## **GABAAR-Mediated Currents**

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Propofol or barbiturate application at low concentrations increases desensitization and slows deactivation of GABAinduced current and propofol/barbiturate at high concentrations directly elicits after-responses upon their washout in hippocampal or sensory neurons. It is postulated that the generation of such after-responses is caused by removal of the blockade by anesthetic agents as partial antagonists. However, the increased desensitization was invariably followed by slowdown of deactivation of GABA-induced current, and the after-response may arise as a consequence of extreme slowdown of deactivation following strong desensitization. It is thus possible that propofol and barbiturate can facilitate resensitization of GABA responses. Propofol and barbiturate are useful to treat the alcohol/benzodiazepine withdrawal syndrome. Considering that the slowdown of deactivation following desensitization and the after-response induced by propofol or barbiturate application, the regulatory mechanisms of desensitization/resensitization of GABA<sub>A</sub>R-mediated currents might be important for understanding the treatment of the alcohol/benzodiazepine withdrawal syndrome.

Keywords: GABA ; Desensitization ; Resensitization

# **1.**Alcohol/Benzodiazepine Withdrawal Syndrome and its Treatment by Modulating Desensitization/Resensitization Kinetics of GABA<sub>A</sub>R-Mediated Currents

Alcohol and benzodiazepine are useful to mitigate anxiety through enhancing GABA<sub>4</sub>R-mediated inhibition. However, alcohol and benzodiazepine are known as abused drugs. The alcohol or benzodiazepine withdrawal syndrome appears following a reduction on alcohol or benzodiazepine use after a period of excessive use [1][2][3][4]. The alcohol or benzodiazepine withdrawal symptoms typically include anxiety, sweating, hand tremor and sleep disturbance. The underlying mechanisms involve neuronal adaptations, which are revealed as decreased GABAergic responses <sup>[5]</sup> and enhancement of NMDA responses [6][Z][8][9]. Although the exact mechanism for the reduced responsiveness of GABA<sub>A</sub>Rs remains uncertain, changes in surface GABA<sub>A</sub>R protein level and subunit composition, changes in turnover, recycling, and production rates, degree of phosphorylation and decreased coupling mechanisms between GABA and alcohol/benzodiazepine sites are thought to be involved in the reduced responsiveness [10][11][12][13]. It has recently been demonstrated that the benzodiazepine diazepam caused downregulation of GABAergic inhibition through phospholipase C (PLC $\delta$ )/Ca<sup>2+</sup>/calcineurin signaling pathway [14]. The study showed that overexpression of PRIP-1 suppressed diazepam-dependent activation of PLC $\delta$  and diazepam-dependent downregulation of GABA<sub>A</sub>Rs in HEK293 cells <sup>[14]</sup>, indicating that PRIP-1 acts as an inhibitor by outcompeting the PLC $\delta$  binding to GABA<sub>A</sub>Rs. Because intracellular Ca<sup>2+</sup> and calcineurin activity are increased in PRIP-DKO mice <sup>[15]</sup>, these findings suggest that the diazepam-induced long-term downregulation of GABAergic inhibition is mediated by PLC $\delta$ /Ca<sup>2+</sup>/calcineurin signaling pathway. Nevertheless, it is also true that calcineurin causes resensitization of GABAAR-mediated currents by facilitating their desensitization [15][16]. Given the apparently contradictory behaviors of GABAAR-mediated currents by calcineurin activation, the two different behaviors of GABAAR-mediated currents may depend on whether calcineurin activation occurs before or after activation of GABA<sub>A</sub>Rs.

As for the treatment of the alcohol/benzodiazepine withdrawal syndrome, propofol and barbiturate which enhance GABA<sub>A</sub>R-mediated inhibition are useful. Indeed, it was demonstrated that propofol and barbiturates (pentobarbital and phenobarbital) were effective for the treatment of alcohol withdrawal syndrome <sup>[17][18]</sup>, and barbiturate (pentobarbital) was effective for the treatment of benzodiazepine withdrawal syndrome <sup>[19]</sup>. However, it remains unclear how propofol and barbiturate ameliorate reduced GABA responsiveness in patients with alcohol/benzodiazepine withdrawal syndrome. Although the concentrations of propofol and barbiturates that generated the hump-like current are very high <sup>[20][21][22]</sup> compared to the dose used for treatment of the withdrawal syndrome <sup>[17][18]</sup>, the generation of hump-like GABA<sub>A</sub>R currents itself may suggest the occurrence of resensitization of GABA<sub>A</sub>R-mediated currents. Indeed, the desensitization and deactivation of GABA<sub>A</sub>R-mediated currents are facilitated and slowed, respectively, by propofol/barbiturate at much lower concentrations <sup>[21][22]</sup>. Then, propofol and barbiturate may improve the reduced GABA responsiveness through the

resensitization of GABA<sub>A</sub>R-mediated currents. Therefore, the regulatory mechanisms of desensitization/resensitization of GABA<sub>A</sub>R-mediated currents are important to better understand the benzodiazepine/alcohol withdrawal syndrome and to develop the treatment method.

### 2. Drugs that Cause Desensitization and Resensitization of GABA<sub>A</sub>R-Mediated Currents

The drugs that cause desensitization and resensitization of GABA<sub>A</sub>R-mediated currents are summarized in Table 1. Hump-like GABA<sub>A</sub>R currents after a strong desensitization were seen at the offset of propofol application at a high concentration (600  $\mu$ M) in hippocampal pyramidal neurons <sup>[22]</sup>, etomidate application at a high concentration (1 mM) in rat spinal dorsal horn neurons <sup>[23]</sup>, pentobarbital application at high concentrations (1–3 mM) in frog sensory neurons <sup>[21][22]</sup>, rat hippocampal neurons <sup>[24]</sup> and recombinant GABA<sub>A</sub>Rs <sup>[25][26][27][28][29][30]</sup> or phenobarbital application at a high concentration (10 mM) in rat hippocampal neurons <sup>[24]</sup>, although these were not seen at the offset of GABA application. It is believed that the generation of hump-like currents may be caused by removal of the blockade by anesthetic agents as partial antagonists <sup>[25]</sup>, although their mechanisms remain unclear and the involvement of desensitization is not necessarily denied. To better understand the actions of these anesthetic drugs, the molecular and regulatory mechanisms of desensitization of GABA<sub>A</sub>R-mediated currents might be important.

Table 1. Drugs that modulate GABA responses and directly activate GABA<sub>A</sub>Rs at higher concentrations.

Drugs	Neurons/ Recombinant GABA <sub>A</sub> Rs	Effects	Refs.
Anesthetics			
Propofol	Mouse hippocampal neurons	slows deactivation and increases apparent desensitization of GABA responses at low concentrations and directly elicits after-responses upon washout at high concentrations	[22]
Etomidate	Rat spinal dorsal horn neurons	slows deactivation of GABA responses at low concentrations while directly elicits tail currents upon washout at high concentrations	[23]
Barbiturate			
Pentobarbital	Frog sensory neurons	slows deactivation and increases apparent desensitization of GABA responses at low concentrations, and directly elicits hump currents upon washout at high concentrations	[20, 21]
	Rat hippocampal neurons	slows deactivation and increases apparent desensitization of GABA responses at low concentrations, and directly elicits rebound currents upon washout at high concentrations	[24]
	α1β2γ2L	directly elicits tail currents upon washout at high concentrations	[25, 27]
	α1β3γ2L	slows deactivation and increases apparent desensitization of GABA responses at low concentrations, and directly elicits rebound currents upon washout at high concentrations	[26]
	α1β2γ2S, α6β2γ2S	directly elicits hump currents upon washout at high concentrations	[28]
	β3	increases apparent desensitization of GABA responses and directly elicits rebound currents upon washout at high concentrations	[29]
	α1β3γ2L	directly elicits tail currents upon washout at high concentrations	[30]
Phenobarbital	Rat hippocampal neurons	slows deactivation and increases apparent desensitization of GABA responses at low concentrations, and directly elicits rebound currents upon washout at high concentrations	[24]

### 3. A possible Kinetic Mechanism for Resensitization of $\mathsf{GABA}_{\mathsf{A}}$ Currents

In layer II/III pyramidal cells of the mouse barrel cortex, the deletion of PRIP-1/2 enhanced the desensitization of GABA<sub>A</sub>R-mediated currents but paradoxically induced a hump-like tail-current at the offset of the GABA puff <sup>[15]</sup>, and also resulted in the prolongation of the decay phase of eIPSCs <sup>[31]</sup>. To understand the kinetic mechanisms underlying the generation of the hump-like tail-currents and the prolongation of eIPSCs, these currents were simulated using a model

previously proposed <sup>[32]</sup> (Figure 1). It was examined whether the possible increase in the fast desensitization rate ( $d_2$ ) and the possible decrease in the unbinding rate ( $k_{off}$ ) can lead to the generation of the hump-like tail-current at the offset of the GABA puff. In the simulated wild-type pyramidal cell, GABA<sub>A</sub>R-mediated currents were induced without a hump-like tailcurrent in response to 2-sec GABA puff at 50 µM <sup>[15]</sup>. In contrast, in the simulated PRIP-DKO pyramidal cell, GABA<sub>A</sub>Rmediated currents displayed a prominent desensitization and was followed by a prominent hump-like tail-current <sup>[15]</sup>. Thus, a slowdown of  $k_{off}$  and an acceleration of  $d_2$  resulted in a generation of a hump-like tail-current. Following a sharp decrease in [GABA] at the offset of GABA puff, a sharp decrease in  $d_2$  to a level smaller than the fast de-desensitization (i.e. resensitization) rate constant ( $r_2$ ) occurred to subsequently induce a hump-like tail-current. It can be concluded that a higher calcineurin activity in PRIP-DKO layer III pyramidal cells might have caused a slowdown of  $k_{off}$  and an acceleration of  $d_2$  through the modulation of its GABA concentration dependency, leading to a generation of hump-like tail-currents in PRIP-DKO pyramidal cells. It was also investigated whether the increase in  $d_2$  and the decrease in  $k_{off}$  can also lead to the prolongation of eIPSCs. Simulated IPSCs in PRIP-DKO and the wild-type pyramidal cells that have the half-durations similar to those obtained in the real experiments <sup>[15]</sup> revealed that a prolongation of eIPSCs/eIPSPs in PRIP-DKO pyramidal cells results from resensitization of GABA<sub>A</sub>R-mediated currents, which is brought about by an acceleration of  $d_2$ through the modulation of its [GABA] dependency together with a slowdown of  $k_{off}$ .



**Figure 1.** A kinetic model for a hump-like tail-current. (A) A kinetic model of GABA<sub>A</sub>Rs representing mono- and doubleliganded states, each providing access to open and desensitized states. (B and C) Top; Presumed [GABA] changes created by puff application of GABA with a rectangular pressure pulse through a puff pipette containing 200  $\mu$ M GABA in the extracellular medium was assumed to be diluted 4 times, and the onset and offset of the puff application were assumed to be attenuated with a time constant raging between 0.1 and 0.3 sec. Bottom; superimposed traces of the simulated GABA<sub>A</sub>R-mediated currents under the condition that the attenuation time constant is 0.3 and 0.1 sec (solid and interrupted traces, respectively) in simulated wild-type (B) and PRIP-DKO (C) pyramidal cells. (D) Superimposed traces of a simulated wild-type and PRIP-DKO eIPSC induced by a GABA transient shown on an expanded time scale (inset) with a small maximum conductance. Adopted from <sup>[15]</sup> and <sup>[31]</sup>.

Based on the experimental and simulation studies, the regulatory mechanisms of GABA<sub>A</sub>Rs are schematically depicted (Figure 2).



**Figure 2.** Close, open (resensitized) and desensitized states of GABA<sub>A</sub>Rs. When GABA binds to GABA<sub>A</sub>Rs, the receptors open the pore, and consequently increasing the permeability of the ion pore to Cl<sup>-</sup>. In response to a prolonged application of GABA, GABA<sub>A</sub>Rs are desensitized (*d*) by increased calcineurin activity due to potentiated Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release (CICR) followed by store-operated Ca<sup>2+</sup> entry (SOCE) <sup>[15]</sup>. GABA<sub>A</sub>Rs are resensitized through the de-desensitization (*r*) at the offset of the GABA puff. PRIP outcompetes the PLC $\delta$  in binding to GABA<sub>A</sub>R  $\beta$  subunits <sup>[14]</sup>. *d*: desensitization, *r*: resensitization, RYR: ryanodine receptor, SOCC: store-operated Ca<sup>2+</sup> channel, IP<sub>3</sub>R: inositol trisphosphate receptor.

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