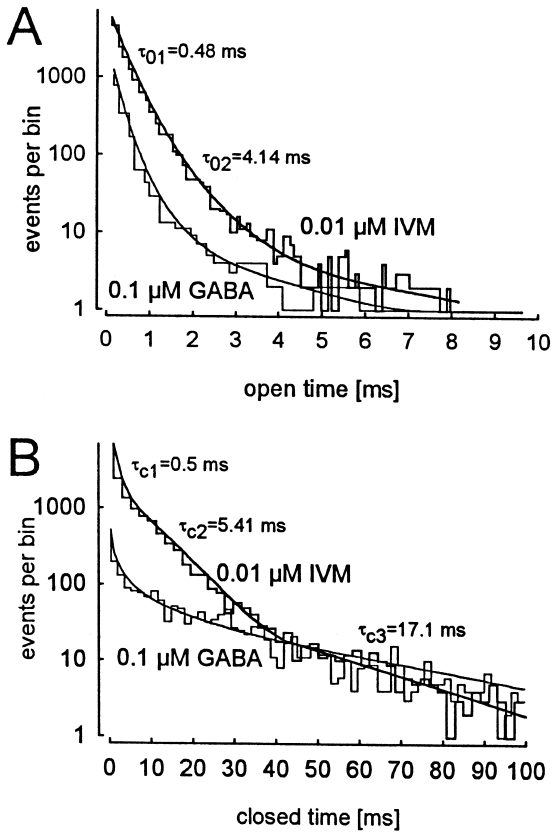

Fig. 4. Open and closed time histograms of single channel currents elicited by 0.1 μM GABA (thin lines) and 0.01 μM ivermectin (thick lines). The values of the time constants correspond to the fits of the data generated after activation with ivermectin. Semi-logarithmic plots. (A) Open time histograms. Binwidth: 0.2 ms. (B) Closed time histograms. Binwidth: 2 ms. IVM = ivermectin.

The respective closed time histograms generated from recordings with the two agonists shared a short time constant with values of $\tau_{c1} = 0.52 \pm 0.19$ ms ($n = 5$) after activation with GABA and $\tau_{c1} = 0.69 \pm 0.25$ ($n = 5$) after activation with ivermectin (Fig. 4B). Also, no statistically significant difference (Student's *t*-test, *p*-value ≤ 0.01) could be detected for the second closed time constants of $\tau_{c2} = 3.46 \pm 2.5$ with GABA and $\tau_{c2} = 5.25 \pm 2.5$ with ivermectin. The presence of additional closed time constants varied from recording to recording and was probably due to different numbers of channels on the patches.

3.3. Ivermectin does not result in potentiation of GABA-elicited currents after co-application

To investigate possible co-agonistic actions of ivermectin, co-application experiments were performed. In Fig. 5A, the typical current trace after activation with 10 mM GABA and the current trace observed after co-application of saturating GABA and ivermectin concentrations are shown. After the initial rise of the current induced by GABA, desensitisation of the receptor in the presence of GABA started within 1 ms. During the initial phase of desensitisation after a few hundred milliseconds, the current rose again to the maximal amplitude initially achieved by GABA after 1 ms. The current from then on was stable and no desensitisation occurred throughout the whole co-application time of 3000 ms. The decline of the current after rapid washout of both agonists showed the same time constants as observed for currents elicited by 10 mM GABA alone. On lowering the ivermectin concentrations co-applied with 10 mM GABA, the effect of reduced desensitisation declined and after co-application of ivermectin concentrations below 1 μM, the elicited currents were indistinguishable from currents elicited with 10 mM GABA alone. For this effect, a half-maximal ivermectin concentration of about 10 μM was determined.

Activation of the channel with 10 μM GABA resulted in currents desensitising with two time constants of about 515 and 4113 ms (Fig. 5B; Krampfl et al., 2000). With this agonist concentration, a current amplitude relative to that with 10 mM GABA of 0.62 ± 0.04 was achieved. Applica-

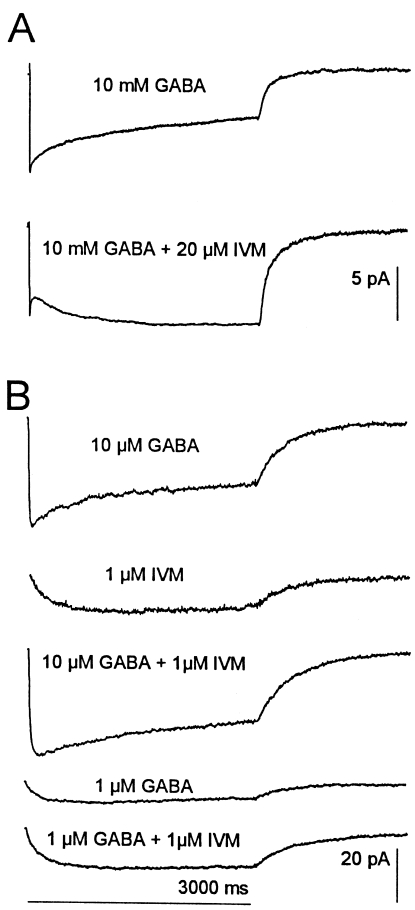

Fig. 5. Co-application of GABA and ivermectin did not result in potentiation of the current. (A) Averaged current traces (10–30 each) after activation of an outside-out patch with 10 mM GABA, 10 mM GABA together with 20 μM ivermectin, and 10 mM GABA together with 1 μM ivermectin. (B) Activation of another outside-out patch with 10 μM GABA, 1 μM ivermectin; 10 μM GABA together with 1 μM ivermectin, 1 μM GABA and 1 μM GABA together with 1 μM ivermectin. IVM = ivermectin.

tion of 1 μM ivermectin elicited a relative current amplitude of 0.31 ± 0.14. Consequently, a co-activating action of ivermectin should result in a relative current amplitude higher than 0.62 using concentrations of 10 μM GABA and 1 μM ivermectin. The current amplitude detected after co-application of both agonists in these concentrations was not significantly higher than the one detected after application of 10 μM GABA alone and followed the time course determined by 10 μM GABA with a decay time slightly longer than the one observed after washout of GABA alone. Application of 1 μM GABA resulted in non-desensitising currents with a maximal relative amplitude of 0.074 ± 0.023. This relative open probability is lower than the respective one of 0.31 achieved with 1 μM ivermectin. After co-application of the two agonists in these concentrations, the resulting current showed the same amplitude and the same rise time elicited by application of 1 μM ivermectin alone. Also, the decays of the currents after washout of both agonists were determined by the agonist eliciting the higher open probability. On further reduction of the ivermectin concentration down to 0.1 μM applied together with 1 μM GABA, no co-agonistic effect of ivermectin could be detected. With all concentrations tested, the resulting current after co-application of GABA and ivermectin was always determined by the concentration of the agonist eliciting the higher relative amplitude.

3.4. Channels consisting of $\alpha_1\beta_2$- or $\alpha_1\gamma_{2S}$-subunits could also be activated by ivermectin

In vivo, the majority of the $GABA_A$-receptors is composed of three different subunits, predominantly $\alpha_x\beta_x\gamma_x$. Recombinant receptors containing only two subunits, namely $\alpha_x\beta_x$ or $\alpha_x\gamma_x$, can also form functional Cl^- channels (Angelotti and Macdonald, 1993; Granja et al., 1997). Receptors consisting of $\alpha_1\beta_2$ and $\alpha_1\gamma_{2S}$ were tested for their ivermectin sensitivity. The density of functional binary receptors on HEK cells was only about 10% of the density of the receptor containing three subunits. Activation of the $\alpha_1\beta_2$-receptor with 10 mM GABA resulted in fast rising currents desensitising to about 40% of the maximal amplitude (Fig. 6A). With 20 μM ivermectin, the same maximal current amplitude could be achieved ($n = 4$) suggesting that the γ-subunit is not necessary for ivermectin sensitivity of GABA receptors.

Receptors composed of $\alpha_1\gamma_{2S}$-subunits also showed a fast rising current but desensitised to less than 40% of the maximal current amplitude (Fig. 6B). At the end of the GABA pulse large reopening currents were visible suggesting an open channel block by GABA. Application of 20 μM ivermectin to these receptors again resulted in activation to the same maximal current amplitude as achieved with 10 mM GABA ($n = 3$). Therefore, also the β-subunit seems not to be responsible for the ivermectin sensitivity of the receptor. The rise time of the current after application of ivermectin was about 10-fold longer with the $\alpha_1\gamma_{2S}$-channel compared to the $\alpha_1\beta_2$-channel indicating different kinetics for ivermectin activation.

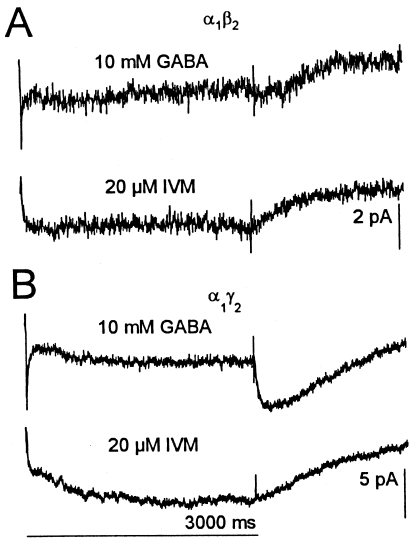

Fig. 6. Receptors consisting of $\alpha_1\beta_2$- or $\alpha_1\gamma_{2S}$-subunits were also sensitive for ivermectin. (A) Averaged current traces (10–30 each) after activation of receptors consisting of $\alpha_1\beta_2$-subunits with 10 mM GABA (upper trace) and 20 μM ivermectin (lower trace). (B) Same experiment as in A with receptors consisting of $\alpha_1\gamma_{2S}$-subunits. IVM = ivermectin.

4. Discussion

4.1. Mechanism of the activating action of ivermectin

Ivermectin activated the GABAergic receptor consisting of the subunits $\alpha_1\beta_2\gamma_{2S}$ as an agonist resulting in different kinetic properties compared to activation of the receptor by GABA. Due to the Hill coefficient of 2.2, determined from the dose–response relationship for GABA, three agonist-binding sites had to be assumed. In contrast, a Hill coefficient of 1.7 was determined from the dose–response relationship for ivermectin, suggesting a minimal number of two binding sites for this agonist. The current rise time with 20 μM ivermectin was about 500 times longer than the respective one after application of the 500-fold concentration of GABA (10 mM) (Fig. 1). Therefore, the rates for binding of ivermectin to the receptor had to be in the same range as the ones for GABA, probably somewhat lower due to one binding step less. The decay time constant after washout of ivermectin is determined by the dissociation rate of the agonists from the receptor. Consequently, to achieve a decay time constant of about 1000 ms, the rate for dissociation of ivermectin from the receptor must be lower than the one for GABA with which the dissociation rate determines the fast decay time constant, τ_{decay1} (Fig. 2). Besides the lengthening of the decay times, a lower rate for dissociation is also necessary to achieve the open probability determined for ivermectin being in the same range as the respective one determined with a 500 times

higher GABA concentration. Since there were no significant differences in the open time histograms showing two open time constants with GABA and ivermectin, at least two different open states, a short and a long one, had to be suggested for both agonists. The rates for opening and closing had to be in the same range for GABA and ivermectin. A principal difference in the kinetic properties detected on activation with ivermectin is that the receptor did not enter desensitised states in contrast to activation by high and intermediate GABA concentrations, falling in line with the observation that the decay time constant with ivermectin alone was monoexponential (Fig. 2). The longer decay time constant, τ_{decay2}, after washout of GABA, is generated by the rates out from the desensitised states entered on activation with GABA.

No co-agonistic effect of ivermectin was detected. Therefore, an independent action with distinct binding sites for GABA and ivermectin at the receptor seems likely. This suggestion is supported by the observation that on co-application of GABA and ivermectin in subsaturating concentrations, the amplitude and time course of the elicited current is determined by the agonist applied in a concentration resulting in a higher current amplitude when applied alone (Fig. 5B). Binding sites distinct from the ones for GABA are also proposed for a lot of other modulatory substances, like barbiturates, benzodiazepines, and steroides (Sieghart, 1995). The effect of abolished desensitisation after co-application of saturating GABA and ivermectin concentrations seemed to be caused by a different mechanism, distinct from the activating one. For this effect, a half-maximal ivermectin concentration of 10 μM was determined in contrast to 2.3 μM for the agonistic effect. Probably there are additional binding sites at the receptor preventing it to enter desensitised states after occupation with ivermectin. In co-application experiments with saturating concentrations of GABA and ivermectin, initial, fast desensitisation of the current was interrupted and subsequently abolished in the presence of ivermectin (Fig. 5A). One could think that ivermectin, with its slower activation, is able to withdraw receptors already occupied by GABA from their desensitised states and to activate them by the agonistic action of ivermectin. In this case, the decay time constant after washout of GABA and ivermectin should be lengthened towards the range observed for application of ivermectin alone (Fig. 1). But in the recordings (Fig. 5A), 10 mM ivermectin did not lengthen the decays when co-applied with 10 mM GABA, supporting the suggestion that this effect is caused via a different mechanism with which ivermectin only prevents the receptors from desensitisation whereas the activation is carried by GABA.

4.2. Comparison with other studies

In the present study, the agonistic action of the insecticide and anthelmintic ivermectin on the rat recombinant $\alpha_1\beta_2\gamma_{2S}$ GABAergic Cl^- channel was investigated at the kinetic level. Former investigations of the actions of ivermectin on GABAergic receptors have been performed on in situ preparations with unknown subunit compositions. Therefore, direct comparisons are difficult since the huge number of subunits found to form GABAergic Cl^- channels result in receptors with different kinetics and pharmacology. Direct activation of Cl^- channels was observed in rat hippocampal neurons (Schönrock and Bormann, 1983). Activation there occurred within 10 s and for washout of the transmitter, 20 min was necessary. Apart from a different receptor type activated in this study, the application to whole cells with interaction of the extremely lipophilic substance with the preparation could also be responsible for these very long washout times. In the present study, ivermectin was applied to outside-out patches using a fast application system in a special recording chamber with a high flow rate of the background solution to ensure rapid changes of the applied solutions. On chick neuronal GABAergic receptors expressed after injection of total mRNA preparations, only a co-agonistic action of ivermectin was described (Sigel and Baur, 1987). It is not known why no currents could be elicited in the absence of GABA but interestingly, inhibition of desensitisation by ivermectin was also observed there. More investigations preferably with molecularly defined receptors have to be performed to enlighten all actions of ivermectin on this group of ligand-gated ion channels.

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