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Effects of propofol on recombinant AMPA receptor channels

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Abstract

The interaction of the anaesthetic propofol with recombinant human AMPA-type glutamate receptor channels was investigated by a patch-clamp study using fast agonist application techniques. Despite the marked effects of propofol on inhibitory synaptic transmission and voltage gated sodium channels, there is also evidence for a specific pharmacological action on AMPA receptors. In our study, we observed a deceleration of AMPA receptor channel desensitization in the prolonged presence of glutamate and propofol that is likely to account for the enhancement of ion currents through AMPA receptor channels observed in previous studies. While there was an increase in the rate and extent of desensitization at glutamate receptor 1, glutamate receptor 2, and glutamate receptor 3 AMPA receptors, no affection of current rise time, peak current amplitude, and deactivation properties was observed. Thus, our findings point to an isolated interaction with processes that control desensitization of AMPA receptor channels rather than indicating an interaction with channel opening and closing processes due to agonist binding and unbinding. The pharmacological effect described resembles in part that of compounds like cyclothiazide and aniracetam which are known to interact with channel desensitization.

Keywords: Human AMPA-type receptor; Desensitization; Propofol; Patch-clamp; Fast solution exchange

1. Introduction

The intravenous general anaesthetic propofol acts on a broad variety of molecular targets, like most general anaesthetics are thought to (for review see Franks and Lieb, 1994). A strong and clinically relevant interaction with ligand-gated as well as voltage operated ion channels has been described. Concerning ligand-gated ion channels, the main inhibitory receptor channels ($GABA_A$ receptors and glycine receptors) at the synapses of the brain and spinal cord, were investigated in previous studies (Ahrens et al., 2004; Antkowiak, 1999; Hales and Lambert, 1991; Laube et al., 2002; Mohammadi et al., 2001; Pistis et al., 1997). In an early study, Yamakura et al. (1995) observed differentiated effects of propofol on recombinant glutamate receptor channels. While there was a slight depression of *N*-methyl-D-aspartate (NMDA) receptor currents in the presence of propofol, currents through α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor channels were modulated depending on the AMPA receptor subtype investigated. The current responses of homomeric glutamate receptor 1 channels were enhanced 1.55 fold by 1 mM propofol in this study, while there was a slight depression of currents through glutamate receptor 1–glutamate receptor 2 channels observed at 1 mM propofol, but not at lower concentrations. It has to be noted that slow perfusion techniques were used that allow for steady state measurements but not for the analysis of channel kinetics in the presence of modulators. There was no shift of the dose–response curve of glutamate observed in this study. Thus, the changes of the relative current amplitudes in the presence of propofol cannot be interpreted as changes of the affinity of glutamate to its binding site by propofol. In a study on the modulatory action of propofol on the effects of various chemoconvulsants in vivo, Bansinath et al. (1995) observed that propofol facilitated glutamate mediated effects and acted

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more potently at AMPA related mechanisms than at mechanisms involving kainate or NMDA glutamate receptor subtypes.

AMPA-type receptor channels are the most abundant excitatory ligand-gated receptor channels of the vertebrate central nervous system for fast synaptic transmission. Four cDNA clones of AMPA receptor subunits (glutamate receptor 1–4) have been isolated by molecular cloning (Hollmann and Heinemann, 1994). The molecular diversity of AMPA receptors is increased by alternative splicing and posttranscriptional nuclear RNA editing. Glutamate receptor 1–4 subunits exist in two different forms generated by alternative splicing. They are characterized by the fastest gating kinetics known for ligand-gated ion channel receptors and account for the fast component of excitatory postsynaptic currents. AMPA receptor channel subtypes show fast desensitization with time constants ranging from below 2 ms to not more than 6 ms when studied by fast agonist application techniques (Grosskreutz et al., 2003; Koike et al., 2000; Krampfl et al., 2002a; Lomeli et al., 1994; Mosbacher et al., 1994). Thus, for the investigation of the effect of propofol on AMPA receptor channel kinetics it was crucial to achieve agonist application on the microsecond time-scale. By this approach, the determination of the time course of the development and decay of fast current transients elicited by the agonist glutamate provides a direct measure for kinetic processes like desensitization and deactivation (Franke et al., 1987; Wyllie et al., 1998).

2. Material and methods

2.1. Transient expression of human recombinant AMPA receptors

Human glutamate receptor 1 flop, glutamate receptor 1 flip, glutamate receptor 2 flip GQ (with glycine "G" at the R/G editing site and glutamine "Q" at the Q/R editing site), and glutamate receptor 3 flop cDNAs (a kind gift from Dr. Höger and Dr. Lemaire, BASF, Ludwigshafen, Germany) subcloned each in pcDNA3 expression vectors (Invitrogen, Groningen, The Netherlands) were used for transfection as reported previously (Krampfl et al., 2001). Transformed human embryonic kidney (HEK) 293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5% CO_2/95% air incubator. They were plated on 12 mm glass coverslips coated with poly-L-lysine. Cells were suspended in a buffer used for transfection (in mM: 50 K_2HPO_4, 20 K-Acetate, pH 7.35). For transient transfection of human AMPA receptors, the corresponding cDNAs were added to the suspension (25 μg/ml). A reporter cDNA encoding enhanced green fluorescent protein (EGFP, 10 μg/ml) was used to visually identify transfected cells. For transfection we used an electroporation device by EquiBio (Middlesex, UK). Transfected cells were replated on glass coverslips in DMEM containing 10% FCS and incubated for at least 15 h.

2.2. Electrophysiological recording of macroscopic currents from excised outside-out patches

Patch-clamp measurements were performed on outside-out excised patches for fast application experiments using standard methods (Hamill et al., 1981). Patch pipettes were pulled from borosilicate glass tubes and fire-polished with a horizontal DMZ pipette puller (Zeitz Instruments, Augsburg, Germany). Pipette series resistance was 8–12 MΩ when filled with intracellular solution containing (in mM): 140 KCl, 11 EGTA, 10 HEPES, 10 glucose, 2 $MgCl_2$. The osmolarity was adjusted to 340 mosml^{-1} with mannitol. HEK293 cells were superfused with an extracellular solution containing (in mM): 162 NaCl, 5.3 KCl, 2 $CaCl_2$, 0.67 NaH_2PO_4, 0.22 KH_2PO_4, 15 HEPES, 5.6 glucose. The pH of both solutions was adjusted to 7.3. Sodium-L-glutamate (monohydrate) was obtained from Merck (Eurolab, Darmstadt, Germany).

Data were recorded with an Axopatch 200B patch-clamp amplifier (Axon Instruments, Union City, CA, USA). Ensemble currents were sampled with 20 kHz using a Digidata 1200 Interface and the pCLAMP6 software suit on a PC (Axon Instruments, Union City, CA, US). For further analysis data were filtered at 5 kHz. The holding potential was kept between −40 and −80 mV; in this range, it had no influence on the current kinetics. For statistical analysis the independent Student's two-tailed *t*-test was used. Differences were considered significant at the $p<0.05$ level. All data were given as mean±S.E.M. (n, number of experiments).

2.3. Fast application technique

A piezoelectric-driven theta-glass application pipette was used for application of the neurotransmitter glutamate (10 mM) to excised outside-out membrane patches as previously described (Krampfl et al., 2002a). The piezoelectric translator was from Physik Instrumente (Waldbronn, Germany) and the theta-glass tubing from Hilgenberg (Malsfeld, Germany). Solution delivery through the theta-glass pipette was driven by air pressure. The time for solution exchange was regularly <100 μs as estimated by measurements of liquid junction potentials with a 10-fold difference in ionic strength (Krampfl et al., 2002b). Glutamate was applied with pulses of 1 ms or 50 ms duration with intervals of 30 s between two successive 1 ms or 50 ms pulses. The time for complete exchange of the background and glutamate solution to propofol containing solutions took around 10 s. Typical application protocols were designed with alternating application of test solutions/bath solution containing propofol, and 10 mM glutamate pulses without propofol. By the use of the theta-glass system in combination with a double-barrel solution reservoir connected to each bore of the theta glass, we could easily control both test

and bath solution and perform concentration jump experiments without any constraints. Glass and polytetrafluoroethylene were used as materials for the perfusion system. For the quantitative evaluation 4–12 current traces for each experiment were averaged.

2.4. Solutions

Purified 2,6 diisopropylphenol (propofol) was purchased from Sigma (Deisenhofen, Germany). Propofol was prepared as 1 M stock solution in ethanol, light protected and stored in glass vessels at −20 °C. Concentrations were calculated from the amount injected into the glass vials. Drug-containing vials were vigorously vortexed for 60 min (Ahrens et al., 2004). Glutamate was dissolved directly in the bath solution.

3. Results

In the experiment of Fig. 1, 10 mM glutamate was applied to outside-out patches containing glutamate receptor 2 flip GQ channels. Fast current transients with a 20–80% rise time of 0.3 ms were observed with peak current amplitude of −210 pA. In the prolonged presence of glutamate, the current amplitude decayed due to desensitization to a steady state current amplitude of −11.0 pA. The time course of desensitization was fitted with a single exponential τ_{Des}=4.8 ms. Upon removal of glutamate, fast deactivation due to unbinding of glutamate was observed. When 1 mM propofol was added to the glutamate containing test solution, the peak current amplitude declined slightly to −205 pA while desensitization rate and extent decreased. Thus, τ_{Des} increased to 5.9 ms and the steady state current amplitude in the prolonged presence of glutamate and propofol was −17.2 pA. To test if the effect of propofol can be enhanced by preincubation of the patches with propofol, i.e. by an interaction of propofol with the receptor channels in the unliganded state, we added 1 mM propofol to the bath solution, before application of the test solution containing 10 mM glutamate+1 mM propofol. Under these conditions, the peak current amplitude was −207 pA and τ_{Des} decreased to 6.4 ms. No significant change of the steady state current amplitude occurred.

Upon pulse-wise application of 10 mM glutamate to membrane patches containing recombinant glutamate receptor channels, current transients were recorded with maximum peak current amplitudes of −186.5±53.8 pA (*n*=28) for glutamate receptor 1 flip, −74.9±37.5 pA (*n*=7) for glutamate receptor 1 flop, −92.1±13.7 pA (*n*=49) for glutamate receptor 2 flip GQ, and −156.6±45.3 pA (*n*=11) for glutamate receptor 3 flop. On average, the maximum peak current amplitudes of glutamate receptor 1 flip, glutamate receptor 1 flop, glutamate receptor 2 flip GQ, and glutamate receptor 3 flop receptor channels did not change significantly, neither when propofol was co-applied with glutamate nor when both bath solution and glutamate containing solution contained 1 mM propofol (Fig. 2A). Similarly, we observed no significant effect on the current rise time of either glutamate receptor subtype tested.

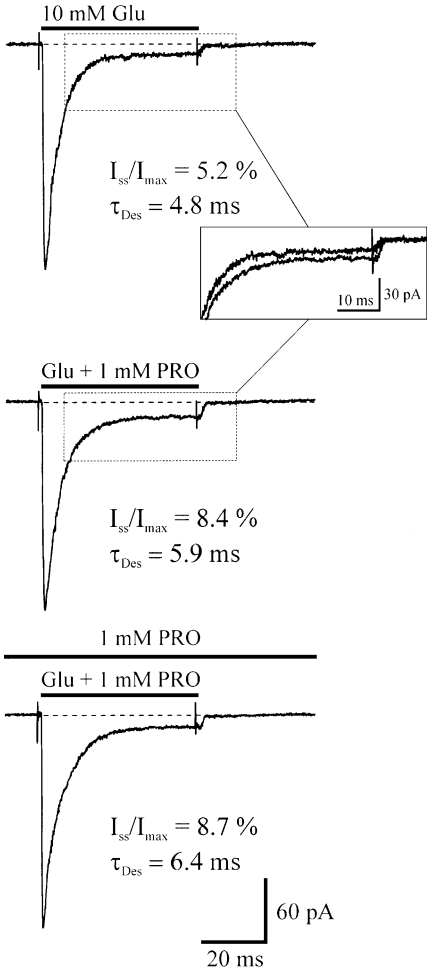

Fig. 1. Current responses of glutamate receptor 2 flip GQ channels after fast application of 10 mM glutamate to an outside-out patch (upper current trace). Time constant of desensitization (τ_{Des}) and relative steady state current (I_{ss}/I_{max}) as given next to the respective current trace. The interrupted line indicates the baseline current level for better estimation of the steady state current amplitude. The smooth grey curves next to the current traces show the respective fits. The inset between the upper and middle current trace shows the overlay of the steady state current in presence or absence of 1 mM propofol on an expanded scale. When 1 mM propofol was added to the glutamate containing test solution, desensitization slowed and the steady state current increased (middle trace). Upon exchanging bath solution to 1 mM propofol containing bath solution no significant further changes of current responses were observed (lower trace). Membrane potential was held at −60 mV.

However, as shown in the experiment of Fig. 1, there was a slight but significant effect on desensitization of glutamate receptor channel currents. Without application of propofol, τ_{Des} was 2.7±0.2 ms (*n*=19) at glutamate receptor 1 flip channels, 2.9±0.6 ms (*n*=7) at glutamate receptor 1 flop channels, 6.1±0.2 ms at glutamate receptor 2 flip GQ channels (*n*=49), and 2.8±0.1 ms (*n*=18) at glutamate receptor 3 flop channels. In the presence of 1 mM propofol,

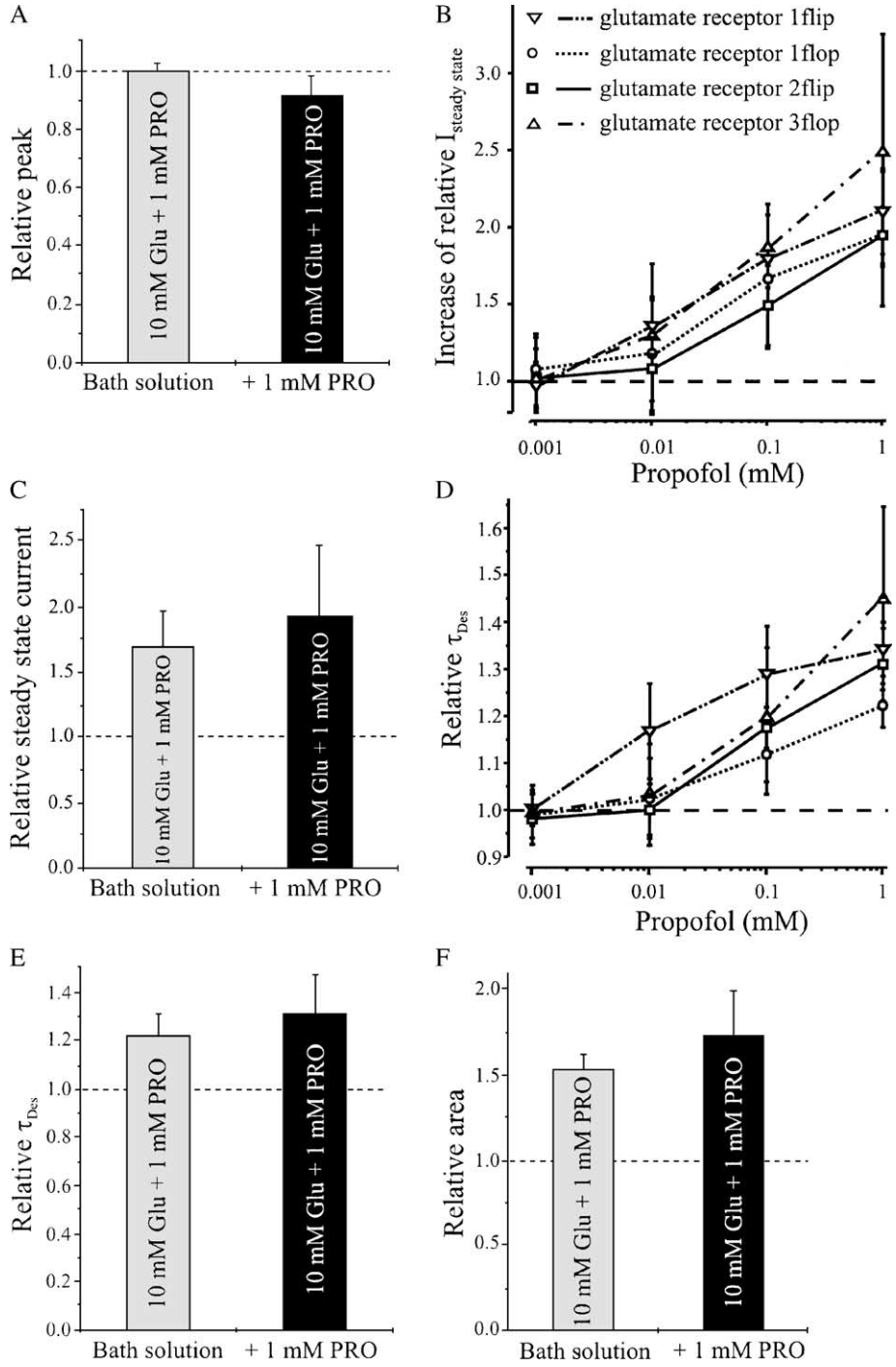

Fig. 2. The diagrams show the main results of fast agonist application experiments with outside-out patches containing glutamate receptor 2 flip GQ channels. The left, light grey column shows the results of the application of 10 mM glutamate+1 mM propofol compared to pulse-wise application of 10 glutamate alone. The black column shows the results when both the glutamate solution and the bath solution contain 1 mM propofol. There were no significant differences between each pair of columns but a tendency to a more pronounced effect especially on relative steady state currents and relative areas. Relative values were normalized to the mean of the control response before and after the respective applications in the presence of propofol (control values indicated by interrupted lines for clarity). (A) Relative peak current amplitudes were not affected significantly. (B) Dose dependency is shown for the relative steady state current amplitude. (C) The respective values for the relative steady state current amplitude at 1 mM propofol. (D) The time constant of desensitization, τ_{Des}, increased with increasing propofol concentration (coapplication data shown). (E) The respective values for τ_{Des} at 1 mM propofol. (F) The increase in the normalized area under the current trace indicates that there is a valuable increase in ion current flow in the presence of 1 mM propofol.

desensitization slowed down to relative values of 1.34±0.11 (n=8), 1.22±0.04 (n=5), 1.31±0.05 (n=8), and 1.45±0.20 (n=5), at glutamate receptor 1 flip, glutamate receptor 1 flop, glutamate receptor 2 flip GQ, and glutamate receptor 3 flop channels, respectively. The increase of the time constant of desensitization, τ_{Des}, reached significance

(p<0.05) at a propofol concentration 0.1 mM for glutamate receptor 1 flip and glutamate receptor 2 flip GQ. For glutamate receptor 1 flop and glutamate receptor 3 flop a tendency towards slower desensitization emerged at 0.1 mM propofol, a significantly slower desensitization was observed at 1 mM propofol. The quality of the fit did not improve when it was performed with two or more exponentials.

Desensitization was completed within 50 ms pulses of 10 mM glutamate. To compare the extent of desensitization without an influence of the different desensitization time constants of the glutamate receptor subtypes investigated, we estimated the extent of desensitization by extrapolation of the exponential fits to infinite time. Normal values of relative steady state current amplitudes were 1.4±0.1% at glutamate receptor 1 flip channels, 1.2±0.2% at glutamate receptor 1 flop channels, 5.8% at glutamate receptor 2 flip GQ channels and 1.4±0.2% at glutamate receptor 3 flop channels. There was a dose-dependent increase in the relative steady state current amplitude at the glutamate receptor subtypes tested. In the presence of 1 mM propofol a relative steady state current amplitude of 1.4±0.1% at glutamate receptor 1 flip channels, 1.2±0.2% at glutamate receptor 1 flop channels, 5.8±0.5% at glutamate receptor 2 flip GQ channels and 1.4±0.2% at glutamate receptor 3 flop channels was observed. Thus, the relative values of the steady state proportion of glutamate receptor currents in the presence of 1 mM propofol compared to control were 2.1±0.3 (n=16), 1.9±0.4 (n=3), 1.9±0.4 (n=19), and 2.5±0.8 (n=4) at glutamate receptor 1 flip, glutamate receptor 1 flop, glutamate receptor 2 flip GQ, and glutamate receptor 3 flop channels, respectively.

We focused in our study especially on glutamate receptor 2 flip GQ channels for several reasons: since the main effect of propofol was an increase of the very low steady state level of glutamate receptor currents, we investigated glutamate receptor 2 flip channels, which had relative steady state current amplitudes of about 6% of maximum peak current amplitudes. Therefore, the relative steady state current amplitude was easily measurable in the outside-out patch experiments of our study. Another reason was the difference in the effects of propofol acting on glutamate receptor 2 containing receptors or on glutamate receptor 1 homomers reported by Yamakura et al. (1995).

The increase of the steady state current in presence of propofol was dose-dependent as shown in Fig. 2B,C for glutamate receptor 2 flip GQ channels. Significance of the increase was observed with p<0.05 at concentrations of =0.1 mM propofol for glutamate receptor subtypes tested. As can be estimated from the dose–response curve of the relative steady state current amplitude of glutamate receptor 2 flip GQ channels in presence of propofol, saturation is not reached within the dose range tested in our experiments (Fig. 2B). To get a measure for the effect of the reduced desensitization (Fig. 2D,E) on ion current flow through AMPA receptor channels, we determined the effect on the area under the current trace (Fig. 2F) as a measure of the respective ion current flow.

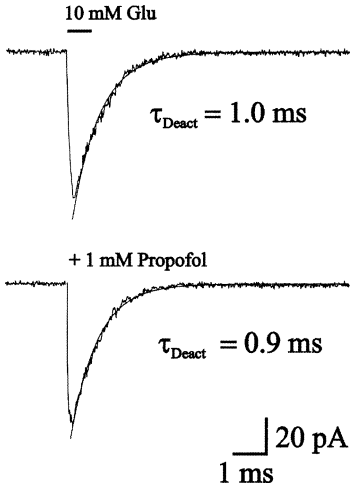

Fig. 3. Analysis of the time constant of deactivation after application of short glutamate pulses of 10 mM glutamate. Upper trace: average current traces of glutamate receptor 2 flip GQ upon application of 1 ms pulses of 10 mM glutamate. The smooth line shows the monoexponential fit of the current decay. Lower trace: after exchange of test solution and bath solution to the 1 mM propofol containing solutions similar current transients were observed (no statistically significant difference). Membrane potential was held at −60 mV.

In additional experiments conducted with glutamate receptor 1 flip channels and glutamate receptor 2 flip GQ channels we tested if there was a measurable effect of propofol on current deactivation. Deactivation describes the current decay after removal of the agonist. To test the time course of deactivation, we elicited current transients by 1 ms pulses of glutamate in the presence/absence of 1 mM propofol. In these experiments, the current decay after reaching the peak current amplitude was similar at both glutamate receptor subtypes tested. For glutamate receptor 1 flip channels, the time constant of deactivation, τ_{Deact}, was 0.91±0.10 (n=4), and for glutamate receptor 2 flip GQ channels 1.10±0.19 ms (n=7). There was no significant change in the presence of 1 mM propofol (Fig. 3).

4. Discussion

We performed a fast application patch-clamp analysis of the interaction of propofol with different subtypes of recombinant AMPA receptor channels. By this approach, a significant effect of propofol on AMPA receptor channel desensitization was revealed (Fig. 2B,C,D,E). As shown comprehensively in the diagrams of Fig. 2B,C,D,E desensitization got slower and the relative steady state current amplitude increased in presence of propofol by a factor of ~2. As a consequence, the current flow as measured by the area under the current trace increased

significantly (Fig. 2E). The effects were dose-dependent and did not reach saturation within the dose range tested (1 μM to 1 mM propofol; Fig. 2B,D). In short, we observed no essential subunit dependence of propofol. No difference was seen in the current rise time, the maximum peak current amplitudes and the deactivation rate (Figs. 1 and 3).

In particular, our results are in line with the results of Yamakura et al. (1995) concerning the increased steady state current amplitude at glutamate receptor 1 receptor channels. Since in that study bath application of agonists/drugs with very low solution exchange kinetics has been used, the currents recorded have to be considered as steady state currents and complete desensitization has to be presumed. Under these conditions, the average enhancement of the current amplitudes by a factor of 1.55 by 1 mM propofol matches the increase of the steady state current observed in our study. However, Yamakura et al. (1995) observed no valuable effect on currents elicited by kainate in case of coexpression of glutamate receptor 1 and glutamate receptor 2 subunits. Contrary to the first glance discrepancy of this observation of a missing effect on glutamate receptor 1/glutamate receptor 2 receptor channels in the previous study and a similar effect on glutamate receptor 1 and glutamate receptor 2 receptor channels in our study, the results are not contradictious. Since kainate elicits a non-desensitizing current at AMPA receptor channels there could be no interaction with desensitization. Again, this observation points to a specific interaction of propofol with glutamate receptor channels desensitization.

The benzoylperidine aniracetam, the benzothiadiazine cyclothiazide, and several related compounds are known to interact specifically with AMPA receptor channels by slowing and reducing desensitization (Bertolino et al., 1993; Johansen et al., 1995; Lawrence et al., 2003; Lei et al., 2001; Partin et al., 1996). Studies on the molecular mechanism of action of these compounds have revealed effects on channel closure due to glutamate unbinding as well as an interaction with the microscopic process that controls desensitization. Covariance of the rates of desensitization and deactivation in studies on AMPA receptor kinetics and the observation of combined modulation of both rates by compounds like cyclothiazide and aniracetam led to the assumption of some kind of coupling of desensitization and deactivation (Partin et al., 1996). However, in our study we did not observe any modulation of deactivation additionally to the reduction of desensitization. There seems to be a combination of direct and indirect effects of these compounds on the desensitization process (Lawrence et al., 2003; Partin et al., 1996). Compounds like aniracetam that enhance AMPAergic synaptic transmission are used clinically as nootropic drugs. The effect of these compounds on glutamate receptor desensitization is much more pronounced than the effects of propofol observed in our study. Accordingly, there should be hardly a contribution of the interaction of propofol with glutamate receptor desensitization to the overall clinical effect of propofol (clinically used concentrations $\ll$0.1 mM). However, the effect revealed in our study seems to account for experimental observations like that described by Bansinath et al. (1995) in their study on the interaction of propofol with the action of several chemoconvulsants.

With respect to the profile of channel interactions of propofol comprising activation, potentiation, and channel block at inhibitory $GABA_A$ and–in part–glycine receptor channels (Ahrens et al., 2004; Antkowiak, 1999; Hales and Lambert, 1991; Laube et al., 2002; Pistis et al., 1997), and block of voltage gated sodium channels (Haeseler et al., 2001; Rehberg and Duch, 1999), there is a marked concordance of the effects of propofol with that of barbiturates like pentobarbital (e.g. Krampfl et al., 2002b; Mohammadi et al., 2004; Pistis et al., 1997). Despite that concordance there is a substantial difference in the action on AMPA receptor channels. For barbiturates, a channel block like mechanism is thought do depress glutamate mediated excitatory neurotransmission within the central nervous system, whereas propofol has positive modulatory effects on AMPA receptor channels.

In conclusion, we could show in our study that there is a pharmacological interaction of propofol in the concentration range $\geq$100 μM with AMPA receptor channels. The main molecular mechanism is a reduction of the rate and extent of desensitization. Presumably, this is the result of an effect on the microscopic process of desensitization. There was no significant evidence for an interaction of propofol with channel opening or closing as indicated by unaltered responses to short pulses of agonist (Fig. 3). The clinical pharmacological effect of propofol can widely be explained by the pronounced enhancement of the inhibitory synaptic transmission and a blockade of voltage gated sodium observed with μM concentrations of propofol. However, our data are of relevance for AMPA receptor pharmacology, reveal the molecular mechanism of action underlying previous experimental observations (Bansinath et al., 1995; Yamakura et al., 1995), and add a new and structurally different compound to the group of positive modulators of AMPA receptor channels (Bufler et al., 2001; Lawrence et al., 2003; Partin et al., 1996).

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