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Block of $GABA_A$ receptor ion channel by penicillin: Electrophysiological and modeling insights toward the mechanism

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ABSTRACT

$GABA_A$ receptors ($GABA_AR$) mainly mediate fast inhibitory neurotransmission in the central nervous system. Different classes of modulators target $GABA_AR$ properties. Penicillin G (PNG) belongs to the class of noncompetitive antagonists blocking the open $GABA_AR$ and is a prototype of β-lactam antibiotics. In this study, we combined electrophysiological and modeling approaches to investigate the peculiarities of PNG blockade of GABA-activated currents recorded from isolated rat Purkinje cells and to predict the PNG binding site. Whole-cell patch-clamp recording and fast application system was used in the electrophysiological experiments. PNG block developed after channel activation and increased with membrane depolarization suggesting that the ligand binds within the open channel pore. PNG blocked stationary component of GABA-activated currents in a concentration-dependent manner with IC_{50} value of 1.12 mM at −70 mV. The termination of GABA and PNG co-application was followed by a transient tail current. Protection of the tail current from bicuculline block and dependence of its kinetic parameters on agonist affinity suggest that PNG acts as a sequential open channel blocker that prevents agonist dissociation while the channel remains blocked. We built the $GABA_AR$ models based on nAChR and GLIC structures and performed an unbiased systematic search of the PNG binding site. Monte-Carlo energy minimization was used to find the lowest energy binding modes. We have shown that PNG binds close to the intracellular vestibule. In both models the maximum contribution to the energy of ligand–receptor interactions revealed residues located on the level of 2′, 6′ and 9′ rings formed by a bundle of M2 transmembrane segments, indicating that these residues most likely participate in PNG binding. The predicted structural models support the described mechanism of PNG block.

1. Introduction

The γ-aminobutyric acid type A receptor ($GABA_AR$) is a member of the Cys-loop receptor family of pentameric ligand-gated ion channels, which also comprises excitatory nicotinic acetylcholine receptors (nAChR) and 5-hydroxytryptamine receptors, as well as the inhibitory glycine receptors (Betz, 1990; Connolly and Wafford, 2004). Cys-loop receptors consist of an extracellular domain (ECD) including agonist binding pocket and a transmembrane domain (TMD) forming an ion channel. The TMD of each subunit contains four transmembrane helixes (M1–M4) and a large intracellular loop M3–M4. Five M2 segments form the receptor pore. $GABA_ARs$ are the major inhibitory receptors in the central nervous system (CNS), formed by combination of α_{1-6}, β_{1-3}, γ_{1-3}, ρ_{1-3}, ε, π, δ or θ subunits with the predominant receptor being $\alpha_1\beta_2\gamma_2$ and with a subunit stoichiometry of 2:2:1 (Hevers and Luddens, 1998; Sieghart, 2006). Alpha and beta subunits are necessary for receptor activation by GABA and the γ subunit defines sensitivity to benzodiazepines.

The $GABA_AR$ is a target for many pharmacological compounds of different classes including benzodiazepines, barbiturates, steroids, and noncompetitive antagonists (Cascio, 2006; Sieghart, 2006). The last ones include β-lactam antibiotics, t-butylbicyclophosphorothionate (TBPS), insecticides and picrotoxin (PTX). Despite large structural diversity, these noncompetitive antagonists are believed to bind in different positions within a common "convulsant" binding pocket in the chloride channel lumen (Chen et al., 2006; Olsen, 2006). The amino acid composition of the M2 helixes determines the ion selectivity and conductance properties of the channel (Galzi et al., 1992; Keramidas et al., 2004).

A classic $GABA_AR$ blocker is the convulsant PTX that inhibits GABAergic transmission by channel blockade (Dillon et al., 1995; Newland and Cull-Candy, 1992). Accumulating evidences indicate that a PTX binding site is located within the channel pore near the cytoplasmatic end (Buhr et al., 2001; Erkkila et al., 2008; Ffrench-Constant et al., 1993). Another well-known open channel blocker of $GABA_AR$ is penicillin (Chow and Mathers, 1986; Twyman et al., 1992). While extensive efforts have been made to determine the molecular mechanism of PTX inhibition of $GABA_AR$ channels, paradoxically little

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work has been done on the mechanisms of penicillin interaction with $GABA_A$ channel.

Penicillin G (PNG) is a prototype of β-lactam antibiotics which are the antibacterial agents. However, administration of these drugs can cause toxic side effect on CNS. Epileptogenic properties of PNG were documented since the 1940s (Chow et al., 2005) and since then adverse effects of PNG were thoroughly investigated (Curtis et al., 1972). The widely accepted theory of the pathogenesis of convulsions induced by penicillin and related β-lactam compounds suggests that these drugs suppress GABAergic transmission in the CNS (Chow and Mathers, 1986). At millimolar concentrations, PNG causes a voltage-dependent open channel block of $GABA_AR$ (Fujimoto et al., 1995; Pickles and Simmonds, 1980; Twyman et al., 1992). These data suggest that PNG enters the open pore of $GABA_AR$ and then occludes it. This theory is supported by competition between PNG and PTX for suppression of $GABA_A$ receptors (Bali and Akabas, 2007). It has been demonstrated that PTX inhibits $GABA_AR$ by a noncompetitive, open channel block mechanism (Newland and Cull-Candy, 1992; Olsen, 2006; Yoon et al., 1993). Single channel recordings have shown that PNG reduced mean life-time of the GABA-activated channels in the open state rather than changed the channel conductance (Chow and Mathers, 1986; Twyman et al., 1992) indicating the drug influence on the receptor gating. However, the exact mechanism of PNG interaction with $GABA_AR$ and the site of PNG binding in $GABA_AR$ pore still remain unknown.

In this study, we combined electrophysiological and modeling approaches to investigate the peculiarities of PNG blockade of GABA-activated currents in the isolated cerebellar Purkinje cells and to build the structural model of PNG binding in the pore of $GABA_AR$. We confirm that PNG is an open channel blocker and extend the previous findings by demonstrating that PNG acts as a "sequential blocker", which prevents the channel from closing while blocked. Such mechanism of block is also known as a "foot-in-the door" effect and was described previously for acetylcholine (Adams, 1976; Neher and Steinbach, 1978), NMDA (Benveniste and Mayer, 1995; Vorobjev and Sharonova, 1994) and $GABA_A$ receptors (Kolbaev et al., 2002). We have designed a structural model of PNG binding in the $GABA_AR$ pore. The molecular modeling revealed that the maximum contribution to the energy of ligand–receptor interactions is provided by the residues located on the level of 2′, 6′ and 9′ rings formed by a bundle of M2 transmembrane segments, indicating that these residues most likely participate in PNG binding. The proposed model indicates that the binding sites of PNG and PTX are overlapping.

2. Results

2.1. Inhibition of GABA-induced currents by penicillin

All recorded Purkinje cells (n = 46) displayed responses to GABA which were modulated by PNG. Fig. 3A illustrates the inhibitory effects of penicillin on currents activated by 5 μM GABA. Penicillin (0.1–10 mM) applied together with GABA, at −70 mV suppressed the responses to GABA, measured at steady state, in a concentration-dependent manner. The effect of PNG developed quickly, and was easily reversible. By itself, penicillin (up to 10 mM) had no action on resting currents (data not shown). Application of PNG in the absence of GABA did not change the peak response of subsequent GABA applications (not shown), suggesting that the compounds can only access the binding site in the open state. When GABA receptors were activated by 5 μM GABA the IC_{50} value for PNG inhibition of GABA-induced currents was 1.1 ± 0.12 mM and Hill coefficient was 0.84 ± 0.10 ($n = 6$) (Fig. 3B).

2.2. Penicillin-induced inhibition is more potent at high vs low GABA concentrations

The degree of block produced by a fixed concentration of an open channel blocker is expected to increase with agonist concentration

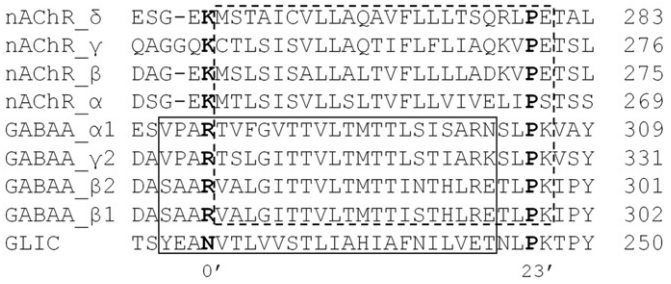

Fig. 1. Sequence alignment of M2 segment of nACh, GAB_AA and GLIC receptors. Sequences taken from the Protein Data Bank with PDB codes: nAChR 2BG9 and GLIC 2XQ3 and from the UniProt database with ascension numbers $GABA_A$_α1 P14867, $GABA_A$_β2 P47870, $GABA_A$_β1 P18505, and $GABA_A$_γ2 P18507. Sequences aligned relative to the highly conserved Arg 0′ and Lys 0′ ($GABA_A$ and nAChR) or Pro 23′ ($GABA_A$, nAChR and GLIC) residues highlighted in bold. Last residue number is shown. Dashed and solid line rectangles underline the difference in M2 amino acid composition in the $GABA_A$_nAChR and $GABA_A$_GLIC models.

because this allows the blocker increased access to its binding site (Ascher et al., 1979). The influence of agonist concentration on the extent of blockade was determined by measuring the inhibition (by 1 mM PNG) of currents evoked by increasing concentration of GABA (Fig. 4A). The amplitudes of currents were measured at the end of drug co-application. PNG inhibition was tested at GABA concentrations from 3 to 300 μM. The inhibition was GABA concentration-dependent, being larger at higher concentrations of GABA (Fig. 4B). Penicillin inhibited the steady-state component of current induced by 3 μM GABA to 75 ± 6.1% of control (n = 4), to 49 ± 2.7% for current induced by 10 μM GABA (n = 4), and to 36 ± 6.2% (n = 4) at GABA concentrations of 100 μM (Fig. 4C). The comparison of concentration–response curve for GABA in control and during co-application with 1 mM PNG shows that the blocker both inhibited the maximal GABA current and shifted dose–response curve to the left (Fig. 4B). The concentration–response curve for GABA experienced a leftward shift: from 15.5 μM in control conditions to 6.0 μM in the presence of 1 mM PNG (n = 4). The shift in GABA dose–response curves fits well to open channel blocking action of PNG.

2.3. Penicillin block at different holding potentials

To determine the voltage dependence of PNG effect, PNG was co-applied with GABA at several membrane potentials. Fig. 5A illustrates the effect of 1 mM PNG on the current elicited by 5 μM GABA at −110, −90, −70, −50, −30, −10, +10, and +30 mV. The GABA current–voltage relationship was roughly linear in the absence of PNG, but showed significant inward rectification in the presence of PNG (Fig. 5B). Thus, the block of GABA-induced currents was greater at more positive holding potentials. At 1 mM, PNG depressed stationary GABA current by 49 ± 6% at −110 mV and by 87 ± 1% at +30 mV.

A voltage-dependent effect suggests that action of a drug is located within the channel pore of the receptor. Therefore, we analyzed the results according to a simple one-site blockade model (Woodhull, 1973). The voltage dependence of the block is shown in Fig. 5C. The proportion of blocked current increased with membrane depolarization and reached the maximum of ~90% block at +30 mV. Using Woodhull model, we estimated the electrical depth of the PNG binding site in $GABA_AR$ δ = 0.39 ± 0.03 and respective $K_D(0)$ = 200 ± 20 μM (see Experimental methods).

2.4. Penicillin block prevents the channel closure and agonist dissociation

The termination of GABA and PNG co-application was followed by transient increase in the inward current ("rebound" or "tail" current) (Fig. 3A). The onset of this current coincided with the initiation of perfusion with normal extracellular solution. The amplitude and kinetics