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Interplay between ionotropic receptors modulates inhibitory synaptic strength

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The essence of neuronal function is to generate outputs in response to synaptic potentials. Synaptic integration at a synapse determines neuronal outputs in the CNS. In a recent study, we describe that excitatory and inhibitory transmitter-gated channels physically crosstalk each other at the cellular and molecular level. Increased membrane expression of ATP P2X4 receptors by using an interference peptide competing with the intracellular endocytosis motif enhances neuronal excitability, which is further enhanced by reciprocal interaction between post-synaptic ATP- and GABA-gated channels. Molecular interaction is supported by experiments of co-immunoprecipitation and mutagenesis of P2X4 subunit. Two amino acids in the intracellular carboxyl tail of P2X4 subunit appears to be responsible for this crosstalk. Our recent study provides molecular and electrophysiological evidence for physical interaction between excitatory and inhibitory receptors that appears to be crucial in determining synaptic strength at central synapses.

Key words: synapse, ligand-gated channels, P2X, GABA-A receptors, plasticity, crosstalk, trafficking, ATP, GABA, interaction

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Fast synaptic transmission between neurons is achieved through the release of one or more neurotransmitters from the same presynaptic terminal, resulting in the activation of different classes of ligand-gated ion channels co-localized at the same post-synaptic site.[1-4] Although it has been long believed that each receptor type acts independently of the other, recent studies have revealed that ATP-gated channels crosstalk with cys loop receptors in recombinant expression and cell culture preparations.[5-12] Of particular interest is that an excitatory neurotransmitter, ATP is always considered a co-transmitter and is released either with an inhibitory neurotransmitter, GABA,[4,13-15] or with an excitatory neurotransmitter, glutamate in the CNS.[16,17] Therefore, the process of synaptic integration at mixed synapses is potentially complicated by the presence of ATP P2X receptors.

Our recent study clearly demonstrates the physical and functional interactions between excitatory ATP P2X4 and inhibitory $GABA_A$ receptors at the cellular and molecular levels.[18] Their interactions appear to be critical in regulating synaptic strength at the synaptic level and, as a result, neuronal excitability (**Fig. 1**). A series of experiments, including co-immunoprecipitation, peptide-based pull downs, mutagenesis and overexpression of peptides in heterogeneous system provides converging evidence for a physical interaction between a specific intracellular motif (Tyr 374, Val 375) within the C-terminal tail of P2X4 subunits and $GABA_A$ β subunits. In addition, our prior studies show that the main intracellular loop of mainly $GABA_{A/c}$ β or ρ subunits are involved in the coupling with P2X2 or P2X3 receptors.[7,8,12] Furthermore, a recent FRET study confirms the close proximity of the C-terminal tail of P2X2 subunits and intracellular loop of $GABA_A$ subunits.[19] It is thus believed that the C-terminal tail of P2X subunits interacts directly with the main intracellular loop of subunits of cys loop receptor family, including $GABA_A$, nicotinic or 5-HT3 receptors. Importantly, the interaction

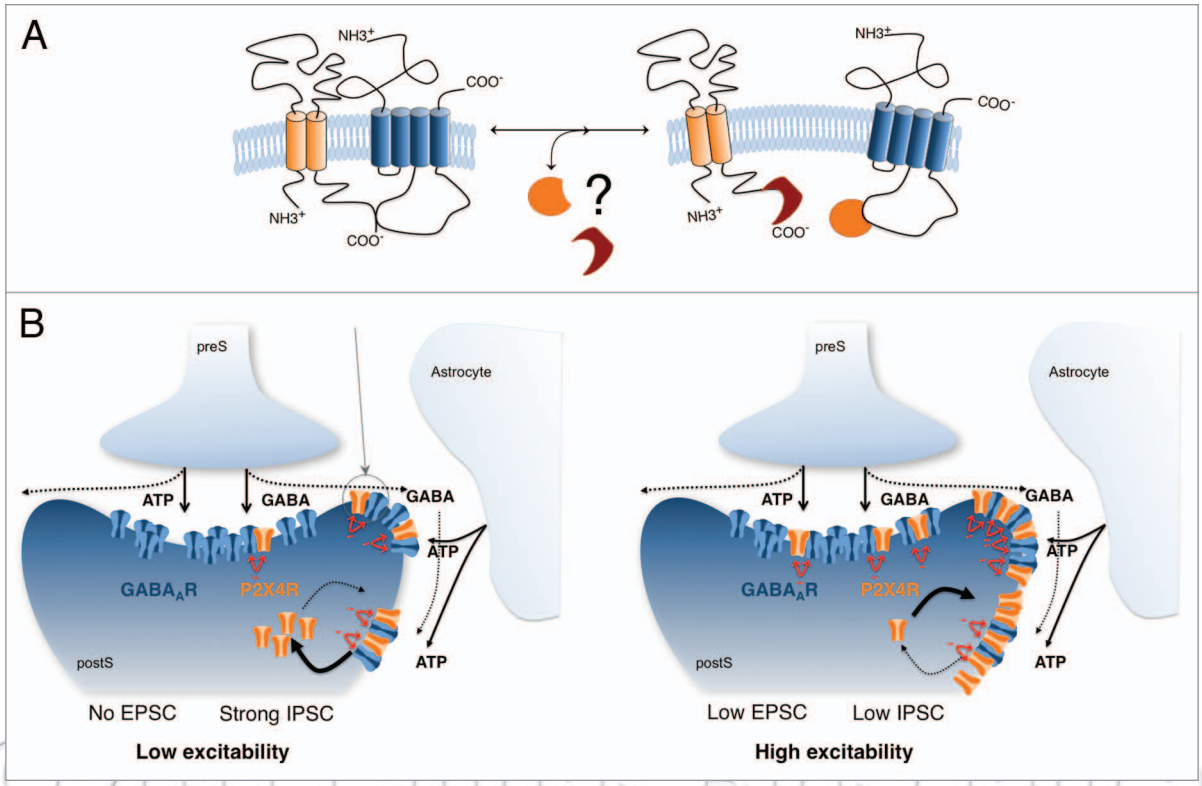

Figure 1. Interaction between P2X4 and $GABA_A$ receptors at a central synapse. (A) Schematic diagrams to describe the interaction between P2X4 and $GABA_A$ subunits. Unknown proteins and/or factors may regulate or initiate physical coupling between the C-terminal tail of P2X and the second intracellular loop of $GABA_A$ subunits. If this is the case, the cross-inhibition would be regulated by these factors. (B) Surface trafficking of P2X4 receptors and subsequent interaction with $GABA_A$ receptors at synapses and/or peri/extra synaptic sites downregulate inhibitory synaptic inputs. Additionally, this interaction may alter trafficking of other receptors, including $GABA_A$ receptors.

motif identified in P2X4 subunit is different from that identified in P2X3. These identified motifs are absent in P2X2 subunit. Likewise, the interacting regions of cys loop receptors, including GABA, nicotinic and 5-HT3 subunits show no primary sequence homology. It thus appears that the interaction between P2X and cys loop receptors is subunit-specific. In addition to the direct interaction between two receptors, undefined interacting proteins and/or regulatory factors may trigger or alter this negative interaction since these receptors appear to act independently in some neuronal populations[20] (**Fig. 1**).

P2X4-$GABA_A$ receptor interaction results in an instantaneous and reciprocal current inhibition in recombinant expression system. In other words the amplitude of the currents evoked by concomitant application of ATP and GABA is significantly smaller than the predicted sum of the responses to separate application of ATP and GABA. Although P2X4 and $GABA_A$ receptors form separate channels in recombinant expression system, the co-activation of both receptors results in non-additive responses due to reciprocal inhibition of both channel types. It should be emphasized that this current occlusion is abrogated by mutation of Tyr^{374} and/or Val^{375} as well as by intracellular administration of a peptide corresponding to amino acid 372–377 region of P2X4 receptor (YV6). This peptide appears to bind to GABA β subunits, which in turn occludes the binding of the P2X4 receptors.

Among P2X receptors, P2X4-containing receptors constitutively cycle into and out of the membrane in a dynamin-dependent mechanism. As a result, P2X4 receptors appear to be predominantly retained in intracellular compartments.[21] Blockade of P2X4 receptor internalization with a site-specific peptide increases surface expression of P2X4 subunits and the mean amplitude of ATP responses. Furthermore, increased surface expression of P2X4 receptors induces a decrease in the frequency and the amplitude of GABAergic post-synaptic currents. This depression of GABAergic currents is abolished following intracellular administration of YV6 peptide that disrupts interaction between P2X4 and $GABA_A$ receptors. We think that the physical interaction between P2X4 and $GABA_A$ receptors at the synaptic level plays an essential role in regulating inhibitory synaptic strength.

Since spontaneous P2X receptor-mediated postsynaptic currents are scarce following blockade of P2X4 receptor internalization, P2X4 receptors appear to be located mainly at peri/extrasynaptic areas in the CNS. If it is the case, P2X4 receptors may play a role in the modulation of synaptic transmission. For instance, P2X4 would preferentially interact with neighboring synaptic $GABA_A$ receptors at GABAergic synapses in the VMH. Indeed, in-depth analysis of individual sIPSCs following blockade of the interaction reveals two distinct GABAergic synaptic currents

(**Figs. 2A and B**): 34.8 ± 4% of sIPSCs have a fast decay time course of 10 ± 1.3 ms (n = 11 neurons) and 65.2 ± 4% of sIPSCs have a slow decay phase of 21.2 ± 1.9 ms (n = 11 neurons). As the composition of $GABA_A$ subunits determines the subcellular localization as well as biophysical properties, including decay time phase of $GABA_A$ receptors,[22-24] we may speculate that $GABA_A$ receptors having a slow decay phase are physically coupled with P2X4 receptors. In fact, the desensitization of αβ- or αβδ-containing $GABA_A$ receptors is slower than that of αβγ-containing $GABA_A$ receptors.[25] These αβγ-containing receptors appear to be located at synaptic sites, whereas δ subunit-containing receptors or αβ-containing receptors without γ subunits are found at extrasynaptic sites.[26] Furthermore, native α2- or α3 subunit-containing $GABA_A$ receptors decay more slowly than α1-expressing $GABA_A$ receptors.[27] It is thus possible that P2X4 receptors would interact with synaptic $GABA_A$ receptors containing α2 or α3, β and γ subunits. This is, somewhat, consistent with prior studies showing that extrasynaptic $GABA_A$ receptors do contain neither α2 nor α3.[28-30] Given that the interaction between P2X and GABA receptors influences receptor trafficking,[7,19] the expression of P2X4 receptors on the membrane would be critical in altering targeting of $GABA_A$ receptors in favor of a peri/extra synaptic location.

In summary, the observed crosstalk between excitatory and inhibitory ligand-gated channels appears to be a novel form of short-term synaptic plasticity at central synapses. P2X4 subunit-mediated fine tuning of GABAergic transmission would contribute to the regulation of synaptic strength, thereby regulating neuronal outputs in neural circuits fundamental to feeding behavior in particular and likely in brain.

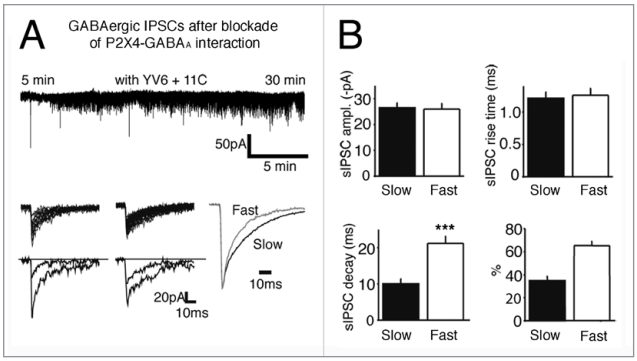

Figure 2. Disruption of the interaction between P2X4 and $GABA_A$ receptors increases GABAergic synaptic transmission and reveal two populations of sIPSCs. (A) Recording samples of the baseline GABAergic synaptic activity in the presence of both 11C and YV6 in the patch pipette. Analysis of individual sIPSCs recorded in the presence of 11C and YV6 revealed that there were two distinct $GABA_A$ receptor-mediated sIPSCs (fast vs. slow components). ~two third of sIPSCs had a slow decay phase. Bottom part: Superimposition of traces of sIPSCs (left, fast sIPSCs; middle, slow sIPSCs; right, normalized traces of average amplitude of sIPSCs to shows the difference in the decay time course of sIPSCs). Hp = -70 mV. (B) Summary of the basic characteristics of slow and fast sIPSCs recorded from 11 different SF-1 GFP-positive neurons (***p < 0.0001).

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