Molecular and Regulatory Mechanisms of Desensitization and Resensitization of GABA<sub>A</sub> Receptors with a Special Reference to Propofol/Barbiturate

Youngnam Kang<sup>1,2</sup>,*, Mitsuru Saito<sup>3</sup> and Hiroki Toyoda<sup>4,*</sup>

<sup>1</sup> Department of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, Osaka 565-0871, Japan
<sup>2</sup> Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul 110-749, Korea
<sup>3</sup> Department of Oral Physiology, Graduate School of Medical and Dental Sciences, Kagoshima University, Sakuragaoka 8-35-1, Kagoshima 890-8544, Japan; mtrsaito@dent.kagoshima-u.ac.jp
<sup>4</sup> Department of Neuroscience and Oral Physiology, Osaka University Graduate School of Dentistry, Osaka 565-0871, Japan

* Correspondence: kang@dent.osaka-u.ac.jp (Y.K.); toyoda@dent.osaka-u.ac.jp (H.T.)

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Abstract: It is known that desensitization of GABA<sub>A</sub> receptor (GABA<sub>A</sub>R)-mediated currents is paradoxically correlated with the slowdown of their deactivation, i.e., resensitization. It has been shown that an upregulation of calcineurin enhances the desensitization of GABA<sub>A</sub>R-mediated currents but paradoxically prolongs the decay phase of inhibitory postsynaptic currents/potentials without appreciable diminution of their amplitudes. The paradoxical correlation between desensitization and resensitization of GABA<sub>A</sub>R-mediated currents can be more clearly seen in response to a prolonged application of GABA to allow more desensitization, instead of brief pulse used in previous studies. Indeed, hump-like GABA<sub>A</sub>R currents were produced after a strong desensitization at the offset of a prolonged puff application of GABA in pyramidal cells of the barrel cortex, in which calcineurin activity was enhanced by deleting phospholipase C-related catalytically inactive proteins to enhance the desensitization/resensitization of GABA<sub>A</sub>R-mediated currents. Hump-like GABA<sub>A</sub>R currents were also evoked at the offset of propofol or barbiturate applications in hippocampal or sensory neurons, but not GABA applications. Propofol and barbiturate are useful to treat benzodiazepine/alcohol withdrawal syndrome, suggesting that regulatory mechanisms of desensitization/resensitization of GABA<sub>A</sub>R-mediated currents are important in understanding benzodiazepine/alcohol withdrawal syndrome. In this review, we will discuss the molecular and regulatory mechanisms underlying the desensitization and resensitization of GABA<sub>A</sub>R-mediated currents and their functional significances.

Keywords: GABA<sub>A</sub> receptor; desensitization; resensitization

1. Introduction

Ligand-gated channels open in response to the neurotransmitter binding but also close (desensitize) for long periods with the agonist still bound [1,2]. It is demonstrated that desensitization of GABA<sub>A</sub> receptor (GABA<sub>A</sub>R)-mediated currents is paradoxically correlated with the slowdown of their deactivation, i.e., resensitization [3]. Desensitization tends to prolong inhibitory currents and keeps the transmitter in the bound state of GABA<sub>A</sub>Rs. The rate at which the receptors enter the desensitization state will affect the shape of inhibitory currents [4–6].
The desensitization of GABAₐR-mediated currents is modulated by various signal transductions. The PKA-mediated phosphorylation modulates the desensitization of GABAₐRs in chick cortical neurons [7], rat sympathetic ganglion neurons [8], rat cerebellar granule neurons [9], and recombinant GABAₐRs [10]. The PKC- and PKG-mediated phosphorylation decreases the fast component of desensitization in recombinant α₁β₁ GABAₐRs [11] and rat cerebellar granule cells [9], respectively. CaMKII (Ca²⁺/calmodulin-dependent protein kinase II) decreased the desensitization of GABAₐR-mediated currents in rat spinal dorsal horn neurons [12], while calcineurin enhanced the desensitization of GABAₐR-mediated currents in rat hippocampal neurons [13]. Calcineurin directly binds to the intracellular loop of the GABAₐR γ2 subunit, thereby dephosphorylating the receptor [14]. Interestingly, it is reported that the desensitization of GABAₐR-mediated currents, which is caused by the enhanced calcineurin activity, paradoxically prolongs the decay phase of inhibitory postsynaptic currents/potentials without appreciable diminution of their amplitudes [4].

The paradoxical correlation between desensitization and resensitization of GABAₐR-mediated currents can be seen in response to a brief pulse in previous studies [3,4]. However, this relationship can be more clearly seen in response to a prolonged application of GABA for enough time to allow full desensitization. Indeed, hump-like GABAₐR currents were produced after a strong desensitization at the offset of puff applications of GABA for 2 s in pyramidal cells of the barrel cortex in the phospholipase C-related catalytically inactive proteins (PRIP-1/2) double-knockout (PRIP-DKO) mice [15]. In these neurons, the increased calcineurin activity due to the potentiated Ca²⁺-induced Ca²⁺ release (CICR) and store-operated Ca²⁺ entry (SOCE) enhances the desensitization of GABAₐR-mediated currents and subsequently causes resensitization of GABAₐR-mediated currents [15].

GABARAP (GABAₐR-associated protein) plays an important role in intracellular trafficking/clustering of GABAₐRs [16,17] and the clustered GABAₐRs display lower apparent affinity for GABA, faster deactivation, and slower desensitization [18]. The kinases and molecules involved in desensitization and resensitization (slowdown of deactivation) of GABAₐR-mediated currents are summarized in Table 1.

| Kinases/ Molecules | Neuron/Recombinant GABAₐRs | Effects | References |
|------------------|---------------------------|---------|------------|
| PKA              | Chick cortical neurons    | increases desensitization | [7]        |
|                  | Rat sympathetic ganglion neurons | decreases peak amplitude and increases fast desensitization | [8]        |
|                  | Rat cerebellar granule cells | decreases fast desensitization | [9]        |
| PKC              | α₁β₁γ₂S, α₁β₃γ₂LS        | increases desensitization and slows deactivation | [10]       |
| PKG              | α₁β₁                        | decreases fast desensitization | [11]       |
| CaMKII           | Rat cerebellar granule cells | decreases fast desensitization | [9]        |
| Calcineurin      | Rat hippocampal horn neurons | increases desensitization and slows deactivation | [4]        |
| PRIP             | Mouse cortical pyramidal neurons | PRIP deletion increases desensitization and generates hump-like currents through increased calcineurin activity | [15]       |
| GABARAP          | α₁β₂γ₂L                     | promotes clustering of GABAₐRs, facilitates deactivation, and slows desensitization | [18]       |
Hump-like GABAAR currents after a strong desensitization were also seen at the offset of propofol applications at a high concentration (600 µM) in hippocampal pyramidal neurons [19], etomidate applications at a high concentration (1 mM) in rat spinal dorsal horn neurons [20], pentobarbital applications at high concentrations (1–3 mM) in frog sensory neurons [21,22], rat hippocampal neurons [23], and recombinant GABAARs [24–29] or phenobarbital applications at a high concentration (10 mM) in rat hippocampal neurons [23], although these were not seen at the offset of GABA applications. Drugs that cause desensitization and resensitization of GABAAR-mediated currents are summarized in Table 2. It is believed that the generation of hump-like currents may be caused by the removal of the blockade by anesthetic agents as partial antagonists [24], although their mechanisms remain unclear and the involvement of desensitization is not necessarily denied. Propofol and barbiturate are clinically used for treatment of benzodiazepine/alcohol withdrawal syndrome [30–32]. Considering that hump-like GABAAR currents that are seen after a strong desensitization or blockade were evoked at the offset of propofol or barbiturate applications, the regulatory mechanisms of desensitization/resensitization of GABAAR-mediated currents might be important for understanding benzodiazepine/alcohol withdrawal syndrome. Here, we discuss the molecular and regulatory mechanisms underlying the desensitization and resensitization of GABAAR-mediated currents in neurons of PRIP-DKO mice and their functional significances.

| Drugs   | Neurons/Recombinant GABAARs | 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                                                                                                                                                                                                                     | [19]   |
| Etomidate | Rat spinal dorsal horn neurons | slows deactivation of GABA responses at low concentrations while directly eliciting tail currents upon washout at high concentrations                                                                                                                                                                                                                                                | [20]   |
| 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                                                                                                                                                                                                                    | [21,22]|
|           | 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                                                                                                                                                                                                                           | [23]   |
|           | α1β2γ2L                     | directly elicits tail currents upon washout at high concentrations                                                                                                                                                                                                                                                                                                                             | [24,26]|
|           | α1β3γ2L                     | slows deactivation and increases apparent desensitization of GABA responses at low concentrations and directly elicits rebound currents upon washout at high concentrations                                                                                                                                                                                                                                         | [25]   |
|           | α1β2γ2S, α6β2γ2S            | directly elicits hump currents upon washout at high concentrations                                                                                                                                                                                                                                                                                                                              | [27]   |
|           | β3                          | increases apparent desensitization of GABA responses and directly elicits rebound currents upon washout at high concentrations                                                                                                                                                                                                                                                                | [28]   |
|           | α1β3γ2L                     | directly elicits tail currents upon washout at high concentrations                                                                                                                                                                                                                                                                                                                              | [29]   |
| 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                                                                                                                                                                                                                  | [23]   |
2. PRIP-1/2 are Involved in Desensitization and Resensitization of GABA<sub>A</sub>R-Mediated Currents

PRIP-1/2 are involved in the membrane trafficking of GABA<sub>A</sub>Rs and the regulation of intracellular Ca<sup>2+</sup> stores [16,17]. Thus, it was investigated whether and how the deletion of PRIP-1/2 affects GABA<sub>A</sub>R-mediated currents evoked by puff applications of GABA in layer III pyramidal cells of the barrel cortex. It was found that 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 (Figure 1) [15]. Thus, it is likely that PRIP-1/2 are involved in the desensitization and resensitization of GABA<sub>A</sub>R-mediated currents. Although similar tail-currents were observed following the removal of propofol [19], etomidate [20], pentobarbital [21–29], and phenobarbital [23], it was the first report on such hump-like tail-currents that were induced by GABA itself.

![Figure 1](image-url) **Figure 1.** GABA<sub>A</sub>R-mediated currents evoked by GABA puff applications in wild-type and PRIP-DKO pyramidal cells. (A and B) Sample traces of GABA<sub>A</sub>R-mediated currents evoked at 0 mV in wild-type and PRIP-DKO pyramidal cells dialyzed with 5 mM EGTA, respectively, by puff application (4 and 6 psi) of GABA for 2 s. a, b, and c are the peak amplitude, the amplitude at the offset of the puff application, and the peak amplitude after the offset of the puff application, respectively. # and § are the durations at half amplitudes of desensitized component ((a + b)/2) and of tail-currents, respectively. (C) The relationship between the desensitization degree [Ds = (a – b)/a] of the GABA<sub>A</sub>R-mediated currents and half-duration of the tail-current (§) induced by a puff with 4 psi. †: p <0.01. (D) The relationship between the half-desensitization time of the GABA<sub>A</sub>R-mediated currents (#) and half-duration of the tail-current (§) induced by a puff with 4 psi. †: p <0.01. Adopted from [15].

3. [Ca<sup>2+</sup>]<sub>i</sub> Dependence of Desensitization and Resensitization of GABA<sub>A</sub>R-Mediated Currents and Their Abolishment by a Calcineurin Inhibitor

It is well known that the desensitization of GABA<sub>A</sub>R-mediated currents is accelerated by increases in [Ca<sup>2+</sup>]<sub>i</sub> [33,34]. As expected, it was clearly demonstrated that both the acceleration of desensitization of GABA<sub>A</sub>R-mediated currents and the generation of the hump-like tail-currents were caused by increases in [Ca<sup>2+</sup>]<sub>i</sub> [15]. Consistent with the idea that desensitization is mechanistically related to the deactivation of GABA<sub>A</sub>R-mediated currents [3], the progress of desensitization of GABA<sub>A</sub>R-mediated currents was invariably accompanied by the enhancement of the hump-like tail-currents [15]. These results suggested that the deletion of PRIP-1/2 results in an enhancement of the desensitization and resensitization of GABA<sub>A</sub>R-mediated currents through increases in [Ca<sup>2+</sup>]<sub>i</sub>. The involvement of CICR and the following SOCE in both the desensitization of GABA<sub>A</sub>R-mediated
currents and the generation of the hump-like tail-currents in PRIP-DKO pyramidal cells was also demonstrated by an intracellular application of ruthenium red [15].

It has been demonstrated that a calcineurin inhibitor, cyclosporin A-cyclophilin A complex, suppressed the desensitization of GABAₐR-mediated currents in acutely dissociated hippocampal neurons [13]. It has also been reported that the inhibition of calcineurin increased the rate of GABA unbinding from GABAₐRs [4]. Consistent with these previous studies, the bath application of a calcineurin inhibitor, fenvalerate, alleviated the desensitization of GABAₐR-mediated currents and markedly decreased the hump-like tail-currents [15]. Thus, it is likely that the hump-like tail-currents in PRIP-DKO pyramidal cells were generated as a result of an acceleration of desensitization of GABAₐR-mediated currents coupled with a slowdown of the GABA unbinding, which was mediated by Ca²⁺-dependent activation of calcineurin. Furthermore, Ca²⁺ imaging revealed that CICR and the following SOCE were more potent in PRIP-DKO pyramidal cells than in wild-type pyramidal cells [15]. Taken together, these results strongly suggest that the enhancement of desensitization and resensitization of GABAₐR-mediated currents in PRIP-DKO pyramidal cells was largely mediated by the upregulation of Ca²⁺-dependent activity of calcineurin due to the potentiation of CICR followed by SOCE.

4. Deletion of PRIP-1/2 Prolongs eIPSCs in Layer II/III Pyramidal Cells

The differences in the kinetic properties of GABAₐR-mediated currents between pyramidal cells of wild-type and PRIP-DKO mice should be reflected in the difference in inhibitory postsynaptic responses. Then, it was investigated how inhibitory postsynaptic responses reflect the changes in the kinetic properties of the GABAₐR-mediated currents in layer III pyramidal cells of the PRIP-DKO barrel cortex.

It was found that the deletion of PRIP-1/2 resulted in the prolongation of the decay phase of inhibitory postsynaptic currents/potentials (IPSCs/IPSPs) in layer II/III pyramidal cells evoked by stimulation of layer III (Figure 2), leaving the overall features of miniature IPSCs unchanged [35]. These observations suggest that the prolongation of inhibitory synaptic actions is likely to result from an enhancement of desensitization followed by an enhanced resensitization of GABAₐR-mediated currents. It has been reported that the PRIP-DKO mice exhibited a reduced expression of synaptic GABAₐRs containing γ2 subunits by 40% in hippocampal neurons [36] and by 18% in cerebellar granule cells [37] as a consequence of the lack of binding between PRIP-1/2 and GABAₐR-associated protein [38]. The mean peak amplitudes of the IPSCs and IPSPs in the PRIP-DKO pyramidal cells were not significantly different from those in the wild-type pyramidal cells. In any case, the amplitude of eIPSPs would not be increased by deletion of PRIP-1/2 [35]. Then, an increase in duration instead of amplitude of eIPSPs is likely to be caused in PRIP-DKO mice.
Figure 2. Evoked IPSCs (eIPSCs) in wild-type and PRIP-DKO pyramidal cells. (A and B) Superimposed sample traces of IPSCs evoked by stimulation with 1.0–1.5 times threshold (1.0–1.5 Th) in wild-type (A) and PRIP-DKO pyramidal cells (B). (C) The mean 10%-90% rise times of IPSCs evoked by stimulation with 1.2 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7) and those evoked by stimulation with 1.4 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7). †: p <0.01. (D) The mean times-to-peak of IPSCs evoked by stimulation with 1.2 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7) and those evoked by stimulation with 1.4 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7). †: p <0.01. (E) The mean half-durations of IPSCs evoked by stimulation with 1.2 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7) and those evoked by stimulation with 1.4 Th in wild-type (n = 8) and PRIP-DKO pyramidal cells (n = 7). †: p <0.01. Adopted from [35].

5. A Possible Kinetic Mechanism Underlying the Generation of the Hump-Like Tail-Currents and the Prolongation of eIPSCs

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 previously proposed model [3] (Figure 3). It was examined whether the possible increase in the fast desensitization rate (d2) and the possible decrease in the unbinding rate (koff) can lead to a generation of the hump-like tail-current at the offset of the GABA puff.
Figure 3. A kinetic model for a hump-like tail-current. (A) A kinetic model of GABA_ARs representing mono- and double-ligated 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 µM 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 ranging between 0.1 and 0.3 s. Bottom; superimposed traces of the simulated GABA_AR-mediated currents under the condition that the attenuation time constant is 0.3 and 0.1 s (solid and interrupted traces, respectively) in simulated wild-type (B) and PRIP-DKO (C) pyramidal cells. The rate constants were as follows (in s\(^{-1}\)): \(k_{on} = 15 \mu M^{-1}\), \(\beta_2 = 2500\), \(\alpha_2 = 142\), \(r_2 = 50\), \(\beta_1 = 200\), \(\alpha_1 = 1100\), \(r_1 = 0.35\), \(d_1 = 6\), \(q = 1 \times 10^{-8} \mu M^{-1}\), and \(p = 1\). The values of \(k_{off}\) in WT and PRIP-DKO GABA_ARs were 90 and 30 s\(^{-1}\), respectively. The value of \(d_{max}\) in WT and PRIP-DKO GABA_ARs was 3600. The values of \(k_h\) in WT and PRIP-DKO GABA_ARs were 2000 and 200, respectively. (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. The rate constants were as follows (in s\(^{-1}\)): \(k_{on} = 20 \mu M^{-1}\), \(\beta_2 = 2500\), \(\alpha_2 = 195\), \(r_2 = 55\), \(\beta_1 = 100\), \(\alpha_1 = 600\), \(r_1 = 0.35\), \(d_1 = 11\), \(q = 1 \times 10^{-8} \mu M^{-1}\), \(p = 0\), and \(d_{max} = 3100\). The values of \(k_{off}\) in WT and PRIP-DKO GABA_ARs were 550 and 410 s\(^{-1}\), respectively. The value of \(d_{max}\) in WT and PRIP-DKO GABA_ARs was 310. The values of \(k_h\) in WT and PRIP-DKO GABA_ARs were 2000 and 150, respectively. Adopted from [15] and [35].

It is known that GABA binding affinity was much larger in the desensitized GABA_ARs compared to the non-desensitized GABA_ARs and the binding affinity of the desensitized GABA_ARs increased depending on the concentration of the pre-applied GABA as was the case with the degree of desensitization of GABA_AR-mediated currents [39]. Then, when the probability of being in the desensitized state (D_{fast}) for GABA_ARs was increased by increasing GABA concentration ([GABA]) or during the 2 s puff application of GABA, D_{fast} would be further recruited, leaving Open_{2} unchanged. Thus, it is reasonable to assume that the \(d_2\), but not \(\beta_2\), increase in a manner dependent on [GABA] [15,39]. Because Bound_{2}, which is bifurcated into Open_{2} and D_{fast}, increases in a manner dependent on [GABA], the idea was incorporated in this model by defining \(d_2\) as follows;

\[
d_2 = \frac{d_{max}}{1 + \left(\frac{K_h}{[GABA]}\right)^n}
\]

where \(d_{max}\) is the maximum desensitization rate, \(K_h\) is the [GABA] that yields the half maximum desensitization rate, and \(n\) is the Hill coefficient [15]. It was assumed that calcineurin increased \(d_2\)
by increasing its [GABA] dependency through a reduction of $k_h$, and the $d_2$ and $k_{off}$ were changed between the simulated wild-type and PRIP-DKO pyramidal cells. These changes were comparable to those caused by the activation of calcineurin reported previously [4,13].

In this simulation, the onset and offset of the 2 s puff application of GABA were assumed to be attenuated with a time constant ranging between 0.1 and 0.3 s. In the simulated wild-type pyramidal cell, GABA$_A$R-mediated currents were induced without a hump-like tail-current in response to 2 s GABA puff at 50 µM [15]. In contrast, in the simulated PRIP-DKO pyramidal cell, GABA$_A$R-mediated currents displayed a prominent desensitization and were followed by a prominent hump-like tail-current [15]. 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. Indeed, decreases in the decay time constant at the offset of GABA puff pulse from 0.3 to 0.1 sec decreased the half-duration of the hump-like tail-current, leaving its amplitude almost unchanged [15]. Only PRIP-DKO pyramidal cells, but not wild-type pyramidal cells, displayed hump-like tail-currents in response to the same GABA puff that may have decayed slowly. These observations clearly indicate that the generation of the hump-like tail-current reflects kinetic differences between GABA$_A$R-mediated currents in wild-type and PRIP-DKO pyramidal cells. Taken together, 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.

Because there were no significant differences in the single-channel current and the number of GABA$_A$Rs between eIPSCs in PRIP-DKO and wild-type pyramidal cells [35], it can be 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 half-durations similar to those obtained in the real experiments [35] revealed that a prolongation of eIPSCs/eIPSPs in PRIP-DKO pyramidal cells results from resensitization of GABA$_A$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 $k_{off}$. The finding of a negative skewness coefficient in PRIP-DKO eIPSCs obtained by the nonstationary variance analysis [35] is consistent with the occurrence of de-desensitization (resensitization) of GABA$_A$R-mediated currents during the decay phase of PRIP-DKO eIPSCs.

Based on the experimental and simulation studies, the regulatory mechanisms of GABA$_A$Rs are schematically depicted (Figure 4).
Figure 4. Close, open (resensitized), and desensitized states of GABA_{A}Rs. When GABA binds to GABA_{A}Rs, the receptors open the pore and consequently increase the permeability of the ion pore to Cl\textsuperscript{-}. In response to a prolonged application of GABA, GABA_{A}Rs are desensitized (\textit{d}) by increased calcineurin activity due to potentiated Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release (CICR) followed by store-operated Ca\textsuperscript{2+} entry (SOCE) [15]. GABA_{A}Rs are resensitized through de-desensitization (\textit{r}) at the offset of the GABA puff. PRIP outcompetes the PLC\textgreek{d} in binding to GABA_{A}R \beta subunits [40]. \textit{d}: desensitization, \textit{r}: resensitization, RYR: ryanodine receptor, SOCC: store-operated Ca\textsuperscript{2+} channel, IP\textsubscript{3}R: inositol trisphosphate receptor.

6. Physiological Significance of Desensitization and Resensitization of GABA_{A}R-Mediated Currents

A single whisker deflection elicits an excitation in a subset of layer IV neurons within a single barrel-related column [41], which subsequently causes an excitation in layer II/III in the same column and then spreads horizontally into neighboring columns [42,43]. The spatio-temporal profile of the excitation spread in layer II/III evoked by stimulation of layer IV was narrower and faster in the barrel cortex of the PRIP-DKO mice compared to the wild-type mice [35]. Such a horizontal excitation spread in layer II/III seems to be strictly controlled by GABA_{A}R-mediated lateral inhibition [42,44,45]. Indeed, bicuculline application abolished such a difference in the spatio-temporal profile of the excitation spread in layer II/III between the two genotypes [35]. It is reported that the PRIP-DKO mice exhibited a greater decrease in performance in the rotarod test [36], which is commonly used to assess the sensorimotor integration [46]. Then, the enhanced phasic inhibition caused by the PRIP-1/2 deletion would suppress the inter-columnar integration in the barrel cortex, consequently decreasing spatial recognition. Further studies are required to clarify the roles of PRIP-1/2 in sensorimotor processing in the barrel cortex.

7. Clinical Significance of Desensitization and Resensitization of GABA_{A}R-Mediated Currents

Central nervous system depressants slow brain activity, making them useful for treating anxiety, panic, and sleep disorders. Alcohol and benzodiazepine are useful to mitigate anxiety through enhancing GABA_{A}R-mediated inhibition. However, alcohol and benzodiazepine are known as abused drugs. Alcohol or benzodiazepine withdrawal syndrome appears following a reduction in alcohol or benzodiazepine use after a period of excessive use [47–50]. 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 [51] and enhancement of NMDA responses [52–55]. Although the exact mechanism for the reduced responsiveness of GABA_{A}Rs remains uncertain, changes in surface GABA_{A}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 [56–59]. It has recently been demonstrated that the benzodiazepine diazepam caused downregulation of GABAergic inhibition through the phospholipase C (PLCδ)/Ca²⁺/calcineurin signaling pathway [40]. The study showed that overexpression of PRIP-1 suppressed diazepam-dependent activation of PLCδ and diazepam-dependent downregulation of GABAARs in HEK293 cells [40], indicating that PRIP-1 acts as an inhibitor by outcompeting the PLCδ binding to GABAARs. Because intracellular Ca²⁺ and calcineurin activity are increased in PRIP-DKO mice [15], these findings suggest that the diazepam-induced long-term downregulation of GABAergic inhibition is mediated by the PLCδ/Ca²⁺/calcineurin signaling pathway. Nevertheless, it is also true that calcineurin causes resensitization of GABAAR-mediated currents by facilitating their desensitization [4,15]. 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 GABAARs.

As for the treatment of benzodiazepine/alcohol withdrawal syndrome, propofol and barbiturate which enhance GABAAR-mediated inhibition are useful. Indeed, it was demonstrated that propofol and barbiturates (pentobarbital and phenobarbital) were effective for the treatment of alcohol withdrawal syndrome [30,32] and barbiturate (pentobarbital) was effective for the treatment of benzodiazepine withdrawal syndrome [60]. However, it remains unclear how propofol and barbiturate ameliorate reduced GABA responsiveness in patients with benzodiazepine/alcohol withdrawal syndrome. Although the concentrations of propofol and barbiturates that generated the hump-like current are very high [19,21,22] compared to the dose used for treatment of the withdrawal syndrome [30,32], the generation of hump-like GABAAR currents itself may suggest the occurrence of resensitization of GABAAR-mediated currents. Indeed, the desensitization and deactivation of GABAAR-mediated currents are facilitated and slowed, respectively, by propofol/barbiturate at much lower concentrations [19,22]. Then, propofol and barbiturate may improve the reduced GABA responsiveness through the resensitization of GABAAR-mediated currents. Therefore, the regulatory mechanisms of desensitization/resensitization of GABAAR-mediated currents are important to better understand benzodiazepine/alcohol withdrawal syndrome and to develop the treatment method.

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Abbreviations

| Abbreviation | Description                                      |
|--------------|--------------------------------------------------|
| CaMKII       | Ca²⁺/calmodulin-dependent protein kinase II      |
| CICR         | Ca²⁺-induced Ca²⁺ release                        |
| DKO          | double-knockout                                  |
| GABAAR       | GABAAR receptor                                  |
| GABARAP      | GABAAR-associated protein                        |
| IPSC         | inhibitory postsynaptic current                  |
| IPSP         | inhibitory postsynaptic potential                |
| NMDA         | N-methyl-D-aspartate                             |
| PLC          | phospholipase C                                  |
| PRIP         | phospholipase C-related catalytically inactive protein |
| RYR          | ryanodine receptor                               |
| SOCC         | store-operated Ca²⁺ channel                     |
| SOCE         | store-operated Ca²⁺ entry                        |
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