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Inhibitory interaction between $P2X_4$ and $GABA_C$ $\rho1$ receptors

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ABSTRACT

Reciprocal functional inhibition between P2X and $GABA_{A/C}$ receptors represents a novel mechanism fine-tuning neuronal excitability. However, the participating receptors and underlying mechanisms are not fully understood. $P2X_4$ receptor is widely found in neurons that express $GABA_C$ $\rho1$ receptor. Thus, we co-expressed $P2X_4$ and $\rho1$ receptors in HEK293 cells and, using patch-clamp recording, examined whether they have mutual functional inhibition. Currents evoked by simultaneous application of ATP and GABA ($I_{ATP+GABA}$) were significantly smaller compared to the addition of I_{ATP} and I_{GABA}. Furthermore, I_{ATP} were strongly suppressed during $\rho1$ receptor activation. Similarly, I_{GABA} were greatly attenuated during $P2X_4$ receptor activation. Such mutual inhibition was absent in cells only expressing $P2X_4$ or $\rho1$ receptor. Taken together, these functional data support negative cross-talk between $P2X_4$ and $\rho1$ receptors.

Neurotransmitters ATP and GABA contribute to fast cell-to-cell communication in the nervous system by activating their ionotropic receptors, P2X and $GABA_{A/C}$, respectively [1–5]. P2X receptors are cationic channels assembled as homo/hetero-trimers from seven P2X subunits ($P2X_1$–$P2X_7$). Each subunit comprises intracellular N- and C-termini, two transmembrane domains and a large extracellular loop (Fig. 1A). Activation of P2X receptors results in membrane depolarization and/or elevation in intracellular Ca^{2+} concentration. On the other hand, $GABA_{A/C}$ receptors are homo/hetero-pentamers with each subunit composed of extracellular N- and C-termini and four transmembrane domains (Fig. 1B). $GABA_{A/C}$ receptors function as Cl^- channels and activation mainly leads to membrane hyperpolarization. The $GABA_C$ subtype receptors form from three subunits ($\rho1$–$\rho3$). Therefore, P2X and $GABA_{A/C}$ receptors fundamentally differ in both structure and function, and therefore were long considered to operate independently.

However, a recurring theme in recent years is that P2X receptors interact with distinctive ionotropic neurotransmitter receptors, including $GABA_A$ receptors [6–8], nACh receptors [9–13], serotonin receptors [14,15]. $P2X_4$ receptor is widely expressed throughout the nervous system [16–22], including neurons or regions that also express $\rho1$ $GABA_C$ receptor, such as bipolar cells of retina [23–29], neurons of spinal cord [30,31], neurons of pyramidal and granule layer of hippocampus [32–34], superficial gray layer of superior colliculus [27,35] and dorsal lateral geniculate nucleus [27,36–38]. Coexistence of neuronal $P2X_4$ and $\rho1$ receptors raises an interesting question of whether they talk to each other. However, expression of multiple P2X and $GABA_C$ receptors and current lack of means to separate them render intriguingly difficult to address such a question. Studies of molecularly defined receptors in heterologous cells have been proved useful in dissecting the interacting receptors and underlying mechanisms. Thus, previous studies show that $P2X_2$ receptor can interact with $GABA_C$ $\rho1$ receptor and $GABA_A$ receptors containing several types of α and β subunits [39,40], nACh $\alpha3\beta4$ and $\alpha4\beta2$ receptors [13,41], and 5-HT_{3A} receptor [15]. A recent study has demonstrated that $P2X_3$ and $P2X_{2/3}$ receptors interact with $GABA_A$ $\alpha2\beta3$ receptor [8]. In the present study, we co-expressed $P2X_4$ and GABA $\rho1$ receptors in HEK293 cells and, using patch clamp recording, investigated whether they communicate with each other. Our data provide functional evidence suggesting an inhibitory interaction between $P2X_4$ and $\rho1$ receptors.

Materials and methods

Molecular and cell biology. Clones encoding rat $P2X_2$, $P2X_4$, and $P2X_6$ subunits with a C-terminal EE epitope and rat $\rho1$ subunit with a Myc or FLAG epitope were used. HEK293 cell culture and transfection with plasmids were described previously [42].

Patch clamp recordings. Whole cell current recordings were performed using an Axopatch 200B amplifier or an EPC-10 HEKA amplifier at a holding potential of −60 mV. Patch pipette was fabricated from borosilicate glass capillaries. Series resistance (2–15 MΩ) was compensated (60–70%) [43]. Extracellular solution contained (in mM) 147 NaCl, 2 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, and 13 glucose. Intracellular solution contained (in mM) 145 NaCl, 10 EDTA and 10 HEPES. Both solutions were maintained pH7.3 and 300–315 mOsm/l. Agonists were applied with 2–4 min intervals via a RSC160 system (Bio-Logic Science Instruments). Agonist concentra-

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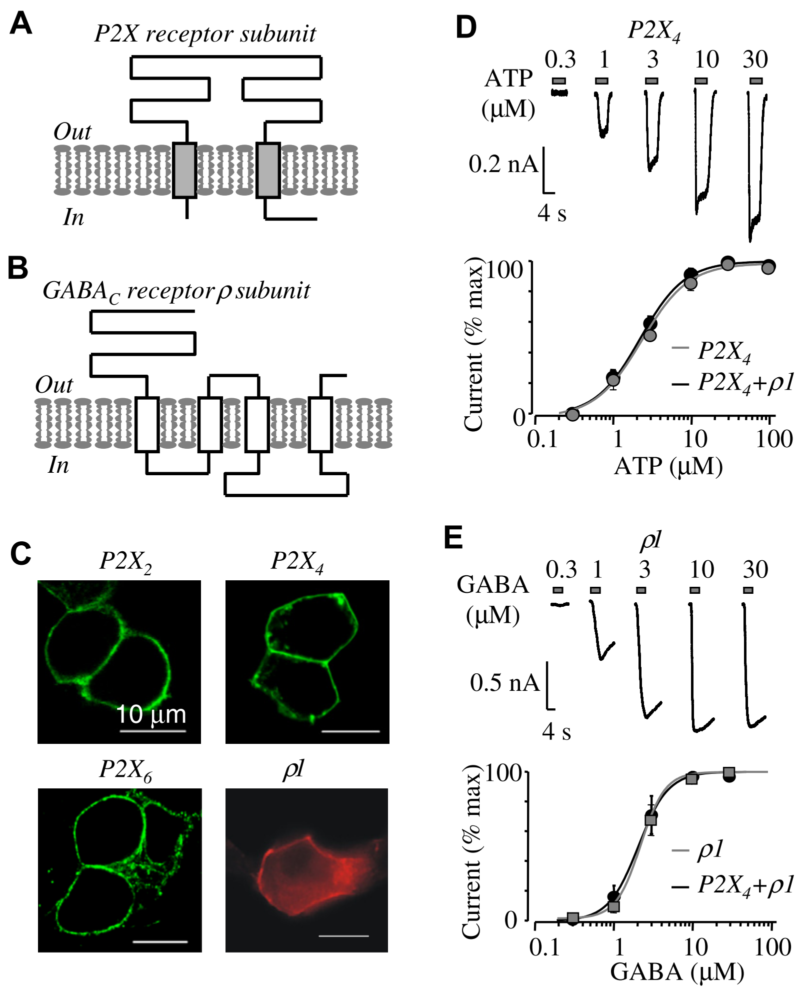

Fig. 1. Characterization of P2X and ρ1 receptors. (A,B) Schematics illustrating P2X subunit (A) or $GABA_C$ ρ subunit (B). (C) Images showing immunostaining in cells expressing the indicated receptors. (D,E) Representative currents evoked by ATP or GABA, and ATP concentration-current curves for $P2X_4$ alone ($n = 6$) or $P2X_4$ and ρ1 ($n = 6$) (D), and GABA concentration-current curves for ρ1 alone ($n = 13$) or $P2X_4$ and ρ1 ($n = 5$) (E).

tion-current curves were fit to the Hill equation: $I/I_{max} = 100/(1 + (EC_{50}/[A])^n)$, where I represents currents expressed as percentage of the maximal currents, n is the Hill coefficient, and EC_{50} is the agonist concentration evoking 50% of the maximal current. EC_{50} and n values were obtained by fitting the data from individual cells. All the figures, where appropriate, show mean ± SEM values and curves fitted to the pooled data. Statistical analysis was performed using Student's t-test.

Immunocytochemistry. Transfected cells were stained as detailed previously [42] using primary mouse anti-EE antibody (1:1000; BAbCo), anti-cMyc (1:1000; Santa Cruz) or anti-FLAG antibody (1:1000; Sigma), and secondary rabbit fluorescein isothiocyanate-conjugated anti-mouse IgG antibody (1:200; Sigma) or goat Cy3 anti-mouse IgG antibody (1:1000; Stratech Scientific).

Co-immunoprecipitation and Western blotting. These experiments were performed as described previously [44]. In brief, transfected cells were lysed in 200 μl lysis buffer (50 mM Tris–HCl pH8.0, 150 mM NaCl, 2 mM EGTA, 1% Triton X-100, and 5% glycerol, supplemented with a cocktail of protease inhibitors (Roche)). Cell lysate was mixed with 20 μl pre-equilibrated Ezview red anti-FLAG M2 affinity beads (Sigma), and agitated at 4 °C for 2 h. Proteins were resolved on 10% SDS–PAGE gels and transferred to nitrocellular membranes. After blocked with 5% non-fat milk in TBST solution (10 mM Tris–HCl pH 8.0, 150 mM NaCl, and 0.05% Tween 20), membranes were incubated for 1 h with primary rabbit anti-EE antibody (1:2000; Bethyl) and then incubated for 50 min with horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:2000; Santa Cruz). Proteins were visualized using supersignal west pico chemiluminescent substrates according to the manufacturer's protocol (Pierce) and captured on Kodak X-ray films.

Results

Characterization of expression and properties of P2X and ρ1 receptors

We first examined by immunocytochemistry protein expression in cells expressing individual receptors. Cells expressing EE-tagged $P2X_2$, $P2X_4$ or $P2X_6$ receptors were strongly labelled using anti-EE antibody. Similarly, cells expressing Myc-tagged ρ1 receptor were stained using anti-Myc antibody (Fig. 1C). The immunoreactivity was predominately localized to the plasma membrane. No immunostaining was observed in non-transfected cells (data not shown). Next, we measured, using patch-clamp recording, agonist-evoked currents in those cells. ATP evoked strong currents in cells expressing $P2X_2$ or $P2X_4$ receptor (Fig. 1D) in a concentration-dependent manner, but no current in cells expressing $P2X_6$ receptor ($n = 10$). The ATP EC_{50} values were 7.2 and 2.4 μM for $P2X_2$ and $P2X_4$ receptors, respectively (Table 1). None of these cells responded to GABA. Conversely, GABA but not ATP elicited currents in cells expressing ρ1 receptor (Fig. 1E). The GABA EC_{50} value is 2.8 μM (Table 1). $P2X_4$

Table 1
Summary of agonist sensitivity of P2X and ρ1 receptors

Receptors	ATP EC_{50}; *n* (No. of cells)	GABA EC_{50}; *n* (No. of cells)
$P2X_2$	7.2 ± 0.6 μM; 1.8 ± 0.3 (3)	
$P2X_4$	2.4 ± 0.3 μM; 1.6 ± 0.4 (6)	
ρ1		2.8 ± 0.3 μM; 3.3 ± 0.3 (13)
$P2X_2$ + ρ1	12.7 ± 3.4 μM; 2.4 ± 0.3 (4)	3.6 ± 1.0 μM; 3.1 ± 0.3 (4)
$P2X_4$ + ρ1	2.7 ± 0.4 μM; 1.4 ± 0.09 (6)	2.5 ± 0.4 μM; 3.6 ± 0.4 (5)

Note. No significant difference in ATP EC_{50} values between $P2X_2$ and $P2X_2$ + ρ1, between $P2X_4$ and $P2X_4$ + ρ1, or in GABA EC_{50} values between ρ1, ρ1 + $P2X_2$, and ρ1 + $P2X_4$.

receptors exhibited fast activation and modest deactivation kinetics whereas ρ1 receptor activation and particularly deactivation were noticeably slow (Fig. 1D–E). In summary, these properties are similar to those reported previously [17,18,28,42,45,46].

Secondly, we examined functional expression and agonist sensitivity in cells co-expressing P2X and ρ1 receptors. Cells co-expressing $P2X_2$ or $P2X_4$ with ρ1 receptors responded nicely to both ATP and GABA. The mean currents evoked by ATP (I_{ATP}) or GABA (I_{GABA}) were not significantly different from those in cells expressing only P2X (I_{ATP}) or ρ1 receptor (I_{GABA}) (Supplementary Figure 1, and data not shown). We also performed co-immunoprecipitation to test whether P2X and ρ1 receptors show protein–protein interaction. Indeed, we observed such interaction in cells co-expressing $P2X_2$ and ρ1, or $P2X_4$ and ρ1, but not $P2X_4$ and empty vector (Supplementary Figure 2). As summarized in Table 1 and Fig. 1D–E, co-expression had no significant effect on the agonist sensitivity for P2X and ρ1 receptors.

Non-additive currents evoked by simultaneous activation of P2X and ρ1 receptors

We employed the widely used protocol to study functional interaction between P2X and ρ1 receptors. The principal assumption is that currents from simultaneous activation of two receptors are additive if they function independently. In cells co-expressing $P2X_2$ and ρ1, currents evoked by co-application of saturating concentrations of 100 μM ATP and 10 μM GABA ($I_{ATP+GABA}$) were 3.8 ± 0.3 nA ($n = 4$), which were significantly lower than predicted (4.8 ± 0.4 nA; $n = 4$; $p < 0.05$; Fig. 2A), confirming the negative cross-talk between $P2X_2$ and ρ1 receptors [39]. In cells co-expressing $P2X_4$ and ρ1 receptors, $I_{ATP+GABA}$ (3.1 ± 0.3 nA, $n = 5$) were approximately 65% of predicted currents (4.6 ± 0.6 nA, $n = 5$; $p < 0.01$) (Fig. 2B), indicating occurrence of inhibitory interaction between $P2X_4$ and ρ1 receptors. In cells co-expressing $P2X_6$ and ρ1 receptors where there was no detectable I_{ATP}, however, $I_{ATP+GABA}$ (1.6 ± 0.3 nA) were virtually the same as I_{GABA} (1.7 ± 0.3 nA, $n = 4$).

Reciprocal inhibition between $P2X_4$ and ρ1 receptors

We further characterized the inhibitory interaction between $P2X_4$ and ρ1 receptors by comparing the currents from activation of one receptor in the absence and presence of activation of the other receptor. The I_{ATP} were markedly attenuated during ρ1 receptor activation (Fig. 3A). Similarly, the I_{GABA} were strongly reduced during $P2X_4$ receptor activation (Fig. 3B). These results suggest the existence of reciprocal inhibition.

No allosteric or indirect modulation of $P2X_4$ receptor by GABA or ρ1 receptor by ATP

Inhibition of ρ1 receptor by ATP could result from direct allosteric modulation as reported for NMDA receptors [47] or indirect modulation via endogenous P2Y receptors in HEK293 cells [48]. Similar mechanisms may be responsible for inhibition of $P2X_4$ receptor by GABA [49]. To examine these possibilities, we compared $I_{ATP+GABA}$ with I_{ATP} in cells expressing $P2X_4$ alone, or with I_{GABA} *in cells expressing ρ1 alone. In cells expressing $P2X_4$ alone,* $I_{ATP+GABA}$ (1.2 ± 0.1 nA) were not different from I_{ATP} (1.3 ± 0.1 nA, $n = 3$; $p > 0.5$) (Fig. 3C). In cells expressing ρ1 alone, $I_{ATP+GABA}$

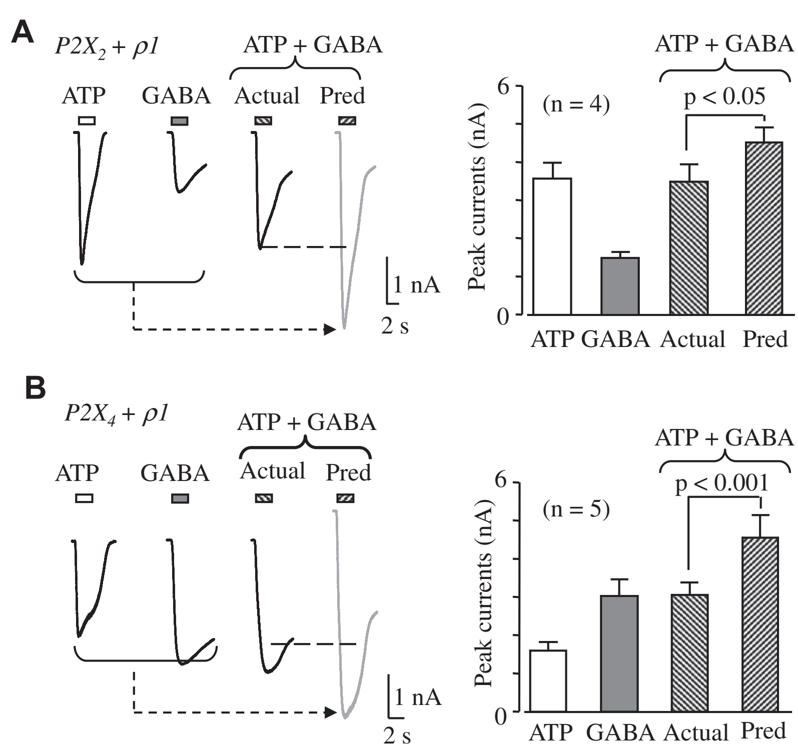

Fig. 2. Non-additive currents of co-activation of P2X and ρ1 receptors. *Left*, Representative currents in a cell co-expressing $P2X_2$ and ρ1 (A) or $P2X_4$ and ρ1 (B) evoked by 100 μM ATP, 10 μM GABA, or both agonists. The predicted currents (Pred) are arithmetic addition of the currents by separate application of ATP and GABA in the same cells. *Right*, Summary of agonist-evoked peak currents from all the cells.

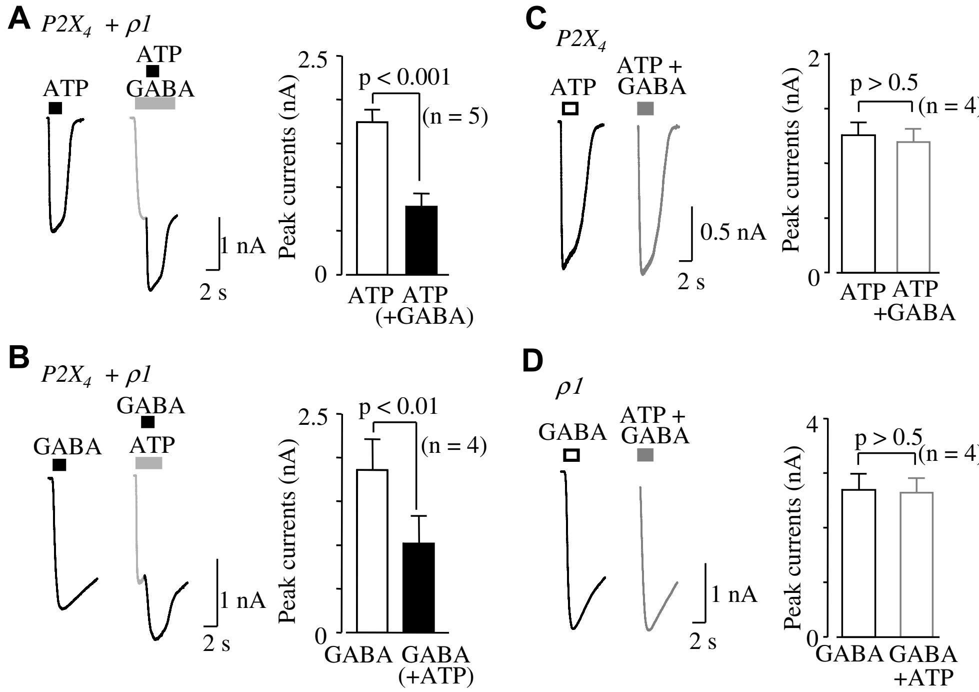

Fig. 3. Reciprocal inhibition between $P2X_4$ and ρ1 receptors. (A,B) *Left*, Representative currents in cells co-expressing $P2X_4$ and ρ1 evoked by 100 μM ATP in the absence or presence of ρ1 receptor activation (A), or by 10 μM GABA in the absence or presence of $P2X_4$ receptor activation (B). *Right*, Summary of agonist-evoked currents. (C, D), *Left*, Representative currents evoked by 100 μM ATP or co-application of 100 μM ATP and 10 μM GABA in a cell expressing $P2X_4$ alone (C), or evoked by 10 μM GABA or co-application of 10 μM GABA and 100 μM ATP in a cell expressing ρ1 alone (D). *Right*, Summary of currents from all the cells.

(2.7 ± 0.3 nA) were the same as I_{GABA} (2.7 ± 0.3 pA; $n = 4$) (Fig. 3D). Furthermore, there was no noticeable difference in the kinetics of activation, inactivation and deactivation (Fig. 3C–D). These results largely exclude the possibilities that direct allosteric modulation and indirect G-protein coupled receptor-mediated modulation contribute to the inhibitory interaction between $P2X_4$ and ρ1 receptors.

Biased inhibition of $P2X_4$ and ρ1 receptors by cross-receptor interaction

To investigate whether receptor activation and/or expression influences the functional interaction, we repeated the above experiments in cells co-expressing $P2X_4$ and ρ1 receptors that showed small currents in response to submaximal concentrations of ATP (10 μM) and GABA (3 μM). The mean I_{ATP} and I_{GABA} were 0.8 ± 0.2 nA and 0.5 ± 0.2 nA ($n = 5$), respectively. In those cells, receptor co-activation still resulted in significant non-additive currents (Fig. 4A) and the I_{ATP} were strongly inhibited during ρ1 receptor activation (Fig. 4B). However, the I_{GABA} were not suppressed during $P2X_4$ receptor activation (Fig. 4C). The results suggest that the cross-receptor interaction is biased, showing stronger inhibition of $P2X_4$ receptor.

Discussion

This study has shown that co-activation of $P2X_4$ and ρ1 receptors produced non-additive currents and such occlusion appears to result from reciprocal inhibition. Co-immunoprecipitation provides additional supporting evidence for their interaction. Taken together, the data show an inhibitory interaction between $P2X_4$ and ρ1 receptors.

Previous studies illustrate co-existence of $P2X_4$ and ρ1 receptors in many neurons and regions in the CNS (see introductory paragraph), suggesting that they may interact as the P2X and $GABA_A$ receptors in peripheral neurons [6–8]. However, $P2X_4$ receptor is

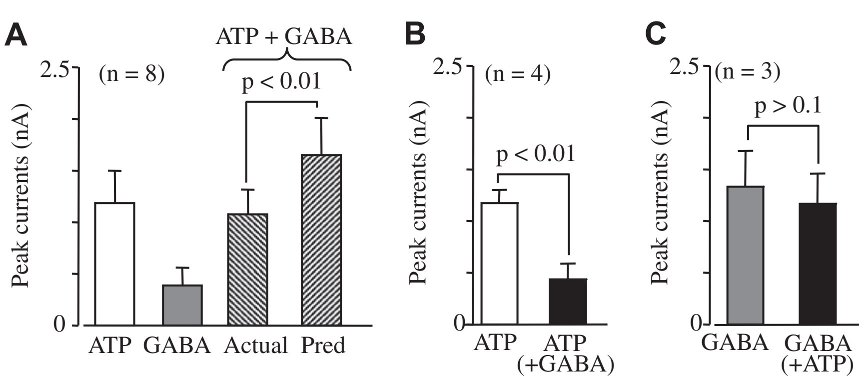

Fig. 4. Biased inhibition of $P2X_4$ and ρ1 receptors by cross-receptor interaction. (A) Summary of currents evoked by 10 μM ATP, 3 μM GABA, or co-application of both in cells expressing $P2X_4$ and ρ1 receptors. (B,C) Summary of currents evoked by ATP in the absence or presence of ρ1 receptor activation (B) and currents evoked by GABA in the absence or presence of $P2X_4$ receptor activation (C) in cells expressing $P2X_4$ and ρ1 receptors.

often co-expressed with several other P2X receptors [5] and currently no tools, including pharmacology [4], are available to distinguish them. Thus it remains intriguingly difficult to explore the interaction of native $P2X_4$ and ρ1 receptors. We used heterologous expression cells and provided functional evidence for inhibitory interaction between $P2X_4$ and ρ1 receptors (Fig. 2). The interaction between $P2X_4$ and ρ1 receptors is reciprocal as the one between $P2X_2$ and ρ1 receptors (Fig. 3) [39]. Nonetheless, the interaction between $P2X_4$ and ρ1 receptors seems biased, resulting in stronger inhibition of $P2X_4$ receptor (Fig. 4). Such information will help to better understand the P2X receptors interacting with $GABA_{A/C}$ receptors and, more importantly, to appreciate the physiology of P2X receptors.

There are two candidate explanations for the inhibitory interaction between $P2X_4$ and ρ1 receptors. The first one is that two receptors are closely associated and ion flux through one receptor inhibits activation of the other, as proposed for the interactions between P2X and $GABA_A$ receptors [6] and between P2X and nACh receptors [10]. The second possibility is that two receptors are coupled so that activation of one receptor induces allosteric conformational change in the other and consequently inhibits its activation. Such a mechanism governs the interactions between P2X and $GABA_{A/C}$ receptors [8,39,40], between $P2X_2$ and $5HT_{3A}$ receptors [15], and between $P2X_2$ and nACh receptors [13,41]. The protein–protein interaction between $P2X_4$ and ρ1 receptors, as shown by co-immunoprecipitation (Supplementary Figure 2), is compatible with both interpretations, and further studies are thus needed to distinguish them.

In summary, we provide functional evidence for an inhibitory interaction between $P2X_4$ and $GABA_C$ ρ1 receptors. These two receptors are widely expressed in the central nervous system, and therefore such negative cross-talk implies a potential novel mechanism modulating neuronal signaling mediated by these receptors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.07.096.

References

[1] B.S. Khakh, Molecular physiology of P2X receptors and ATP signaling at synapses, Nat. Rev. Neurosci. 2 (2001) 165–174.
[2] S.J. Moss, T.G. Smart, Constructing inhibitory synapses, Nat. Rev. Neurosci. 2 (2001) 240–250.
[3] D. Zhang, Z.H. Pan, M. Awobuluyi, S.A. Lipton, Structure and function of GABA(C) receptors: a comparison of native versus recombinant receptors, Trends Pharmacol. Sci. 22 (2001) 121–132.
[4] R.A. North, Molecular physiology of P2X receptors, Physiol. Rev. 82 (2002) 1013–10167.
[5] B.S. Khakh, R.A. North, P2X receptors as cell-surface ATP sensors in health and disease, Nature 442 (2006) 527–532.
[6] E. Sokolova, A. Nistri, R. Giniatullin, Negative cross talk between anionic GABAA and cationic P2X ionotropic receptors of rat dorsal root ganglion neurons, J. Neurosci. 21 (2001) 4958–4968.
[7] R. Karanjia, L.M. García-Hernández, M. Miranda-Morales, N. Somani, R. Espinosa-Luna, L.M. Montaño, C. Barajas-López, Cross-inhibitory interactions between GABAA and P2X channels in myenteric neurons, Eur. J. Neurosci. 23 (2006) 3259–3268.
[8] E. Toulmé, D. Blais, C. Léger, M. Landry, M. Garret, P. Séguéla, E. Boué-Grabot, An intracellular motif of P2X(3) receptors is required for functional cross-talk with GABA(A) receptors in nociceptive DRG neurons, J. Neurochem. 102 (2007) 1357–1368.
[9] K. Nakazawa, ATP-activated current and its interaction with acetylcholine-activated current in rat sympathetic neurons, J. Neurosci. 14 (1994) 740–750.
[10] C. Barajas-López, R. Espinosa-Luna, Y. Zhu, Functional interactions between nicotinic and P2X channels in short-term cultures of guinea-pig submucosal neurons, J. Physiol. 513 (1998) 671–683.
[11] T.J. Searl, R.S. Redman, E.M. Silinsky, Mutual occlusion of P2X ATP receptors and nicotinic receptors on sympathetic neurons of the guinea-pig, J. Physiol. 510 (1998) 783–791.
[12] X. Zhou, J.J. Galligan, Non-additive interaction between nicotinic cholinergic and P2X purine receptors in guinea-pig enteric neurons in culture, J. Physiol. 513 (1998) 685–697.
[13] B.S. Khakh, X. Zhou, J. Sydes, J.J. Galligan, H.A. Lester, State-dependent cross-inhibition between transmitter-gated cation channels, Nature 406 (2000) 405–410.
[14] C. Barajas-López, L.M. Montaño, R. Espinosa-Luna, Inhibitory interactions between 5-HT3 and P2X channels in submucosal neurons, Am. J. Physiol. Gastrointest. Liver Physiol. 283 (2002) G1238–G1248.
[15] E. Boué-Grabot, C. Barajas-López, Y. Chakfe, D. Blais, D. Bélanger, M.B. Emerit, P. Séguéla, Intracellular cross talk and physical interaction between two classes of neurotransmitter-gated channels, J. Neurosci. 23 (2003) 1246–1253.
[16] X. Bo, Y. Zhang, M. Nassar, G. Burnstock, R. Schoepfer, A P2X purinoceptor cDNA conferring a novel pharmacological profile, FEBS Lett. 375 (1995) 129–133.
[17] G. Buell, C. Lewis, G. Collo, R.A. North, A. Surprenant, An antagonist-insensitive P2X receptor expressed in epithelia and brain, EMBO J. 15 (1996) 55–62.
[18] G. Collo, R.A. North, E. Kawashima, E. Merlo-Pich, S. Neidhart, A. Surprenant, G. Buell, Cloning of P2X5 and P2X6 receptors and the distribution and properties of an extended family of ATP-gated ion channels, J. Neurosci. 16 (1996) 2495–2507.
[19] U. Brändle, E. Guenther, C. Irrle, T.H. Wheeler-Schilling, Gene expression of the P2X receptors in the rat retina, Brain Res. Mol. Brain Res. 59 (1998) 269–272.
[20] R. Jabs, E. Guenther, K. Marquordt, T.H. Wheeler-Schilling, Evidence for P2X(3), P2X(4), P2X(5) but not for P2X(7) containing purinergic receptors in Müller cells of the rat retina, Brain Res. Mol. Brain Res. 76 (2000) 205–210.
[21] T.H. Wheeler-Schilling, K. Marquordt, K. Kohler, R. Jabs, E. Guenther, Expression of purinergic receptors in bipolar cells of the rat retina, Brain Res. Mol. Brain Res. 76 (2000) 415–418.
[22] T.H. Wheeler-Schilling, K. Marquordt, K. Kohler, E. Guenther, R. Jabs, Identification of purinergic receptors in retinal ganglion cells, Brain Res. Mol. Brain Res. 92 (2001) 177–180.
[23] R. Enz, J.H. Brandstätter, H. Wässle, J. Bormann, Immunocytochemical localization of the GABAc receptor rho subunits in the mammalian retina, J. Neurosci. 16 (1996) 4479–4490.
[24] H.H. Yeh, E.V. Grigorenko, M.L. Veruki, Correlation between a bicuculline-resistant response to GABA and GABAA receptor rho 1 subunit expression in single rat retinal bipolar cells, Vis. Neurosci. 13 (1996) 283–292.
[25] E.L. Fletcher, P. Koulen, H. Wässle, GABAA and GABAC receptors on mammalian rod bipolar cells, J. Comp. Neurol. 396 (1998) 351–365.
[26] P. Koulen, J.H. Brandstätter, R. Enz, J. Bormann, H. Wässle, Synaptic clustering of GABA(C) receptor rho-subunits in the rat retina, Eur. J. Neurosci. 10 (1998) 115–127.
[27] K. Wegelius, M. Pasternack, J.O. Hiltunen, C. Rivera, K. Kaila, M. Saarma, M. Reeben, Distribution of GABA receptor rho subunit transcripts in the rat brain, Eur. J. Neurosci. 10 (1998) 350–357.
[28] R. Enz, G.R. Cutting, GABAC receptor rho subunits are heterogeneously expressed in the human CNS and form homo- and heterooligomers with distinct physical properties, Eur. J. Neurosci. 11 (1999) 41–50.
[29] A. Greka, S.A. Lipton, D. Zhang, Expression of GABA(C) receptor rho1 and rho2 subunits during development of the mouse retina, Eur. J. Neurosci. 12 (2000) 3575–3582.
[30] A. Rozzo, M. Armellin, J. Franzot, C. Chiaruttini, A. Nistri, E. Tongiorgi, Expression and dendritic mRNA localization of GABAC receptor rho1 and rho2 subunits in developing rat brain and spinal cord, Eur. J. Neurosci. 15 (2002) 1747–1758.
[31] W. Zheng, W. Xie, J. Zhang, J.A. Strong, L. Wang, L. Yu, M. Xu, L. Lu, Function of gamma-aminobutyric acid receptor/channel rho 1 subunits in spinal cord, J. Biol. Chem. 278 (2003) 48321–48329.
[32] F. Didelon, M. Sciancalepore, N. Savic, M. Mladinic, A. Bradbury, E. Cherubini, Gamma-aminobutyric acid A rho receptor subunits in the developing rat hippocampus, J. Neurosci. Res. 67 (2002) 739–744.
[33] B. Liu, N. Hattori, B. Jiang, Y. Nakayama, N.Y. Zhang, B. Wu, K. Kitagawa, M. Taketo, H. Matsuda, C. Inagaki, Single cell RT-PCR demonstrates differential expression of GABAC receptor rho subunits in rat hippocampal pyramidal and granule cells, Brain Res. Mol. Brain Res. 123 (2004) 1–6.
[34] A. Alakuijala, J. Alakuijala, M. Pasternack, Evidence for a functional role of GABA receptors in the rat mature hippocampus, Eur. J. Neurosci. 23 (2006) 514–520.
[35] S. Kirischuk, J. Akyeli, R. Iosub, R. Grantyn, Pre- and postsynaptic contribution of GABAC receptors to GABAergic synaptic transmission in rat collicular slices and cultures, Eur. J. Neurosci. 18 (2003) 752–758.
[36] E. Boue-Grabot, M. Roudbaraki, L. Bascles, G. Tramu, B. Bloch, M. Garret, Expression of GABA receptor rho subunits in rat brain, J. Neurochem. 70 (1998) 899–907.
[37] M. Schmidt, M. Boller, G. Ozen, W.C. Hall, Disinhibition in rat superior colliculus mediated by GABAc receptors, J. Neurosci. 21 (2001) 691–699.

[38] K. Schlicker, M. Boller, M. Schmidt, GABAC receptor mediated inhibition in acutely isolated neurons of the rat dorsal lateral geniculate nucleus, Brain Res. Bull. 63 (2004) 91–97.
[39] E. Boué-Grabot, M.B. Emerit, E. Toulmé, P. Séguéla, M. Garret, Cross-talk and co-trafficking between rho1/GABA receptors and ATP-gated channels, J. Biol. Chem. 279 (2004) 6967–6975.
[40] E. Boué-Grabot, E. Toulmé, M.B. Emerit, M. Garret, Subunit-specific coupling between gamma-aminobutyric acid type A and P2X2 receptor channels, J. Biol. Chem. 279 (2004) 52517–52525.
[41] B.S. Khakh, J.A. Fisher, R. Nashmi, D.N. Bowser, H.A. Lester, An angstrom scale interaction between plasma membrane ATP-gated P2X2 and alpha4beta2 nicotinic channels measured with fluorescence resonance energy transfer and total internal reflection fluorescence microscopy, J. Neurosci. 25 (2005) 6911–6920.
[42] L.-H. Jiang, F. Rassendren, A. Mackenzie, Y.H. Zhang, A. Surprenant, R.A. North, *N*-Methyl-D-glucamine and propidium dyes utilize different permeation pathways at rat P2X(7) receptors, Am. J. Physiol. Cell Physiol. 289 (2005) C1295–C1302.
[43] V. Spelta, L.-H. Jiang, A. Surprenant, R.A. North, Kinetics of antagonists at $P2X_{2/3}$ receptors, Br. J. Pharmacol. 135 (2002) 1524–1530.
[44] Z.Z. Mei, R. Xia, D.J. Beech, L.-H. Jiang, Intracellular coiled-coil domain engaged in subunit interaction and assembly of melastatin-related transient receptor potential channel 2, J. Biol. Chem. 281 (2006) 38748–38756.
[45] D. Zhang, Z.H. Pan, X. Zhang, A.D. Brideau, S.A. Lipton, Cloning of a gamma-aminobutyric acid type C receptor subunit in rat retina with a methionine residue critical for picrotoxinin channel block, Proc. Natl. Acad. Sci. USA 92 (1995) 11756–11760.
[46] N. Filippova, A. Sedelnikova, W.J. Tyler, T.L. Whitworth, H. Fortinberry, D.S. Weiss, Recombinant GABA(C) receptors expressed in rat hippocampal neurons after infection with an adenovirus containing the human rho1 subunit, J. Physiol. 535 (2001) 145–153.
[47] S. Ortinau, B. Laube, H. Zimmermann, ATP inhibits NMDA receptors after heterologous expression and in cultured hippocampal neurons and attenuates NMDA-mediated neurotoxicity, J. Neurosci. 23 (2003) 4996–5003.
[48] J.B. Schachter, S.M. Sromek, R.A. Nicholas, T.K. Harden, HEK293 human embryonic kidney cells endogenously express the P2Y1 and P2Y2 receptors, Neuropharmacology 36 (1997) 1181–1187.
[49] E. Sokolova, A. Nistri, R. Giniatullin, The ATP-mediated fast current of rat dorsal root ganglion neurons is a novel effector for GABA(B) receptor activation, Neurosci. Lett. 338 (2003) 181–184.