Interplay between ionotropic receptors modulates inhibitory synaptic strength

Young-Hwan Jo1 and Eric Boué-Grabot2,3,*
1Department of Medicine; Albert Einstein College of Medicine; Bronx, NY USA; 2Univ. Bordeaux; Institut des Maladies Neurodégénératives; UMR 5293; Bordeaux, France; 3CNRS; Institut des Maladies Neurodégénératives; UMR 5293; Bordeaux, France

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.

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, 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/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 P2X4 subunits and intracellular loop of GABA_A subunits.19 It is thus believed that the C-terminal tail of P2X4 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

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

Submitted: 07/08/11
Accepted: 07/08/11
DOI: 10.4161/cib.4.6.17291
*Correspondence to: Eric Boué-Grabot; Email: eric.boue-grabot@u-bordeaux2.fr

Addendum to: Jo YH, Donier E, Martinez A, Garret M, Toulmé E, Boué-Grabot E. Crosstalk between P2X4 and GABA_A receptors determines synaptic efficacy at a central synapse. J Biol Chem 2011; 286:19993-20004; PMID: 21482824; DOI: 10.1074/jbc.M111.31324

www.landesbioscience.com Communicative & Integrative Biology 706

©2011 Landes Bioscience
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 GABAA receptors. We think that the physical interaction between P2X4 and GABAA 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 GABAA 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.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Interaction between P2X4 and GABA<sub>α</sub> receptors at a central synapse. (A) Schematic diagrams to describe the interaction between P2X4 and GABA<sub>α</sub> 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<sub>α</sub> 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<sub>α</sub> receptors at synapses and/or peri/extra synaptic sites downregulate inhibitory synaptic inputs. Additionally, this interaction may alter trafficking of other receptors, including GABA<sub>α</sub> receptors.
GABA A receptors is slower than that of αβγ-containing GABA A receptors. 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. Furthermore, native α2- or α3 subunit-containing GABA A receptors decay more slowly than α1-expressing GABA A receptors. 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. Given that the interaction between P2X and GABA receptors influences receptor trafficking, 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. P2X 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.

References
1. Zander JF, Munster-Wandowski A, Brunk I, Palmer I, Gomez-Lima G, Heinemann U, et al. Synaptic and vesicular coexistence of VGLUT and VGAT in selected excitatory and inhibitory synapses. J Neurosci 2010; 30:7634-45; PMID:20519538; DOI:10.1523/JNEUROSCI.1041-10.2010.
2. Wojcik SM, Katarzyniak S, Guillien I, Frauf E, Rossmund G, Bross N, et al. A shared vesicular carrier allows synaptic corelease of GABA and glycine. Neuron 2006; 50:575-87; PMID:16701208; DOI:10.1016/j.neuron.2006.04.016.
3. Ren J, Qin C, Hu F, Tan J, Qiu L, Zhao S, et al. Hubenula “cholinergic” neurons co-release glutamate and acetylcholine and activate postsynaptic neurons via distinct transmission modes. Neuron 2017; 69:445-52; PMID:23135256; DOI:10.1016/j.neuron.2010.12.038.
4. Jo YH, Schlüchter R. Synaptic corelease of ATP and GABA in cultured spinal neurons. Nat Neurosci 1999; 2:241-5; PMID:10195216; DOI:10.1038/6544.
5. Barajas-Lopez E, Espinosa-Luna R, Zha Y. Functional interactions between nicotinic and P2X2 channels in short-term cultures of guinea-pig submucosal neuons. J Physiol 1998; 513:671-83; PMID:9824709; DOI:10.1111/j.1469-7793.1998.671ba.x.
6. Boul-Grabot E, Barajas-Lopez C, Chakfe Y, Blais D, Belanger D, Emerit MB, et al. Intracellular cross talk and physical interaction between two classes of neurotransmitter-gated channels. J Neurosci 2003; 23:1246-53; PMID:12598613.
7. Boul-Grabot E, Emerit MB, Touline E, Seguela P, Garret M. Cross-talk and co-trafficking between rho/GABA receptors and ATP-gated channels. J Biol Chem 2004; 279:6067-75; PMID:14660627; DOI:10.1074/jbc.M307772200.
8. Boul-Grabot E, Touline E, Emerit MB, Garret M. Subunit-specific coupling between gamma-amino butyric acid type A and P2X2 receptor channels. J Biol Chem 2004; 279:52517-25; PMID:15456793; DOI:10.1074/jbc.M410223200.
9. Karanja R, Garcia-Hernandez LM, Miranda-Morales M, Somani N, Espinosa-Luna R, Montano LM, et al. Cross-inhibitory interactions between GABA A and P2X2 channels in myenteric neurons. Eur J Neurosci 2006; 23:3259-68; PMID:16820016; DOI:10.1111/j.1460-9568.2006.04861.x.
10. Khakh BS, Boul-Grabot E, Touline E, Emerit MB, Garret M. ATP-gated cation channels (P2X purinoceptors) in trigeminal mesencephalic nucleus neurons of the rat. J Physiol 1997; 498:709-15; PMID:9051882.
11. Khakh BS, Zhou X, Sydes J, Galligan JJ, Lester HA. Stain-dependent cross-inhibition between transmitter-gated channel receptors. Nature 2000; 406:405-6; PMID:10935636; DOI:10.1038/35019066.
12. Touline E, Blais D, Leger C, Landry M, Garret M, Seguela P, et al. An intracellular motif of P2X3 receptors is required for functional cross-talk with GABA A receptors in nociceptive DRG neurons. J Neurochem 2007; 102:1357-68; PMID:17498217; DOI:10.1111/j.1471-4159.2007.04640.x.
13. Hugel S, Schlüchter R. Presynaptic P2X receptors facilitate inhibitory GABAergic transmission between cultured rat spinal dorsal horn neurons. J Neurosci 2000; 20:121-30; PMID:10704486.
14. Jo YH, Role LW. Cholinergic modulation of purinergic and GABAergic co-transmission at in vitro hypothalamic synapses. J Neurophysiol 2001; 85:2501-8; PMID:12442429; DOI:10.1152/jn.00352.2002.
15. Jo YH, Role LW. Coordinate release of ATP and GABA at in vitro synapses of lateral hypothalamic neurons. J Neurosci 2002; 22:4794-804; PMID:12077176.
16. Mori M, Fleuss C, Gahwiler BH, Gerber U. Fast synaptic transmission mediated by P2X receptors in CA3 pyramidal cells of rat hippocampal slice cultures. J Physiol 2001; 535:115-23; PMID:11507162; DOI:10.1111/j.1469-7793.2001.t01-1-00115.x.
17. Rubio ME, Soto F. Distinct Localization of P2X receptors at excitatory postsynaptic specializations. J Neurosci 2001; 21:641-53; PMID:1160443.
18. Jo YH, Donier E, Martinez A, Garret M, Touline E, Boul-Grabot E. Cross-talk between P2X4 and [gamma]-aminobutyric acid type A receptors determines synaptic efficacy at a central synapse. J Biol Chem 2002; 278:19989-20004; PMID:12482824; DOI:10.1074/jbc.M111.23124.
19. Shirvashita AN, Triller A, Sieghart W, Sarto-Jackson I. Regulation of GABA(A) receptor dynamics by interaction with purinergic P2X(2) receptors. J Biol Chem 2011; 286:14495-6; PMID:21343285; DOI:10.1074/jbc.M110.165282.
20. Khakh BS, Fisher JA, Nashmi R, Bowser DN, Lester HA. An angstrom scale interaction between plasma membrane ATP-gated P2X2 and alpha7 nicotinic channels measured with fluorescence resonance energy transfer and total internal reflection fluorescence microscopy. J Neurosci 2005; 25:6911-20; PMID:16033901; DOI:10.1523/JNEUROSCI.0561-05.2005.
21. Bohanovic LK, Royle SJ, Murrell-Lagnado RD. P2X receptor trafficking in neurons is subunit specific. J Neurosci 2002; 22:4814-24; PMID:12077178.
22. Ortmann PI, Lu C, Takagaki K, Fu Z, Vicini S. Expression of distinct alpha subunits of GABA A receptor regulates inhibitory synaptic strength. J Neurophysiol 2004; 92:1718-27; PMID:1502896; DOI:10.1152/jn.00243.2004.
23. Kokxma JJ, Fritschi JM, Mack V, Van Kesteren RE, Brussaard AB. Differential GABA<sub>A</sub> receptor clustering determines GABA synaptic plasticity in rat oxytocin neurons around parturition and the onset of lactation. Mol Cell Neurosci 2005; 28:128-40; PMID:15607948; DOI:10.1016/j.mcn.2004.09.002.

24. Belelli D, Harrison NL, Maguire J, Macdonald RL, Walker MC, Cope DW. Extrasynaptic GABA<sub>A</sub> receptors: form, pharmacology and function. J Neurosci 2009; 29:12757-63; PMID:19828786; DOI:10.1523/JNEUROSCI.3340-09.2009.

25. Bianchi MT, Macdonald RL. Slow phases of GABA(A) receptor desensitization: structural determinants and possible relevance for synaptic function. J Physiol 2002; 544:3-18; PMID:12356876; DOI:10.1113/jphysiol.2002.020255.

26. Glykys J, Mody I. The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. J Physiol 2007; 582:1163-78; PMID:17525114; DOI:10.1113/jphysiol.2007.134460.

27. Brussaard AB, Kirs KS, Baker RE, Willems WP, Leyting-Vermeulen JW, Voorn P, et al. Plasticity in fast synaptic inhibition of adult oxytocin neurons caused by switch in GABA(A) receptor subunit expression. Neuron 1997; 19:1103-14; PMID:9390523; DOI:10.1016/S0896-6273(00)80401-8.

28. Herd MB, Hayworth-White AR, Wafford TW, Voorn P, Lambert JJ, et al. The expression of GABA<sub>A</sub> beta subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. J Physiol 2008; 586:989-1004; PMID:18079158; DOI:10.1113/jphysiol.2007.146746.

29. Peden DR, Petitjean CM, Herd MB, Durakoglugil MS, Rosahl TW, Wafford K, et al. Developmental maturation of synaptic and extrasynaptic GABA<sub>A</sub> receptors in mouse thalamic ventrobasal neurones. J Physiol 2008; 583:965-87; PMID:17063661; DOI:10.1113/jphysiol.2007.145375.

30. Chandra D, Jia F, Liang L, Peng Z, Suryanarayanan A, Werner DF, et al. GABA<sub>A</sub> receptor alpha4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. Proc Natl Acad Sci USA 2006; 103:15230-3; PMID:17005728; DOI:10.1073/pnas.0604304103.