Differential Assembly of Rat Purinergic P2X$_7$ Receptor in Immune Cells of the Brain and Periphery

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ATP-gated P2X$_7$ purinoceptors are found in most immune cells of the periphery and the brain where their activation leads to multiple downstream events such as cell permeabilization, apoptosis, and/or cytokine release. P2X$_7$ receptors do not form heteromeric receptors with any of the other six P2X subunits, and it is not known what type of homomeric assemblies the P2X$_7$ subunit makes. We constructed and purified an ectodomain protein of the rat P2X$_7$ receptor (amino acids 60–233) and used this to generate a monoclonal antibody (Ab) with which to probe P2X$_7$ receptors in central and peripheral immune cells. In HEK cells expressing rat P2X$_7$ receptors, the Ab increased the maximum current evoked by BzATP by 3–8-fold with a 5-fold leftward shift in EC$_{50}$ concentration. This Ab recognized only a non-denatured, multimeric form of the receptor on blue native-PAGE but did not recognize the denatured form on SDS-PAGE. A C-terminal polyclonal P2X$_7$ Ab recognized both monomeric subunits on SDS-PAGE and a multimeric complex on blue native-PAGE in this heterologous expression system. With Western blotting using these two Abs, native P2X$_7$ receptors in peritoneal macrophage and bone marrow cells are shown to exist as a strongly bound multimeric complex, whereas P2X$_7$ receptors in brain glia and/or astrocytes appear to form only as monomeric subunits.

Like most neurotransmitters, extracellular ATP activates both metabotropic (G protein-coupled) receptors, the P2Y receptor family, and ionotropic (ligand-gated) receptors, the P2X receptor family (1). When compared with other ionotropic receptors (e.g. nicotinic), which are primarily or solely expressed in nerve and muscle, P2X receptors are unusual in their wide spread expression in both excitable and non-excitable cells (1–3). There are seven P2X receptors, six of which (P2X$_1$–P2X$_6$) are found in brain and peripheral neurons; additionally, mRNA for P2X$_1$, P2X$_2$, and the non-neuronal P2X$_{17}$ receptor are prominent in many immune cells, particularly monocytes, macrophage, bone marrow, and brain microglia (1–3).

The P2X$_7$ receptor shows 40–45% amino acid identity with any one of the other P2X receptors; it shares the overall membrane topology of two transmembrane domains, intracellular N and C termini, and the large ectodomain with conservation of the 10 extracellular cysteine residues, although its C-terminal is some 200 amino acids longer than other P2X receptors (1–3). However, the functional sequelae of P2X$_7$ receptor activation differ strikingly from other P2X receptors. Activation of P2X$_7$ receptors expressed in mammalian cells, such as HEK$^*$ or CHO cells, opens both a small cationic channel and a large (up to 900 Da) dye-permeable pore, followed within seconds by extensive membrane blebbing (4, 5); similar events occur upon activation of native P2X$_7$ receptors in some but not all immune cells expressing this receptor (6–8). The mechanism(s) underlying formation of the permeabilizing pore and other downstream events such as membrane blebbing are not known but the intracellular C-terminal domain is required (4). Moreover, whereas each of the other P2X receptors is capable of forming heteromeric proteins with various other subunits in a specific pattern, the P2X$_7$ subunit cannot heteropolymerize with any of the other subunits (9), even with the P2X$_2$ receptor with which it co-exists in monocytes, bone marrow, and many endothelia and epithelia (4, 10).

Biochemical evidence to date favors a trimeric stoichiometry for P2X$_7$ receptors expressed in oocytes although higher multiples of a trimeric architecture cannot be ruled out (11, 12). Other biochemical, biophysical, and mutagenesis data based on experiments with homomeric P2X$_7$ receptors are compatible with either a trimeric or tetrameric stoichiometry (13, 14, 15). No information yet exists on the multimeric nature of native P2X$_7$ receptors. We have expressed and purified a protein comprising the extracellular domain of the rat P2X$_7$ receptor and have used this protein to generate an ectodomain-specific monoclonal Ab to better probe membrane localization of native P2X$_7$ receptors in both peripheral and brain immune cells. Our studies have revealed striking differences in P2X$_7$ receptor assembly in cells of the peripheral immune system and the brain immune system.

EXPERIMENTAL PROCEDURES

Expression and Purification of P2X$_7$ Receptor Ectodomain

A bacterial expression vector, designated pP2X$_7$ecto, was constructed by ligation of a 792-base pair fragment from a polymerase chain reaction product, comprising amino acids 60–232 of rat P2X$_7$ (GenBank$^\text{TM}$ NM019256) into vector PET32 (Novagen). The plasmid construct was verified by DNA sequencing with BigDye terminator cycle sequencing kit (PE Applied Biosystems). BL21(DE3) Escherichia coli transformed with pP2X$_7$ecto was used for overexpression of ecto-P2X$_7$ protein. After overnight induction of protein expression by 1 mM IPTG (isopropyl-$\beta$-D-thiogalactopyranoside), the culture was centrifuged at 5,000 × g for 10 min. The bacterial pellet was resuspended (1.50 of the culture volume) in lysis buffer (50 mM Tris, pH 8.0, 200 mM NaCl, 2% Triton X-100). The cell lysate was loaded onto a 40 ml protein A Sepharose column equilibrated with 20 mM Tris, pH 8.0, 150 mM NaCl, 50% glycerol, and 0.5% Triton X-100. After extensive washing with the same buffer the preparative column was eluted with 20 mM Tris, pH 8.0, containing 500 mM NaCl. The eluted fractions were concentrated to 20 ml and loaded onto a 40 ml blue native-PAGE (BN-PAGE) gel. Sodium dodecyl sulfate (SDS-PAGE) gels were stained with Coomassie Blue to identify P2X$_7$ subunit bands. The P2X$_7$ protein was excised from the gel and eluted using a range of buffers containing different concentrations of urea.

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$^*$ The abbreviations used are: HEK, human embryonic kidney; BzATP, benzoyl-benzoyl-ATP; Ab, antibody; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; BN-PAGE, blue native-PAGE; PVDF, polyvinylidene difluoride; Trx, thioredoxin.
**Generation of Monoclonal Antibodies against Ecto-P2X7 Protein**

Monoclonal antibodies were generated against ecto-P2X7 protein by Sheffield Hybridomas (University of Sheffield). Primary screening was performed by immunohistochemistry on stably transfected HEK cells and by ELISA. Ecto-P2X7Ab was purified from hybridoma supernatant on a protein G-Sepharose affinity chromatography column.

**Preparation of Whole Cell Lysates and Membrane Fractions**

**From HEK Cells**—HEK cells stably transfected with rP2X7, or rP2X2 (5) were used for these experiments. To prepare whole-cell lysates, the cells were freeze-thawed three times and sonicated. For isolation of membrane fractions, the cells were grown in T75 flasks, washed with ice-cold PBS, and harvested. After brief centrifugation, the cells were resuspended in hypotonic lysis buffer containing 10 mM Tris-HCl (pH 7.5) and protease inhibitors (Complete®). After incubation on ice for 10 min, the cell suspension was homogenized by a Dounce homogenizer, adjusted to 155 mM NaCl, and subjected to ultracentrifugation at 60,000 × g for 1 h at 4 °C. The pellet (membrane fraction) was solubilized in solubilization buffer (20 mM dodecylmaltoside in PBS containing protease inhibitor) for 3 h at 4 °C.

**From Native Tissue**—Adult Wistar rats (43–45 days old) were used in all experiments. They were killed by excess CO2, brains rapidly removed, and placed in ice-cold PBS containing protease inhibitor. The cerebellum and hippocampi were dissected out and 400–500 mg were removed, and placed in ice-cold PBS containing protease inhibitor. The extracts were centrifuged for 30 min at 13,000 g. The resulting supernatant was supplemented with BN sample buffer to a final concentration of 10% glycerol, 0.2% Coomassie Blue R-250, and 50 mM Bis-Tris adjusted with HCl to pH 7.0. For the cathode buffer, 5 mg/ml of Coomassie Blue R-250 was used in 50 mM Tricine, 15 mM Bis-Tris, pH 7.0. Molecular weight standards (Combibek II, Roche Molecular Biochemicals) were applied to two different lanes on both borders of the gel. Either linear polyacrylamide gels from 4 to 15% or 7.5% (w/v) gels without stacking gel were performed at 4 °C and were transferred to PVDF membranes and immunoblotted with appropriate antibody. All experiments were repeated 3–7 times with similar results obtained throughout.

**Immunohistochemistry**

Rats were deeply anesthetized with halothane and perfused transaurically with 4% paraformaldehyde in 0.1 M PBS. Brains were removed, postfixed for a further 3 h, and placed in 30% sucrose in PBS to allow (6 h) brains to sink. The brains were cut parasagittally at a thickness of 40 μm and collected as free-floating sections in 0.1 M Tris-buffered saline (TBS, pH 7.4). Sections were rinsed, incubated in TBS containing 40% methanol, and 0.1% H2O2 for 15 min before rinsing with TBS. Following blocking of nonspecific sites with a solution containing either 5% goat serum (GS, for polyclonal antibodies) or 5% horse serum (HS; for monoclonal antibodies) in TBS with 0.3% Triton X-100 for 1 h, sections were incubated for 4 days at 4 °C in the blocking solution containing primary Ab (0.15 μg/ml). Control staining was performed either in the presence of corresponding IgG given at the same concentration as primary Abs or in the absence of primary Abs. Following several rinses in TBS, sections treated with polyclonal antibodies were incubated in peroxidase goat anti-rabbit immunoglobulin and those treated with monoclonal antibodies were incubated in rat- and mouse-adsorbed peroxidase horse anti-mouse immunoglobulin at a dilution of 1:400 of TBS with either 2% GS or HS, 0.3% Triton-X-100, 0.25% bovine serum albumin for 4 h at room temperature. Bound peroxidase was visualized by immersion of the sections in chromogen solution for 3–5 min. A similar procedure was used for perinuclear macrophage or bone marrow cells but incubation in antibodies (20 μg/ml) was made overnight. For HEK cells, Zamboni’s fixative (10–20 min) was applied, blocked in 5% goat serum in PBS with 2% Triton X-100 (30 min), and incubated with primary Ab for 2–3 h at room temperature or overnight at 4 °C. Cells were rinsed, and the appropriate secondary Ab applied for 1 h. Non-permeabilized cells were fixed with Zamboni’s fixative (10 min), blocked in 5% goat serum in PBS (30 min), and incubated with primary Ab for 2 h at room temperature, rinsed, incubated with secondary Ab for 1 h. The following primary Abs were used: rabbit polyclonal anti-P2X7 (10 μg/ml); anti-P2X2 (0.6 μg/ml), anti-P2X2 (0.6 μg/ml), anti-P2X3 (0.6 μg/ml), all from Alomone Labs, mouse monoclonal anti-EYMPME (2 μg/ml; from BabCO, Richmond, CA) and rat ecto-P2X7Ab (0.12 μg/ml).

**Electrophysiology**

Standard whole cell recordings were carried out as described previously (4, 5). Concentration-response curves were obtained in stably transfected HEK293 cells expressing rat P2X7, by applying sequentially increasing concentrations of BzATP for 3–4 s duration at 1.5-min intervals. Concentration-response curves were obtained before and after 1- or 3-h incubation with 0.62 or 4 μg/ml ecto-P2X7Ab at 37 °C.

**RESULTS**

**Purification of Ecto-P2X7 Protein**

Fig. 1 shows the sequential steps resulting in the purified ectodomain protein. The thioredoxin (Trx) fusion protein can be seen as a broad band appearing at the expected size of ~50 kDa (Fig. 1, lane 2). The N-terminal His tag then allowed affinity purification by Ni2+-NTA-agarose affinity chromatography, resulting in a 90% pure preparation after this single step (Fig. 1,
lanes 3). Thrombin digestion then resulted in the two major protein bands (Trx and ecto-P2X7) seen in Fig. 1, lane 4. Finally the thrombin-digested sample was further purified by gel filtration chromatography to remove Trx and increase purity of ecto-rP2X7. This step yielded a highly pure protein seen in the Coomassie-stained SDS-PAGE gel as the single sharp band at the expected size of 31 kDa (Fig. 1, lane 3). The thrombin-digested sample was then used to generate the ecto-rP2X7 monoclonal Ab used in the present study.

**Cloned P2X7 Receptors Expressed in HEK Cells**

**Immunohistochemistry**—When the ecto-P2X7 Ab was used, intense membrane-localized immunofluorescence was observed in rP2X7-expressing HEK cells with no immunofluorescence being detected in HEK cells expressing any other rat or human P2X receptor, in particular the human P2X7 receptor (Fig. 2A). We were also unable to detect immunofluorescence in HEK cells expressing the human P2X7 receptor with the polyclonal C-terminal P2X7 receptor Ab although functional expression of this receptor was confirmed by electrophysiological recordings (data not shown). The ecto-Ab-induced immunofluorescence of the rat P2X7 receptor was equally intense in permeabilized and non-permeabilized cells, whereas the C-terminal directed P2X7 receptor Ab effectively stained only permeabilized cells (Fig. 2B).

**Electrophysiology**—To determine whether the ecto-P2X7 Ab altered functional properties of this receptor, we recorded membrane currents in response to the ATP analogue, BzATP, from cells incubated with a negative Ab sample, with the C-terminal P2X7 Ab or with the ecto-P2X7 Ab. Responses recorded from cells incubated with either the negative serum sample or the polyclonal P2X7 Ab were not different from responses recorded from control P2X7-expressing HEK cells (n = 4 for each). However, incubation with ecto-P2X7 Ab for 30 or 60 min greatly increased the maximum current amplitude evoked by BzATP and shifted the EC50 value by ∼5-fold to the left (Fig. 2C). Longer incubation times (up to 3 h) produced no further enhancement of BzATP-evoked currents (n = 3). Thus, whereas the ecto-Ab does not block access of the agonist to its binding site, these results show that a functional Ab receptor interaction clearly occurs in the form of an enhancement of the membrane current and an apparent increase in agonist affinity. Increases in agonist affinity at the P2X7 receptor have previously been observed following several different types of manipulations; for example, decreases in extracellular sodium or chloride or previous exposure to ATP all shift the agonist concentration-response curve significantly to the left (17, 18).

**Western Blotting on SDS-PAGE or BN-PAGE**—We next asked whether we could obtain any insight into the multimeric nature of the heterologously expressed P2X7 receptor by comparing the actions of the ecto-P2X7 Ab and the C-terminal P2X7 Ab in Western blots using both denatured and non-denatured conditions on SDS and BN-PAGE gels. We also used HEK cells expressing rat P2X7 receptors and a C-terminal P2X7 polyclonal Ab as a control. The three antibodies were all highly specific for their respective proteins (Fig. 3). The P2X7 Ab, which we used as a control for specificity, labeled two bands from P2X7-transfected HEK cells, at 68 and 52 kDa (Fig. 3A); these results are the same as previously observed for glycosylated and non-glycosylated P2X7 receptors expressed in either HEK cells or oocytes (19, 20). On SDS-PAGE, the C-terminal P2X7 Ab recognized the denatured, monomeric 79 kDa P2X7 receptor; on BN-PAGE, it labeled an ∼400 kDa non-denatured multimeric form (Fig. 3, A and B, middle panels). However, the ecto-P2X7 Ab did not recognize the denatured P2X7 receptor on SDS-PAGE, but strongly bound to the ∼400 kDa non-denatured form on BN-PAGE (Fig. 3, A and B, right panels). The ecto-P2X7 Ab also detected a minor band at 240 kDa, which was not seen by the C-terminal polyclonal P2X7 Ab (Fig. 3B, middle and right panels). In agreement with our results with immunohistochemistry, neither the C-terminal polyclonal P2X7 Ab nor the ecto-P2X7 Ab detected the human P2X7 receptor on SDS-PAGE or on BN-PAGE (data not shown).

**Native P2X7 Receptors Expressed in Brain, Peritoneal Macrophage, and Bone Marrow**

We then performed similar immunohistochemical and Western blotting experiments on rat bone marrow, peritoneal macrophage, hippocampus, and cerebellum. We examined extracts under non-denatured conditions, strongly denatured (boiling plus β-mercaptoethanol) or mildly denatured (β-mer-
captoethanol only) to investigate whether similar or different association patterns were observed.

**Immunohistochemistry**—Intense immunostaining was observed in large, CD11-positive peritoneal macrophage using either the ecto-P2X7 Ab or the C-terminal polyclonal P2X7 Ab (Fig. 4A). The C-terminal P2X7 Ab produced distinct immunostaining in non-neuronal microglia/astrocyte-like cells in the hippocampus as well as the cerebellum (Fig. 4B). However, no staining of any brain region was observed with the ecto-P2X7 Ab although several different fixation and staining protocols were employed (Fig. 4B).

**Denatured Extracts on SDS-PAGE**—The C-terminal P2X7 Ab detected two bands, at 67 kDa and 79 kDa, from hippocampal and cerebellar extracts; these bands were observed from extracts treated either by strong denaturation (Fig. 5A, left panel) or mild denaturation (Fig. 5B, left panel). The smaller 67-kDa band is consistent with the nonglycosylated form of the P2X7 receptor (12) and was also occasionally observed in HEK cells expressing rat P2X7 receptors. When peritoneal macrophage or bone marrow were strongly denatured, the C-terminal P2X7 Ab detected the 79 kDa band with no evidence of the smaller band (Fig. 5A, left panel); a similar labeling was observed for peritoneal macrophage under mild denaturation (Fig. 5B, left panel). However, it labeled an ~180-kDa band from bone marrow cells under mild treatment with β-mercaptoethanol only (Fig. 5B, left panel). The ecto-P2X7 Ab did not label any of the strongly denatured extracts on SDS-PAGE (Fig. 5A, right panel). Similar to the C-terminal Ab, it also detected the 180-kDa band from bone marrow on SDS-PAGE when only β-mercaptoethanol was used but detected nothing from peritoneal macrophage or brain (Fig. 5B, right panel).

**Non-denatured Extracts on BN-PAGE**—Under non-denaturing conditions on BN-PAGE, the C-terminal Ab detected an ~400-kDa band from both peritoneal macrophage and bone marrow but detected only a 79-kDa band from hippocampus and cerebellar extracts (Fig. 5C, left panel). The ecto-P2X7 Ab detected a ~400-kDa band from both peritoneal macrophage and bone marrow, but labeled nothing from brain extracts (Fig. 5C, right panel).

**DISCUSSION**

The important conclusion from this study is that native P2X7 receptors appear to exist as a strongly bound multimeric complex in peripheral bone marrow and peritoneal macrophage but only as monomeric forms in brain glia-astrocyte cells. Our interpretation rests on our results from immunoblotting of denatured and non-denatured extracts, comparing the actions of a C-terminal polyclonal anti-P2X7 Ab and a monoclonal ectodomain anti-P2X7 Ab on cloned P2X7 receptors expressed in HEK cells and on native P2X7 receptors in the brain and periphery. Differential immunostaining of brain slices and peritoneal macrophage also provided indirect support for our interpretation.

We examined in some detail the specificity of the Abs used in our study by immunofluorescence and by Western blotting on HEK cell lines expressing the range of other P2X receptors as well as untransfected HEK cells and can be quite confident that these Abs are both sensitive and specific for rat heterologously expressed P2X7 receptors (Figs. 2, 3). The ecto-P2X7 Ab effectively bound the functional receptor in HEK cells, acting to enhance the current amplitude and shift the Bz ATP concentration curve to the left. Immunostaining was also the same in both permeabilized and non-permeabilized cells using the ecto-P2X7 Ab, whereas the polyclonal C-terminal P2X7 Ab stained only permeabilized cells. These results are in keeping with much previous work on other P2X receptors, primarily the P2X7 receptor, which have localized the C terminus to the intracellular aspect of the plasma membrane (9, 14, 21), and show that the ecto-P2X7 Ab binds to the extracellular domain of the functional receptor in these cells.

BN-PAGE analysis of membrane proteins has provided much useful information concerning subunit assembly, stoichiometry, and the nature of the multimeric complexes formed by ion channels and neurotransmitter receptors (11, 16, 22). In particular, Schmalzing and co-workers (11, 12) have applied this method to homomeric P2X2, P2X3, and P2X7 receptors expressed in oocytes, where results with denatured and non-denatured P2X7 receptor proteins indicate a trimeric stoichiometry. We have not addressed specifically the issue of stoichiometry in our study but have used BN-PAGE to determine whether native P2X7 receptors form as multimeric complexes. Clearly they do in peritoneal macrophage and bone marrow, as revealed by both C-terminal and ecto-P2X7 Abs, but they do not in brain astrocyte/glia cells (Figs. 3B and 5C). The ~400-kDa band observed on the BN-PAGE is not consistent with a receptor composed of 3 or 4 subunits (see below) and may indicate a receptor composed of 6 or more subunits. However, we favor the equally likely possibility that this 400-kDa complex comprises not only the P2X7 receptor but also one or more interacting proteins. The availability of this multimeric-specific ecto-P2X7 Ab may allow future identification of such putative interacting proteins via affinity columns and subsequent separation steps (see Ref. 23). In any event, the significant conclusion from these experiments is that brain P2X7 receptors exist as simple monomers and do not form a multimeric assembly when fresh extracts are obtained from brain slices. Direct evidence for this conclusion comes from BN-PAGE where the C-terminal P2X7 Ab labeled only a monomeric (79 kDa) band from hippocampal and cerebellar extracts. Further indirect evidence supporting this conclusion comes from the absence of any labeling of these brain extracts on either BN-PAGE or SDS-PAGE by the ecto-P2X7 Ab (Fig. 5) and the lack of immunohistochemical staining of brain slices by the ecto-P2X7 Ab (Fig. 4), whereas the C-terminal Ab readily labeled astrocyte-like cells in brain slices by immunohistochemistry and recognized monomeric receptor forms on SDS-PAGE (as well as on BN-PAGE). These results are consistent with our observations from rat P2X7 receptors expressed in HEK cells, where the C-terminal P2X7 Ab recognized both monomeric and multimeric forms of the receptor whereas the ecto-P2X7 Ab recognized only the multimeric but not the monomeric form of the receptor (Fig. 3).

We also observed lesser differences in labeling patterns between the two antibodies or among the tissues examined that may indicate other tissue-specific biological differences in P2X7 receptor assembly or may simply reflect experimental and/or antibody variations. First, we observed a minor band at about

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**FIG. 3. Ecto-P2X7 Ab recognizes only non-denatured receptor.** Immunoblots from HEK cells stably expressing P2X2 or P2X7 receptors as indicated. Solubilized receptors were immunoblotted with P2X2, C-terminal P2X7, or ecto-P2X7 Abs as indicated. A, Western blots on SDS-PAGE gels. B, Western blots on BN-PAGE gels.
240 kDa in P2X<sub>7</sub>-transfected HEK cells on BN-PAGE with the ecto-P2X<sub>7</sub> Ab, a band that was not observed in native tissues. If we assume that the major 400-kDa band results from a P2X<sub>7</sub>/interacting protein(s) complex, it is possible the 240-kDa band may represent a trimeric receptor lacking associated proteins, as appears to be the case for P2X<sub>7</sub> receptors expressed in oocytes where the main band observed on BN-PAGE is at 240 kDa (12). Second, under mild denaturation with the reducing agent β-mercaptoethanol to break exposed disulfide bonds both Abs now recognized a 180-kDa band from bone marrow extracts on SDS-PAGE (Fig. 5B), whereas the labeling pattern obtained from the other tissues (HEK, peritoneal macrophage, cerebellum, hippocampus) did not change (Fig. 5). Could this 180-kDa band be a dimeric form of the P2X<sub>7</sub> receptor that becomes dissociated from other associated proteins or P2X<sub>7</sub> subunits that are linked by disulfide bonds? Whereas we cannot fully account for these minor differences, taken together with the clear evidence for multimeric assembly in peripheral immune cells in contrast to monomeric assembly in brain astrocyte/glia, it is reasonable to propose that tissue-specific differential assembly of P2X<sub>7</sub> receptor complexes may be the rule rather than the exception.

Recent studies of P2X<sub>7</sub> receptors from human lymphocytes (18, 24) and peripheral blood-derived monocytes (25) have shown that, under non-stimulated conditions or as a result of a polymorphism encoding a point mutation (E496A), much of the P2X<sub>7</sub> receptor protein is intracellular. However, the surface expression, and/or functional expression, could be dramatically increased by alterations in extracellular ions or by differentiation into activated macrophage (25). It seems likely that the monomeric form of the P2X<sub>7</sub> receptor observed from brain extracts may be due to a predominantly intracellular localization; this would be consistent with our results on Western blots of cerebellum and hippocampus where two bands, 67 kDa and 79 kDa, were observed. The smaller band presumably represents the non-glycosylated form of the receptor, a result generally indicating an intracellular and not fully processed or assembled protein.

The physiological significance of the differential assembly of
P2X<sub>7</sub> receptors between brain and peripheral immune cells observed in the present study remains to be determined, but it is likely that they may underlie much of the differences in the functional sequelae of P2X<sub>7</sub> receptor activation that have been reported for various tissues. For example, activation of cloned P2X<sub>7</sub> receptors expressed in HEK cells results in formation of a pore permeable to cations of up to 900 Da (4–6) although expression in oocytes apparently does not (26). Similarly, P2X<sub>7</sub> receptors expressed in HEK cells results in formation of a pore permeable to cations of up to 900 Da (4–6) although expression in oocytes apparently does not (26). Similarly, P2X<sub>7</sub> receptors expressed in HEK cells results in formation of a pore permeable to cations of up to 900 Da (4–6) although expression in oocytes apparently does not (26).

Functional properties of P2X<sub>7</sub> receptors in monocytes and mast cells couple to a dye-permeable pore formation (6, 7) but P2X<sub>7</sub> receptors in B-lymphocytes may or may not depending on the experimental conditions or activation state of the B cells (8, 18, 27, 28). Cultured microglial may or may not depending on the experimental conditions or activation state of the B cells (8, 18, 27, 28). Cultured microglial may or may not depending on the experimental conditions or activation state of the B cells (8, 18, 27, 28). Cultured microglial may or may not depending on the experimental conditions or activation state of the B cells (8, 18, 27, 28). Cultured microglial may or may not depending on the experimental conditions or activation state of the B cells (8, 18, 27, 28). Cultured microglial may or may not depending on the experimental conditions or activation state of the B cells (8, 18, 27, 28). Cultured microglial may or may not depending on the experimental conditions or activation state of the B cells (8, 18, 27, 28).

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