0026-895X/98/060989-05$3.00/0

MOLECULAR PHARMACOLOGY, **54:**989–993 (1998).

Co-Expression of $P2X_1$ and $P2X_5$ Receptor Subunits Reveals a Novel ATP-Gated Ion Channel

GONZALO E. TORRES, WILLIAM R. HAINES, TERRANCE M. EGAN, and MARK M. VOIGT

Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis, Missouri 63104

Received July 31, 1998; Accepted August 25, 1998

This paper is available online at http://www.molpharm.org

ABSTRACT

P2X receptors are a family of ion channels gated by extracellular ATP. Each member of the family can form functional homomeric channels, but only $P2X_2$ and $P2X_3$ have been shown to combine to form a unique heteromeric channel. Data from *in situ* hybridization studies suggest that $P2X_1$ and $P2X_5$ may also co-assemble. In this study, we tested this hypothesis by expressing recombinant $P2X_1$ and $P2X_5$ receptor subunits either individually or together in human embryonic kidney 293 cells. In cells expressing the homomeric $P2X_1$ receptor, 30 μM α,β-methylene ATP (α,β-me-ATP) evoked robust currents that completely desensitized in less than 1 sec, whereas α,β-me-ATP failed to evoke current in cells expressing the homomeric $P2X_5$ receptor. By contrast, α,β-me-ATP evoked biphasic currents with a pronounced nondesensitizing plateau phase in cells that co-expressed both subunits. Further, the EC_{50} for α,β-me-ATP was greater in cells expressing both $P2X_1$ and $P2X_5$ than in cells expressing $P2X_1$ alone (5 and 1.6 μM, respectively). Heteromeric assembly was confirmed using a co-immunoprecipitation assay of epitope-tagged $P2X_1$ and $P2X_5$ subunits. In summary, this study provides biochemical and functional evidence of a novel channel formed by P2X subunit heteropolymerization. This finding suggests that heteromeric subunit assembly constitutes an important mechanism for generating functional diversity of ATP-mediated responses.

P2X receptors are ATP-gated ion channels that mediate a diverse array of physiological actions. They have been found in a variety of tissues, including smooth muscle, peripheral neurons, and the central nervous system (Bean, 1992). To date, seven P2X receptor subunits have been identified by cDNA cloning (Soto *et al.*, 1997). When expressed in either *Xenopus laevis* oocytes or mammalian cells, these cloned receptors form functional homomeric channels that conduct a nonselective cation current in response to extracellular ATP (Burnstock, 1997).

The phenotypes associated with activation of the individual recombinant P2X receptors display distinctive pharmacological and biophysical properties that can be grouped into four classes. First, $P2X_1$ and $P2X_3$ receptors desensitize rapidly and are sensitive to both the agonist α,β-me-ATP and the antagonist PPADS. Second, $P2X_2$ and $P2X_5$ receptors desensitize slowly in response to ATP and are not activated by $\alpha\beta$-me-ATP but are antagonized by PPADS. Third, $P2X_4$ and $P2X_6$ also desensitize slowly but are insensitive to both α,β-me-ATP and PPADS. Finally, $P2X_7$ is much less sensitive to MgATP and is the only P2X receptor reported to be able to form a large ionic "super" pore.

All of these properties have been used to identify the presence of subunits in native tissues. For example, the properties of the native P2X response of rat salivary gland (i.e., slowly desensitizing receptors insensitive to α,β-me-ATP and PPADS) match that of the cloned $P2X_4$ receptor, which is in turn the only known P2X receptor expressed in this tissue (Buell *et al.*, 1996). However, in most cases, the phenotypes observed in native tissues do not closely resemble those reported for the cloned subunits (Edwards *et al.*, 1992; Edwards, 1994). These poor matches suggest that additional subunits might account for these responses. Alternatively, heteropolymerization of P2X subunits might occur in native tissues, as has been demonstrated for $P2X_2$ and $P2X_3$ (Lewis *et al.*, 1995). When co-expressed in HEK 293 cells, these subunits co-assemble to form a novel channel with distinct functional properties similar to those seen in sensory neurons (Khakh *et al.*, 1995; Lewis *et al.*, 1995).

Given the high amino acid homology among the members of the P2X family and the demonstration that several P2X subunits are expressed in the same tissues, it is tempting to speculate that heteromeric receptor complexes are a widespread phenomenon among the P2X receptor family. One possible combination suggested by *in situ* hybridization stud-

Supported by National Institutes of Health Grants HL56236 (T.M.E.) and NS35534 (M.M.V.) and an American Heart Association–Missouri Affiliate predoctoral fellowship (W.R.H). G.E.T. and W.R.H. contributed equally to this work.

ABBREVIATIONS: α,β-me-ATP, α,β-methylene ATP; PPADS, pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid; HA, hemagglutinin; HEK, human embryonic kidney; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TBST, Tris-buffered saline/Tween 20.

ies is a complex of $P2X_1$ and $P2X_5$ that has overlapping patterns of expression in the ventral horn of the spinal cord (Collo *et al.*, 1996). In this report, we demonstrate that co-expression of $P2X_1$ and $P2X_5$ receptor subunits in mammalian cells results in heteromeric ATP-gated channels with unique pharmacological and biophysical properties.

Materials and Methods

DNA constructs. The $P2X_1$ receptor cDNA was cloned from a rat heart cDNA library provided by Dr. M. Tamkun (Vanderbilt University, Nashville, TN). $P2X_5$ receptor cDNA was a gift of Dr. G. Buell (Glaxo Institute for Molecular Biology, Plan-les-Ouates, Geneva, Switzerland). Epitopes were introduced into full-length P2X subunits immediately upstream of the stop codon using polymerase chain reaction. The FLAG epitope (DYKDDDDK) was inserted into $P2X_1$ ($P2X_1$-FLAG) and a HA epitope (YPYDVPDYA) was inserted into $P2X_5$ ($P2X_5$-HA). Epitope-tagged subunits were subcloned into pRK-5 and verified by oligonucleotide sequencing.

Cell culture and transfection. HEK 293 cells were transiently transfected with wild type or epitope-tagged $P2X_1$ and $P2X_5$ receptor cDNAs by incubating the cells with 1 μg of total cDNA mixed with 6 μl of Lipofectamine (GIBCO BRL, Grand Island, NY) in 1 ml of serum-free medium. After 5 hr at 37°, the medium was replaced with minimal essential medium. Transfected cells were analyzed 24–48 hr later. For co-transfections, 0.5 μg of each plasmid was mixed and used in the transfection reaction.

Electrophysiology. A suspension of transiently transfected cells was made by agitating the solution bathing the cells attached to the bottom of a single culture dish using a fire-polished Pasteur pipette. Whole-cell voltage clamp was performed as described previously (Egan *et al.*, 1998; Torres *et al.*, 1998). Whole-cell current was recorded from single cells held at −40 mV using an AxoPatch 200A amplifier and low resistance electrodes (1–2 MΩ) (Axon Instruments, Foster City, CA). Recording pipettes were filled with the following intracellular solution: 150 mM CsCl, 10 mM tetraethylammonium-Cl, 5 mM EGTA, 10 mM HEPES, pH 7.4 with CsOH. The bath solution was 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, 10 mM HEPES, pH 7.4 with NaOH. Drugs were applied by manually moving the electrode and attached cell into the line of flow of solutions exiting one of an array of inlet tubes. Data averages are expressed as mean ± standard error. Each experiment was repeated at least three times. Raw data were analyzed and plotted off-line using IgorPro (Wavemetrics, Lake Oswego, OR). The EC_{50} and Hill slope values (and their 95% confidence limits) were determined from plots of peak current amplitudes versus agonist concentrations using InPlot (GraphPAD Software, San Diego, CA) and pooled data from separate experiments.

Immunoprecipitation and Western blotting. Confluent monolayers of HEK 293 cells in 35-mm dishes were washed three times with phosphate-buffered saline and incubated in solubilization buffer [phosphate-buffered saline (136 mM NaCl, 2.7 mM KCl, 12 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4), 1% nonidet P-40, 1 mM phenymethylsulfonyl fluoride, 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, 10 μg/ml leupeptin] at 4° for 1 hr. Immunoprecipitation was carried out using the M2 anti-FLAG antibody (5 μg/ml) in the presence of 50 μl of Protein G Gamma-Bind agarose. Immunoprecipitates were washed five times with solubilization buffer and resuspended in protein sample buffer. Samples were boiled for 5 min and proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by transfer to nitrocellulose filters. The filters were blocked overnight in TBST (20 mM Tris pH 7.6, 145 mM NaCl, 0.05% Tween 20) containing 2% bovine serum albumin, and incubated for 1 hr with primary antibody (M2 anti-FLAG, 10 μg/ml, or anti-HA 1:1000). After several washes with TBST, filters were incubated with peroxidase -conjugated sheep anti-mouse antibody for 1 hr. Filters were washed extensively in TBST and immunoreactivity was detected with the enhanced chemiluminescence detection kit following the manufacturer's suggestions.

Drugs and supplies. ATP and α,β-me-ATP were obtained from Sigma (St. Louis, MO). Enzymes for cloning and sequencing were obtained from Promega (Madison, WI). Vent DNA polymerase used for polymerase chain reaction-based mutagenesis was purchased from New England Biolabs (Beverly, MA), minimal essential medium, glutamine, fetal bovine serum, lipofectamine, and oligonucleotides were obtained from GIBCO BRL. Gel extraction kit, and plasmid DNA isolation kit were from Qiagen (Valencia, CA). Protein G Gamma-Bind agarose was from Amersham Pharmacia Biotech (Piscataway, NJ), and [^{35}S]dATP for sequencing, enhanced chemiluminescence detection reagents, and anti-mouse IgG/horseradish peroxidase conjugate were from Amersham (Indianapolis IN). M2 anti-FLAG monoclonal antibody was from Kodak (New Haven, CT), and mouse anti-HA antibody was from Babco (Richmond, CA).

Results and Discussion

In situ hybridization and Northern blot studies suggest that $P2X_1$ and $P2X_5$ subunits are possible candidates to co-assemble into functional ATP-gated channels. mRNA for

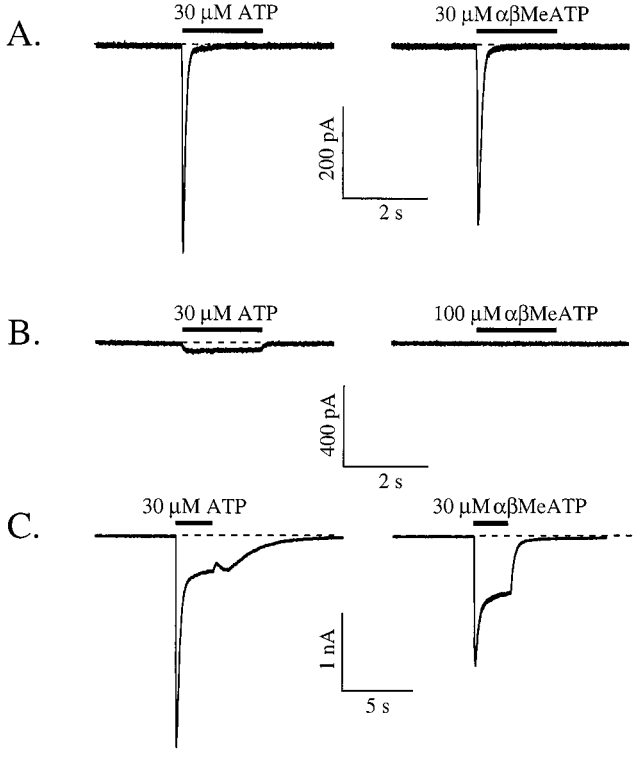

Fig. 1. Co-expression of $P2X_1$ and $P2X_5$ receptor subunits results in a unique phenotype. The agonists ATP and α,β-me-ATP were applied to cells expressing either or both $P2X_1$ and $P2X_5$ receptors. Typical responses are shown. A, A cell transfected with cDNA encoding $P2X_1$ alone showed rapidly desensitizing currents in response to application of either ATP or α,β-me-ATP. B, A different cell transfected with cDNA encoding the $P2X_5$ receptor gave a weak current in response to 30 μM ATP but failed to respond to 100 μM α,β-me-ATP. C, A cell transfected with both cDNAs showed biphasic currents in response to applications of either agonist. ATP caused rapid gating of inward current that quickly fell to a sustained plateau. Removing ATP was always accompanied by generation of an inward "bump" current that decayed slowly over a time course of about 5 sec. The cause of this current component is unknown. The response to 30 μM α,β-me-ATP resembled that evoked by ATP, although this concentration of α,β-me-ATP never generated the bump current seen upon washout of ATP. The different response to α,β-me-ATP of these three typical cells demonstrates the existence of unique phenotypes for $P2X_1$, $P2X_5$, and heteromeric $P2X_1/P2X_5$

both subunits are expressed in heart, sensory ganglia, and spinal cord tissue. Indeed, in cells of the cervical spinal cord, the expression pattern of $P2X_1$ matched that of $P2X_5$ (Collo *et al.*, 1996). Therefore, we examined the possibility that $P2X_1$ and $P2X_5$ subunits can co-assemble into functional channels when co-expressed in HEK 293 cells.

The homomeric channels formed by either $P2X_1$ or $P2X_5$ have distinct pharmacological and biophysical properties. Fig. 1A shows $P2X_1$-mediated currents activated by either ATP or α,β-me-ATP. These currents activated rapidly and underwent fast and complete desensitization. By contrast, ATP-gated currents desensitized slowly in cells that expressed $P2X_5$, and α,β-me-ATP was ineffective (Fig. 1B). We then compared the responses of the homomeric receptors to those seen in cells co-transfected with cDNAs encoding both subunits. In cells co-expressing $P2X_1$ and $P2X_5$ receptors, whole-cell recordings revealed an ion channel whose phenotype differed from those of either homomeric receptors. Like both $P2X_1$ and $P2X_5$, ATP evoked a quickly developing inward current in co-transfected cells held at −40 mV (Fig. 1C). The size of the current depended on the concentration of α,β-me-ATP applied (Fig. 2A). Superficially, the pattern of

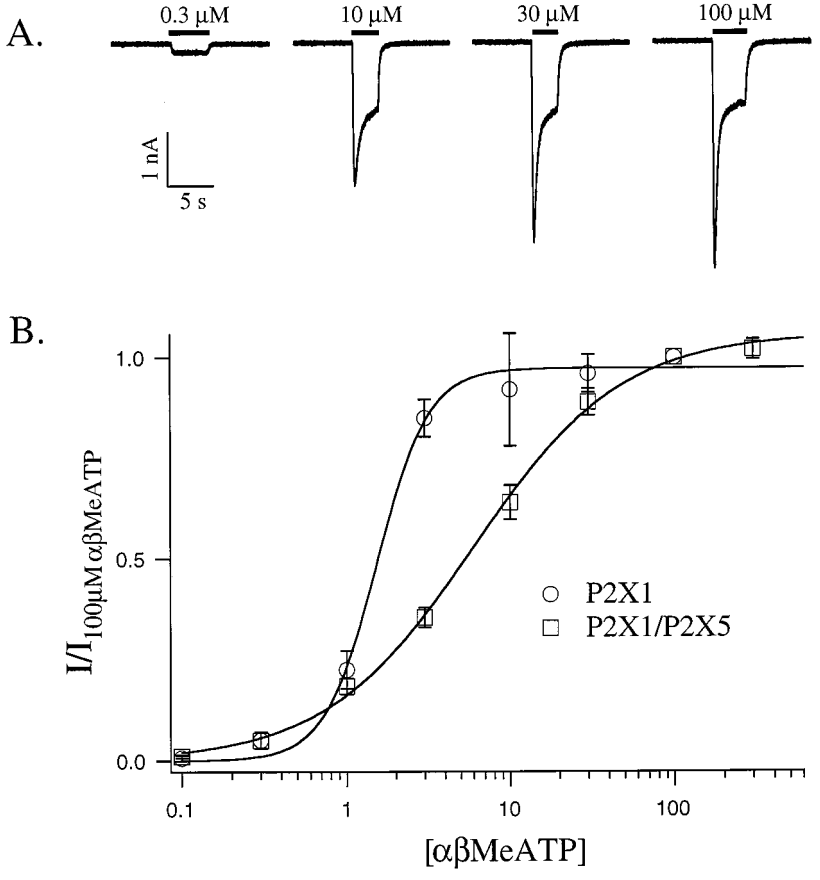

Fig. 2. Dose-response curves for homomeric $P2X_1$ and heteromeric $P2X_1/P2X_5$. A, Raw data traces for application of different concentrations of α, β-me-ATP to a cell expressing heteromeric $P2X1/P2X_5$ receptors. B, Dose-response curves for $P2X_1$ and $P2X_1/P2X_5$ receptors. *Plots*, averaged data obtained from 24 individual cells. In each cell, peak currents evoked by a range of concentrations of α,β-me-ATP were normalized to that obtained with 100 μM α,β-me-ATP. *Points*, average of at least three separate experiments. The solid line is the best fit of the average data to I_{max} $([\alpha,\beta\text{-me-ATP}]^{nH}/([\alpha,\beta\text{-me-ATP}]^{nH} + EC_{50})^{nH})$, where n_H is the Hill coefficient. Cells transfected with cDNA encoding $P2X_5$ receptor alone did not respond to 100–300 μM α,β-me-ATP (three experiments, data not shown).

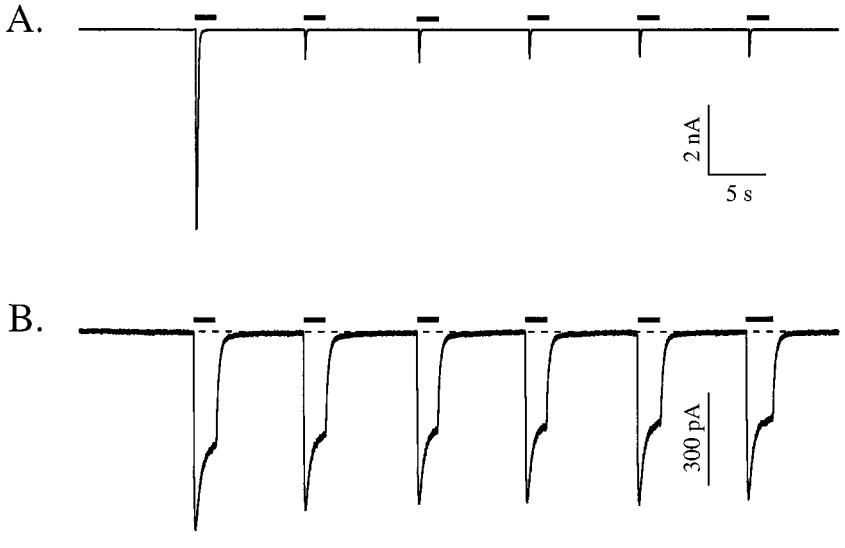

Fig. 3. Peak current amplitude decreases during repeated applications of α,β-me-ATP to cells transfected with $P2X_1$ but not $P2X_1$ and $P2X_5$ receptor subunits cDNAs. α,β-me-ATP (30 μM) was applied for about 1–2 sec once every 10 sec in cells expressing either $P2X_1$ alone or both $P2X_1$ and $P2X_5$. Both traces are shown at the same time scale. A, In cells expressing only $P2X_1$, peak current amplitude was reduced by about 80% after the first agonist application. B, Remarkably less desensitization was seen in cells co-expressing both $P2X_1$ and $P2X_5$.

the response resembled that expected for a combination of currents through homomeric $P2X_1$ and $P2X_5$. That is, the response to ATP was biphasic and consisted of an initial current "spike" (as expected for a homomeric $P2X_1$ response) followed by a smaller sustained plateau current (as expected for a homomeric $P2X_5$ response). However, several lines of data suggest a unique phenotype. First, α,β-me-ATP also evoked a biphasic current, and this would not be expected for a combination of homomeric $P2X_1$ and $P2X_5$ because the latter receptor is insensitive to this drug. Second, cells co-transfected with both $P2X_1$ and $P2X_5$ were less sensitive to α,β-me-ATP than were cells expressing $P2X_1$ (Fig. 2B). Both the EC_{50} (1.6 μM, 1.3–1.9) and the Hill slope (2.6, 1.7–3.5) values of the pooled raw data from cells transfected with $P2X_1$ alone differed from those measured in cells co-transfected with both $P2X_1$ and $P2X_5$ (EC_{50}, 5 μM, 4.3–6.2; n_H, 1.1, 0.9–1.3). This disparity in the Hill slopes could have several different underlying causes: there are fewer α,β-me-ATP responsive subunits than nonresponsive subunits present in the heteromeric assembly, heteromultimerization alters the cooperativity properties of α,β-me-ATP, or that there are different dose-response curves reflecting different subunit stoichiometries that are partially superimposed. In any event, the mechanism(s) involved do not alter the interpretation of the results. Third, the rate of recovery of peak current during repeated applications of α,β-me-ATP was quicker in cells expressing heteromultimeric $P2X_1/P2X_5$ receptors than in those expressing only $P2X_1$. This was shown by applying 30 μM α,β-me-ATP repeatedly for approximately 1–2 sec followed by an 8-sec wash to cells expressing either $P2X_1$ alone or both $P2X_1$ and $P2X_5$ (Fig. 3). This protocol resulted in a profound reduction in peak agonist response after a single application in cells transfected with $P2X_1$ only (Fig. 3A), whereas the current after multiple applications of α,β-me-ATP to HEK 293 cells expressing both subunits were maintained at about 80% of the initial amplitude (Fig. 3B). This latter decrease could reflect either an inherent property of $P2X_1/P2X_5$ heteromers or the presence of a small population of homomeric $P2X_1$ receptors. Taken together, these three findings strongly suggest the formation of heteromultimeric $P2X_1/P2X_5$ receptors

To provide direct demonstration of the heteropolymerization between $P2X_1$ and $P2X_5$ subunits, we performed co-immunoprecipitation experiments. This assay is based on the specific affinity of an anti-tag antibody for an epitope-tagged protein. $P2X_1$ and $P2X_5$ were both tagged with different epitopes and transfected individually or in combination in HEK 293 cells. As seen in Fig. 4A, α,β-me-ATP-gated currents recorded from cells expressing the epitope-tagged P2X subunits alone or in combination were indistinguishable from those recorded from cells expressing the wild-type receptors. In addition, the anti-FLAG and anti-HA antibodies selectively immunoprecipitated $P2X_1$-FLAG or $P2X_5$-HA respectively. No detectable cross-reactivity was found between these two antibodies (Fig. 4B). We then

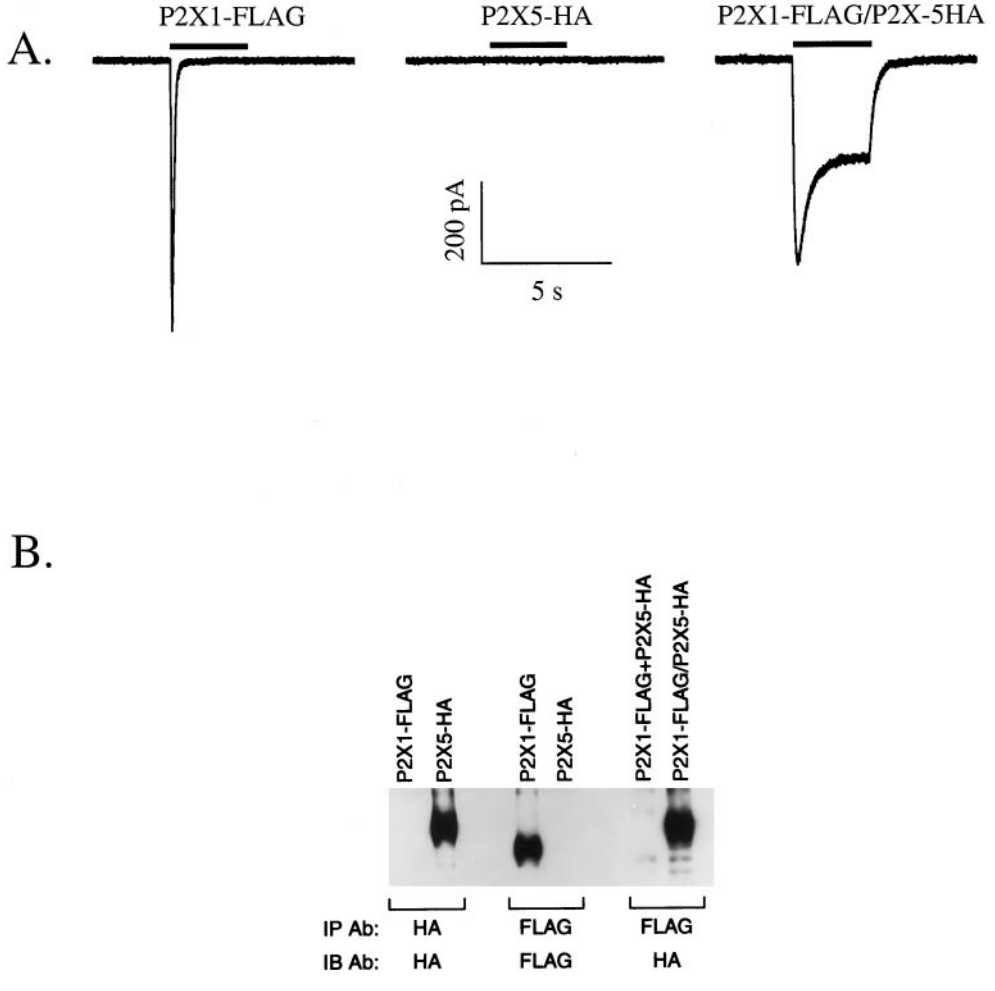

Fig. 4. Co-assembly between $P2X_1$ and $P2X_5$ receptor subunits. A, Functional expression of epitope-tagged $P2X_1$ and $P2X_5$ channels. Whole-cell recordings were obtained from HEK 293 cells transfected with the indicated constructs. The currents were elicited by 30 μM α,β-me-ATP from a holding potential of −40 mV. B, Co-immunoprecipitation of epitope-tagged $P2X_1$ and $P2X_5$. Lysates from HEK 293 cells previously transfected with the indicated constructs were immunoprecipitated, separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to nitrocellulose filters. *Lanes 1* and *2*, lysates were immunoprecipitated and immunoblotted with the anti-HA antibody; *lanes 3* and *4*, lysates were immunoprecipitated and immunoblotted with the anti-FLAG antibody; *lane 5*, lysates from cells expressing individual subunits were mixed, immunoprecipitated with the anti-FLAG antibody, and immunoblotted with the anti-HA antibody; *lane 6*, lysates from cells co-expressing $P2X_1$ and $P2X_5$ were immunoprecipitated with the anti-FLAG antibody and immunoblotted with the anti-HA antibody.

immunoprecipitated one subunit ($P2X_1$-FLAG) and then detected the other subunit ($P2X_5$-HA) by Western blot. Fig. 4B shows the results of the co-immunoprecipitation experiment in cells co-expressing both P2X subunits. After immunoprecipitation of $P2X_1$-FLAG, a strong signal corresponding to $P2X_5$-HA was detected. When lysates from cells expressing either subunit were mixed, no interaction was detected. Although it is possible that the relatively high levels of expression for the two proteins in co-transfected cells promotes nonspecific assembly, these results, taken together with the functional data, support the hypothesis that the two subunits do co-assemble into heteropolymeric assemblies.

Heteropolymerization of channel subunits has been suggested as a means of generating functional and molecular diversity (Green and Millar, 1995). Indeed, the formation of heteromeric channels has been amply demonstrated for many members of the transmitter-gated ion channel family such as nicotinic (Ragozzino *et al.*, 1997; Yu and Role, 1998), and glutamate ionotropic receptors (Boulter *et al.*, 1990), as well as voltage-dependent K^+ channels (Liao *et al.*, 1996; Wischmeyer *et al.*, 1997). In cells that co-express different channel subunits, the occurrence of heteromeric assemblies implies the existence of a variety of channel responses, each of which has potentially unique biophysical characteristics. This array of channel types might be critical for the regulation of cellular processes. ATP-mediated responses through the activation of P2X receptors are also affected by subunit co-assembly. $P2X_2/P2X_3$ and now $P2X_1/P2X_5$ heteromeric channels are good examples of such a heteropolymerization process. Because a wide variety of tissues and cell types express several P2X subunits, other combinations among P2X receptor subunits are likely to occur.

In summary, this report presents several lines of evidence supporting the notion that heteropolymerization occurs between $P2X_1$ and $P2X_5$ receptor subunits. First, whole-cell recordings in HEK-293 cells that co-express P2X1 and P2X5 subunits revealed an α,β-me-ATP-gated ion channel with unique biophysical properties that distinguish the channel from those observed in cells expressing either subunit alone. Second, receptors formed by $P2X_1$ and $P2X_5$ were less sensitive to the agonist α,β me-ATP compared with homomeric $P2X_1$ receptors. Third, peak current amplitude changed little during closely spaced repeated applications of α,β-me-ATP in cells expressing the $P2X_1/P2X_5$ heteromeric channel, which contrasts sharply with the profound decrease in peak current seen for the homomeric $P2X_1$ receptor. In addition, co-immunoprecipitation experiments provide biochemical evidence for protein-protein interaction between these two subunits. Although the results presented in this report do not prove the formation of a heteromeric channel between $P2X_1$ and $P2X_5$ *in vivo*, our findings provide a new P2X phenotype that can be used as a template for elucidating the molecular identities of native P2X receptor channels.

Acknowledgments

We are grateful to Dr. M. Tamkun for providing the rat heart library used to clone $P2X_1$. We also thank Dr. G. Buell for $P2X_5$ cDNA and Drs. R. Mercer and W. Hatfield for their helpful advice on the immunoprecipitation experiments.

References

Bean BP (1992) Pharmacology, and electrophysiology of ATP-activated ion channels. *Trends Pharmacol Sci* **13:**87–90

Boulter J, Hollmann M, O'Shea-Greenfield A, Hartley M, Deneris E, Maron C, and Heinemann S (1990) Molecular cloning, and functional expression of glutamate receptor subunit genes. *Science (Washington DC)* **249:**1033–1037.

Buell G, Lewis C, Collo G, North RA, and Surprenant A (1996) An antagonist-insensitive P2X receptor expressed in epithelia, and brain. *EMBO (Eur Mol Biol Organ) J* **15:**55–62.

Burnstock G (1997) The past, present, and future of purine nucleotides as signaling molecules. *Neuropharmacology* **36:**1127–1139.

Collo G, North RA, Kawashima E, Merlo-Pich E, Neidhart S, Surprenant A, and Buell G (1996) Cloning of P2X5, and P2X6 receptors, and the distribution, and properties of an extended family of ATP-gated ion channels. *J Neurosci* **16:**2495–2507.

Edwards FA (1994) ATP receptors. *Curr Opin Neurobiol* **4:**347–352.

Edwards FA, Gibb AJ, and Colquhoun D (1992) ATP receptor mediated synaptic currents in the central nervous system. *Nature (Lond)* **359:**144–147.

Egan TM, Haines WR, Voigt MM (1998) A domain contributing to the ion channel of ATP-gated P2X2 receptors identified by the substituted cysteine accessibility method. *J Neurosci* **18:**2350–2359.

Green WN and Millar NS (1995) Ion-channel assembly. *Trends Neurosci* **18:**280–287.

Khakh BS, Humphery PPA, and Surprenant A (1995) Electrophysiological properties of the P_{2X}-purinoceptors in rat superior cervical, nodose, and guinea-pig coeliac neurones. *J Physiol (Lond)* **484:**385–395.

Lewis CS, Neidhart C, Holy R, North A, Buell G, and Surprenant A (1995) Coexpression of $P2X_2$, and $P2X_3$ receptors subunits can account for ATP-gated currents in sensory neurons. *Nature (Lond)* **377:**432–435.

Liao YJ, Jan YN, and Jan LY (1996) Heteromultimerization of G-protein-gated inwardly rectifying K+ channel proteins GIRK1, and GIRK2, and their altered expression in weaver brain. *J Neurosci* **16:**7137–7150.

Ragozzino D, Fucile S, Giovannelli A, Grassi F, Mileo AM, Ballivet M, Alema S, and Eusebi F (1997) Functional properties of neuronal nicotinic acetylcholine receptor channels expressed in transfected human cells. *Eur J Neurosci* **9:**480–488.

Soto F, Garcia-Guzman M, and Stuhmer W (1997) Cloned ligand-gated channels activated by extracellular ATP (P2X receptors). *J Membr Biol* **160:**91–100.

Torres GE, Egan TM, and Voigt MM (1998) Topological analysis of the ATP-gated ionotropic P2X2 receptor subunit. *FEBS Lett* **425:**19–23.

Wischmeyer E, Doring F, Wischmeyer E, Spauschus A, Thomzig A, Veh R, and Karschin A (1997) Subunit interactions in the assembly of neuronal Kir3.0 inwardly rectifying K+ channels. *Mol Cell Neurosci* **9:**194–206.

Yu CR and Role LW (1998) Functional contribution of the *alpha* 7 subunit to multiple subtypes of nicotinic receptors in embryonic chick sympathetic neurons. *J Physiol (Lond)* **509:**651–665.

Send reprint requests to: Dr. Mark M. Voigt, Department of Pharmacological and Physiological Science, St. Louis University School of Medicine, 1402 South Grand Blvd. Saint Louis, MO 63104. E-mail: voigtm@slu.edu