ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Research paper Acta Neurobiol Exp 2011, 71: 65–73

Expression of purinergic $P2X_7$ receptor in rat brain during the symptomatic phase of experimental autoimmune encephalomyelitis and after recovery of neurological deficits

Tomasz Grygorowicz[1], Dorota Sulejczak[2], and Lidia Strużyńska[1]*

[1]Laboratory of Pathoneurochemistry, Department of Neurochemistry, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; [2]Department of Experimental Pharmacology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; *Email: lidkas@cmdik.pan.pl

Purinergic ionotropic $P2X_7$ receptor is widely distributed in brain. Strong evidence suggests that this receptor is related to inflammatory and neurodegenerative changes in many pathological states of central nervous system (CNS), including multiple sclerosis (MS). Experimental autoimmune encephalomyelitis (EAE) is the commonly used animal model of MS. In this study we investigate the expression of $P2X_7R$ protein in rat brain in the symptomatic phase of EAE (day 10 post immunization) and after reversion of neurological symptoms (day 20 p.i.).

We found the increased level of $P2X_7R$ protein in brain homogenates of EAE rats in both examined time windows. Immunohistochemical study revealed enhanced receptor's immunoreactivity. Immunoblots done with isolated cellular brain fractions indicated that the $P2X_7R$ overexpression is related to synaptosomal fraction in the symptomatic phase and to the glial (GPV) fraction in the recovery phase of EAE. Concomitantly, we noticed overexpression of astroglial marker GFAP in brain homogenates and astroglial fraction (GPV), so as its enhanced immunoreactivity in brain sections (10 days p.i.) which did not decline to control values in the recovery phase, similarly to $P2X_7R$ expression. Results suggest the involvement of $P2X_7R$-mediated signaling in the pathomechanisms of EAE with the possible relevance of astrocytic pool of cells.

Key words: ATP, astrocytes, brain fractions, EAE, MS, $P2X_7R$, purinergic receptors

INTRODUCTION

Family of purinergic receptors is divided into classes: metabotropic P2Y and ionotropic P2X receptors (Abbracchio and Burnstock 1994). Purinergic receptor $P2X_7$ belongs to ionotropic P2X class. However, it possesses the unique properties and differs from other receptors in this class. Except of two transmembrane domains and extracellular loop, it has a unique, among P2X family, long intracellular domain important for ability of pore formation. Under pathological conditions of prolonged activation or stimulation with high levels of agonist (ATP), it homooligomerizes, and forms non-selective transmembrane pore permeable for molecules up to 900 Da weight (Surprenant et al. 1996). That leads to influx of Na^+ and Ca^{2+} and efflux of K^+ and other molecules and subsequent cell death via necrosis or apoptosis (Di Virgilio et al. 1998, Morelli et al. 2001). Opening of a large conductance $P2X_7R$-pore on some cells may result among others in releasing of glutamate and ATP from the internal stores. Both these substances exhibiting toxic excitatory potential were shown to be released from astrocytes (Duan et al. 2003, Suadicani et al. 2006). $P2X_7R$ activation may also lead to the subsequent activation of multimolecular complex "inflammasome" and inflammatory response (Mingam et al. 2008).

Recently, there is an increasing interest in the role of purinergic signaling, particularly $P2X_7R$-mediated, in the pathophysiology of neurological disorders. Increased expression of $P2X_7R$ was observed in Alzheimer disease (McLarnon et al. 2006), ischemia (Yanagisawa et al. 2008), epilepsy (Kim et al. 2010) and multiple sclerosis (MS; Matute 2007, Sharp et al. 2008).

Correspondence should be addressed to: L. Strużyńska
E-mail: lidkas@cmdik.pan.pl

Received 13 January 2011, accepted 11 February 2011

© 2011 by Polish Neuroscience Society - PTBUN, Nencki Institute of Experimental Biology

MS is a chronic inflammatory and degenerative disease of the CNS characterized by demyelination, oligodendroglial cell death and damage of axons. It is one of the most common reasons of neurological disabilities of young adults (Compston and Coles 2000). Progressive neuronal deficiency causing irreversible disability is the main clinical symptom observed during MS. As it was shown previously, activation of $P2X_7R$ on oligodendrocytes may be involved in the MS pathology contributing to the tissue damage (Matute et al. 2007).

Experimental autoimmune encephalomyelitis (EAE) is a rodent model of MS which is commonly used in research of pathological mechanisms operated in this nervous system's disease. Although EAE/SM is traditionally considered to be related to spinal cord pathology, there are also data confirming pathological changes in brain (Ayers et al. 2004, Wang et al. 2005, Białkowiec-Iskra et al. 2007).

In this work we investigate the level of purinergic $P2X_7R$ expression in brain homogenates of rats subjected to EAE as compared with control, non-immunized animals. We elucidate temporal changes in $P2X_7R$ expression in brains of animals at day 10 post immunization, which is the point of maximal neurological deficits, and at day 20 when the full recovery of neurological symptoms of the disease is achieved. Basing on the evidence that $P2X_7R$ is expressed in many brain cell populations (Duan et al. 2003, Sim et al. 2004), we used different cellular fractions isolated from the whole brain to investigate their contribution to the expression of $P2X_7R$ protein.

METHODS

Animals and immunization procedure

Female Lewis rats (weight 180-210 g) from animals' house at the Mossakowski Medical Research Centre, Polish Academy of Sciences (Warsaw, Poland) were used in all experiments. Experimental autoimmune encephalomyelitis was evoked by intradermal injection of 100 µl inoculum into each footpad under anaesthesia (Narcotan – Halothanum, Zentiva, Prague, Czech Republic). The inoculum contained guinea pigs' spinal cord homogenate in PBS (1:1 ratio) emulsified with Freund's complete adjuvant (CFA, Difco) and 2 mg/ml of Mycobacterium Tuberculosis (Difco H37RA) (Tsumoda and Fujinami 1996, Kwiatkowska-Patzer et al. 2009).

Development of neurological symptoms and lost of body weight were observed daily. Neurological deficits, that occurred were described according to the scale of Kerschensteiner and coworkers (2004): 0 - no symptoms; 1 - limp tail; 2 - hind limb weakness; 3 - hind limb paralysis; 4 - ascending paralysis; 5 - moribund. Animals were sacrificed in different stages of the disease i.e., 10 and 20 days post immunization. All procedures involving animals were approved by the local Experimental Animal Care and Use Committee.

Preparation of glial fraction

Isolation was made according to the method described by Daniels and Vickroy (Daniels and Vickroy 1998). Animals were killed by decapitation and brains were isolated. Than brains were homogenized in isolating medium (0.32 M sucrose, 1 mM EDTA) and centrifuged in 1000×g for 15 min. The supernatant was diluted in SEDH buffer (0.32 M sucrose, 1 mM EDTA, DTT, Hepes pH 7.4). Then it was centrifuged in 5000×g for 15 min and further in 33500×g for 20 min. The deposit was suspended in 12 ml SEDH buffer and centrifuged in the Percoll gradient (20%, 10%, 6%, 0%). GPV fraction was collected from the interphase between 0% - 6%, diluted in 12 ml SEDH buffer and then centrifuged for 20 min in 1000×g. The supernatant was then centrifuged in 33500×g for 20 min. The deposit was suspended in 2 ml SEDH buffer and stored at –20ºC.

Preparation of myelin fraction

Myelin fraction was obtained according to the method described by Norton and Poduslo (1973). Freshly isolated rat brains were homogenized in 0.32 M sucrose and centrifuged at 75000×g for 30 min to obtain crude myelin fraction which was further purified using discontinuous sucrose gradient. Myelin fraction was obtained in interphase between 0.32 M and 0.85 M sucrose. Myelin was rinsed twice by suspending in water and centrifugation at 12000×g for 15 min. Purified fraction was diluted in water with protease inhibitors and stored in -20°C until use for immunoblotting procedure.

Preparation of synaptosomes

Isolation of nerve endings fraction was made according to the method described by Booth and Clark (1978).

Brains were homogenized in sucrose buffer (0.32 M sucrose, 1mM EDTA, 10 mM Tris-HCl pH 7.4) and subsequently centrifuged at 1300×g for 3 min and at 17000×g for 10 min. The pelet was suspended in 1.5 ml medium and centrifuged on Ficoll gradient (12%, 7%, 0%) at 99000×g for 30 min. Synaptosomal fraction obtained between 12% and 7% Ficoll was rinsed in isolating medium and centrifuged at 17000×g for 10 min. The final pellet was suspended in Krebs-Ringer buffer (140 mM NaCl, 5 mM KCl, 10 mM Tris-HCl, 1.3 mM MgSO4 and 1 mM Na2HPO4 pH 7.4) and stored at -20ºC for further experiments.

Western blot analysis

Protein concentration in brain homogenates or GPV/synaptosomal/myelin fractions was measured according to the method of Lowry and coauthors (1951) using bovine albumin as a standard. Samples containing 20 µg of proteins were subjected to SDS-polyacrylamide gel (10%) electrophoresis and examined towards P2X$_7$ receptor or GFAP. Samples transferred to nitrocellulose membranes were incubated overnight (4ºC) with primary polyclonal antibody, anti-P2X$_7$R recognizing the intracellular epitop of amino acids 576-595 (Alomone Labs) in dilution 1:200 or monoclonal antibody anti-GFAP in dilution 1:400. Thereafter the secondary antibody conjugated with HRP (Sigma-Aldrich) in dilution 1:5000 was applied. As an internal standard polyclonal anti-β actin antibody (Sigma-Aldrich) was used.

Bands were visualized with the chemiluminescence ECL kit (Amersham), exposed to Hyperfilm ECL and quantified using densitometry Image Scanner III (GE Healthcare) with Image Quant TL v2005 program.

Immunohistochemical procedure

Animals were anesthetized with a lethal dose of Vetbutal and perfused through the heart with phosphate-buffered saline (PBS pH 7.4) and subsequently with 250 mL of fixative (4% paraformaldehyde in PBS; Sigma-Aldrich). Brains were isolated, postfixed in that same fixative for 1.5 h at room temperature and crioprotected in 30% sucrose in PBS. Frozen brains were cut to 40-micron sections into PBS with sodium azide (0.1%). Sections were stored at -20ºC in antifreezer (30% sucrose, 60% glycol ethylene, 0.05 M PB buffer pH 7.4). Free floating sections were immunostained using primary monoclonal mouse anti-GFAP antibodies (Sigma Aldrich, 1:400) or polyclonal rabbit anti-P2X$_7$R antibodies (Alomone Labs, 1:200). Secondary antibodies were conjugated with HRP (1:1000) and the reaction was visualized using DAB as a chromogen. Images were obtained using BH2-RFCA Olympus fluorescent microscopy and CC12 Soft Imaging System Camera and Cell (Olympus) software.

Statistical analysis

Densitometric analysis was performed using blots obtained from brains of 4 animals in each group. Results of statistical analysis are expressed as mean values ± SD of P2X$_7$R or GFAP relative protein concentration. Inter-group comparison of the results was performed using a one-way analysis of variance (ANOVA) followed by *post-hoc* Dunnett's test. Significance level was set as $p<0.05$.

RESULTS

Animals

Neurological symptoms in animals were scored daily according to the above-described scale (1+ - 5+). In immunized animals we observed neurological deficits characteristic for EAE like progressive development of paralysis of tail and hind limbs and loss of reflexes. Symptoms started to develop at day 7, peaked

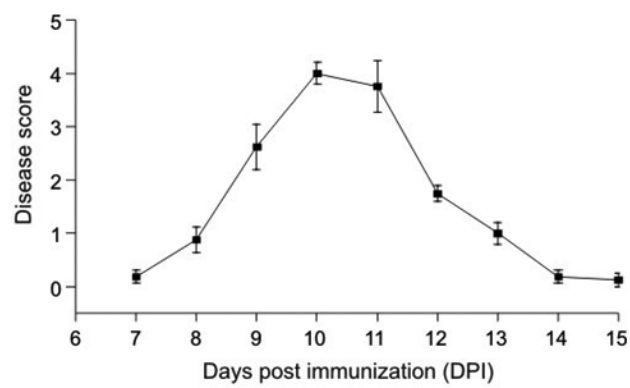

Fig. 1. Scores of the neurological symptoms in animals during the course of the disease. Development of neurological sings was scored as described in the text. The results are means ± SD from 10 animals in each group. Brains were collected at days: 0 (control), 10, and 20 after immunization.

at 10-11 day, and fully recovered at day 15 (Fig. 1). At the peak of the disease animals lost almost 21% of weight.

Animals were sacrificed at day 0 (control, non-immunized), 10 and 20 post immunization to isolate brains for further examination.

Expression of $P2X_7R$

Expression of $P2X_7R$ in brain homogenates of rats subjected to EAE increased significantly in symptomatic phase of the disease (10 d.p.i.) by about 45% relative to control animals. In the late, recovery phase (20 d.p.i.) it remained at similar level (Fig. 2). Although Western blotting method confirm the general tendency of $P2X_7R$ protein to rise significantly within time, the detailed analysis using cellular fractions of brain, showed differences according to the type of cells.

To verify the "cellular origin" of the observed changes in protein expression, we investigated $P2X_7R$ in different brain fractions: glial (GPV), neuronal (synaptosomal) and myelin fraction. In GPV fraction we observed decreased level of receptor's protein in symptomatic phase of EAE whereas during recovery it was significantly increased relative to control animals by

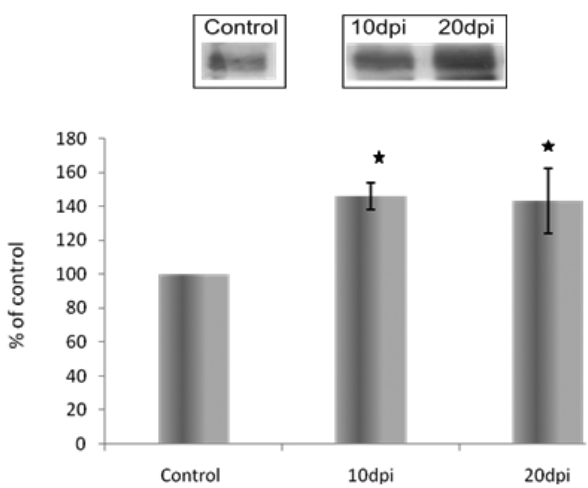

Fig. 2. Expression of $P2X_7$ purinergic receptor's protein in brain homogenates obtained from control and EAE rats in symptomatic (10 d.p.i.) and recovery phase (20 d.p.i.). Representative immunoblot and the graph showing the results of densitometric measurements of 4 independent immunoblots done using 4 distinct brains and expressed as a percentage of control. The relative density was measured against β-actin as an internal standard. *p<0.05 compared with respective control (one-way ANOVA with *post-hoc* Dunnett's test).

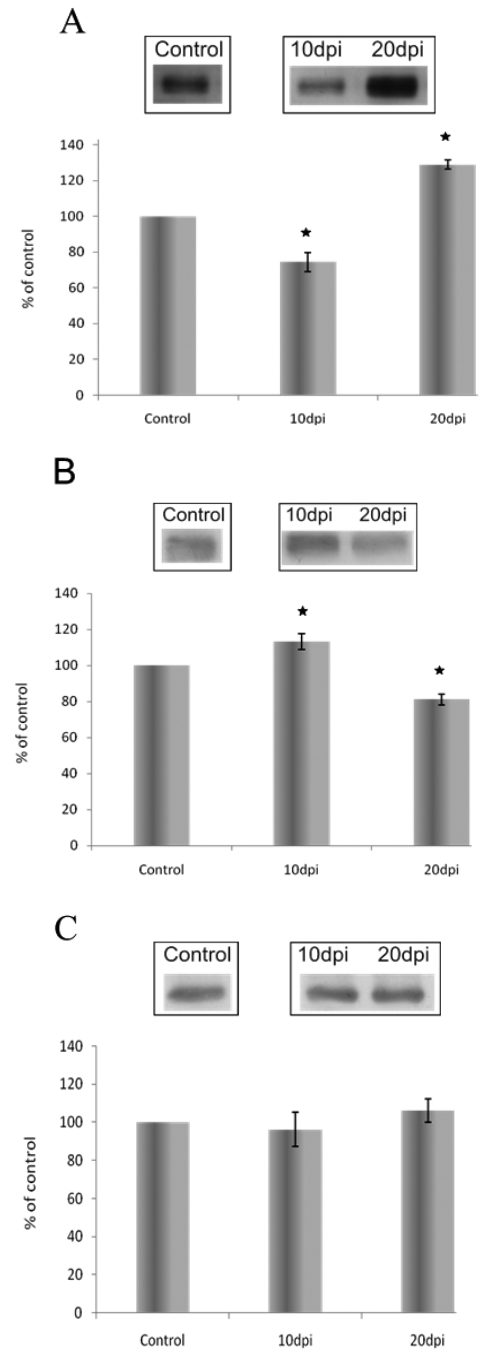

Fig. 3. Expression of $P2X_7$ purinergic receptor's protein in different brain fractions obtained from control and EAE rats in symptomatic (10 d.p.i.) and recovery phase (20 d.p.i.). (A) glial GPV fraction, (B) synaptosomal fraction, (C) myelin fraction. Representative immunoblots and the graphs show the results of densitometric measurements of 3 independent immunoblots done using 3 distinct brain fractions and expressed as a percentage of control. The relative density was measured against β-actin as an internal standard and compared with respective control at *p<0.05 (one-way ANOVA with *post-hoc* Dunnett's test).

about 30% (Fig. 3A). Conversely, in synaptomal fraction we noted small but statistically significant enhancement of relative protein concentration in symptomatic phase (about 15% over control) which further decreased to about 80% of control value in the recovery phase (Fig. 3B). In the case of myelin fraction the level of receptor's protein did not change significantly until day 20 p. i. as compared to the fractions obtained from control animal (Fig. 3C). The overall results suggest the connection of overexpression of P2X$_7$R protein with neurons in symptomatic phase but with glial pool of cells after recovery.

Microscopic analysis of brain slices immunostained towards P2X$_7$R protein revealed changes in its expression during EAE. The increased staining of P2X$_7$R in the frontal cortex in the vicinity of *corpus callosum* of immunized rats was observed in both examined phases of EAE (10 and 20 d.p.i.) as compared to control brains (Fig. 4).

Activation of astroglia during the course of EAE

GFAP is used as a main marker of astroglial activation. In brain homogenates of rats subjected to EAE we observed enhanced expression of this protein both in symptomatic (10 d.p.i.) and recovery (20 d.p.i.) phases by about 50% and 20%, respectively when compared to control (Fig. 5A). The pattern of changes in GFAP immunodensity noticed in astroglial fraction obtained from brains of animals in investigated time points of disease (0, 10, 20 d.p.i.) was slightly different (Fig. 5B). The significant increase in GFAP (about 60% over control) was noticed in recovery phase. It could be observed that GFAP expression did not decrease to the control level while reversing the neurological deficits, neither in homogenates nor in GPV fraction. The quantitative results of immunoblots were confirmed by immunohistochemical analysis. In brain cortex of control rats we observed very weak staining in a small number of astrocytes with thick processes (Fig. 6). The time-dependent increase in the intensity of GFAP immunostaining was observable after immunization. Moreover, it did not reverse during symptomatic recovery. At 20 d.p.i. very strong signal was noticed. Microscopic analysis showed activation of astrocytes which exhibited features of hypertrophy. Described changes in immunoreaction were mostly observed within the frontal motor and somatosensory areas of cortex localized in the close proximity of *corpus callosum*.

DISCUSSION

Considerable evidence suggests that ATP and its purinergic receptor P2X$_7$ are involved in inflammatory reaction and neuronal cell death in many pathological states of CNS (Franke et al. 2006).

Multiple sclerosis is one of CNS pathologies which are traditionally viewed as a "white matter" disease associated with damage to myelin. Indeed, overexpression of P2X$_7$R has been shown to be related to oligodendroglial death in the phase of severe neurological symptoms (Matute et al. 2007). However, it has been already proved that this pathology also involves other types of cells like neurons, astrocytes or microglia. Widespread neuronal and axonal injury in grey matter (Pirko et al. 2007) accompanies pathological changes in white matter compartment. In P2X$_7$R knockout mice the severity of neurological symptoms of the disease as well as axonal damage and astrocyte activation have been significantly reduced (Sharp et al. 2008).

The results of our study showed the enhanced expression of P2X$_7$R protein in brain homogenates of

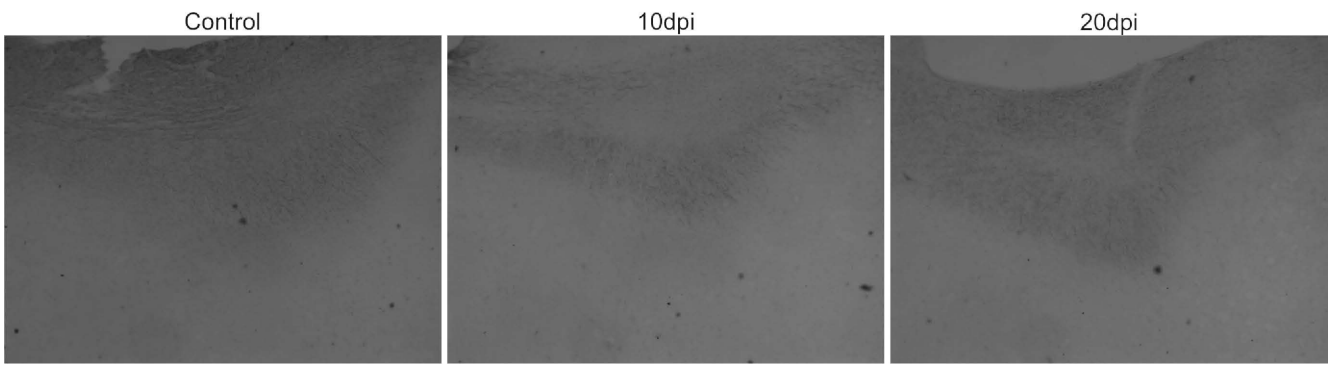

Fig. 4. DAB staining towards P2X$_7$R in brain sections obtained from control and EAE rats at day 10 and 20 post immunization. Magnification ×4.

immunized rats in both examined phases of the disease. This may reflect the involvement of this type of purinergic receptor in the progression of the disease. The immunohistochemical analysis confirmed the semiquantitative results of immunoblotting showing the enhanced immunoreactivity for $P2X_7R$.

While analyzing the expression of receptor's protein using brain fractions, we observed different patterns of changes. In the peak of the disease (10 d.p.i.) the overexpression of $P2X_7R$ was mostly found in synaptosomal fraction, whereas in the recovery phase it was rather connected with glial fraction (GPV). Surprisingly, we did not observed changes in receptor's expression in myelin fraction either at day 10 p.i. or 20 p.i. Previous results of Matute and coworkers (2007) presented the evident connection of $P2X_7R$ with oligodendroglial cell death during the phase of neurological symptoms of the disease. It is possible that these discrepancies arise from different model of study.

In our study we used fractions of high purity therefore the possibility of cross-contamination during fractional $P2X_7R$ immunoblotting in the case of synaptosomal and glia-derived fractions is very low. As it was shown previously, GPVs unlike synaptosomes, express high levels of astrocytic markers – GFAP, glutamine synthetase and carbonic anhydrase (Daniels and Vickroy 1998, Strużyńska 2000, Strużyńska et al. 2001).

However, GFAP is not only specific for astrocytes as a small population of oligodendroglia in rodent brain expresses GFAP (Maglione et al. 2010).

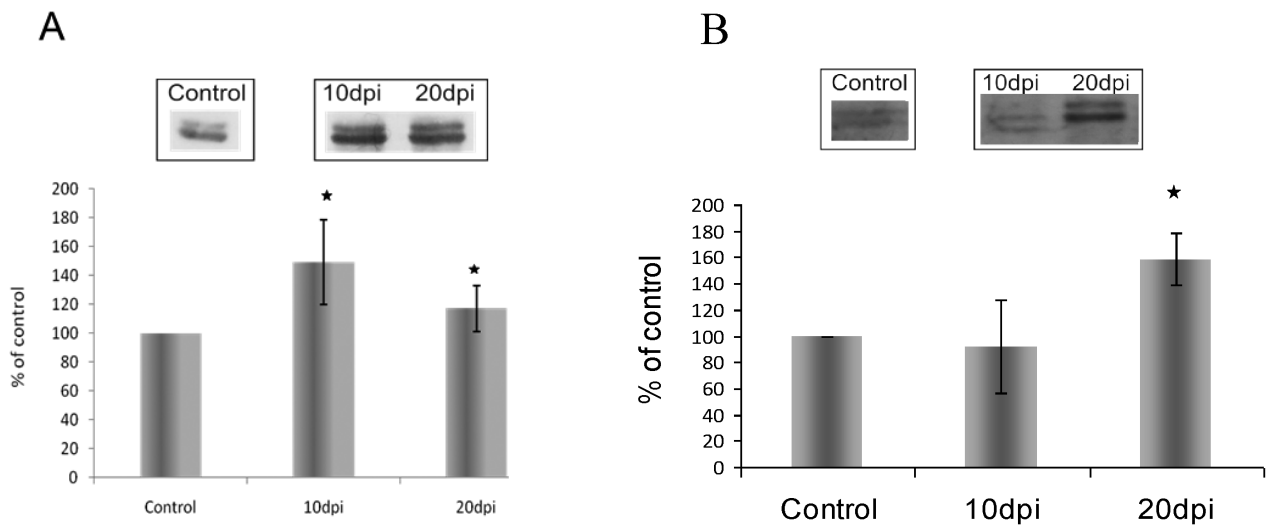

Fig. 5. Expression of GFAP protein in brain homogenates (A) and GPV fraction (B) of control and EAE rats in symptomatic (10 d.p.i.) and recovery phase (20 d.p.i.). Representative immunoblot and the graph indicating the results of densitometric measurements of 4 independent immunoblots in each group of animals done from 4 distinct brains and expressed as a percentage of control. The relative density was measured against β-actin as an internal standard. *$p<0.05$ compared with its corresponding control (one-way ANOVA with *post-hoc* Dunnett's test).

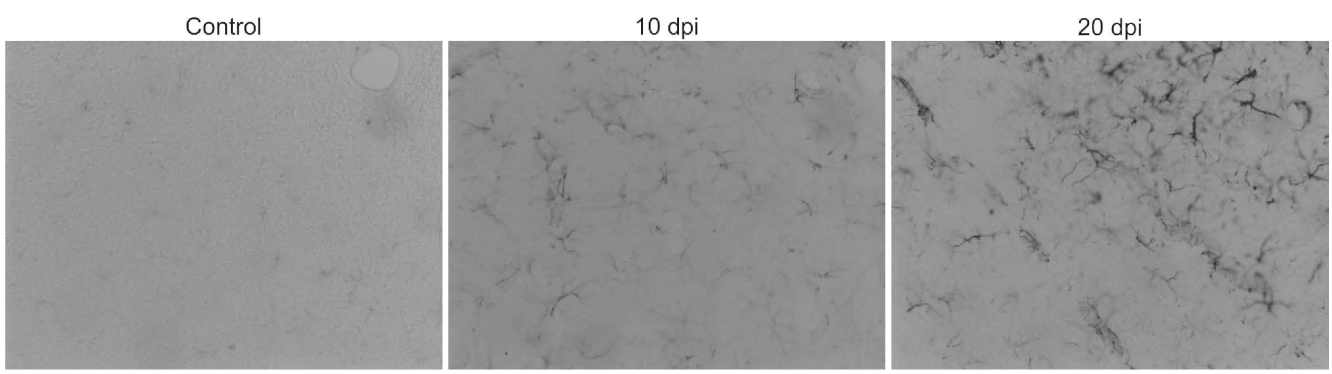

Fig. 6. DAB staining towards GFAP in brain sections obtained from control and EAE rats at day 10 and 20 post immunization. Magnification ×20.

Astrogliosis is the known hallmark of MS/EAE. The overexpression of GFAP was shown to be connected with advanced stages of MS/EAE (Holley et al. 2003, Bannerman et al. 2007) in both white and grey matter of CNS (Pham et al. 2009).

Our results revealed that the overexpression of GFAP observed in symptomatic phase in brain homogenates, still persists after recovery what is evidenced in both homogenates and astroglial fraction. Moreover, parallel to that, the enhancement of P2X$_7$R protein in glial fraction is observed suggesting that astroglial cells may be importantly involved in pathomechanisms of EAE. According to the recent data P2X$_7$R-pore may be the route of glutamate and ATP efflux from glial compartment and can modulate their release (Duan et al. 2003, Hamilton et al. 2008). Thus, astroglia may be regarded as a substantial source of these potentially neurotoxic substances in SM/EAE, not only as a source of proinflammatory cytokines.

It has to be stressed that microglial cells also express P2X$_7$R (Collo et al. 1997). Recently, the role of these receptors located on microglia in neurodegenerative and neuroinflammatory disorders is highlighted due to the involvement in secretion of inflammatory mediators (Lister et al. 2007, Takenouchi et al. 2010). Unfortunately, there is a lack of method for isolating microglial fraction for biochemical assays. By this reason we did not investigate these cells in the present study.

Further it should be stressed that glial activation in neurodegenerative/inflammatory disorders may be of biphasic nature - not only detrimental buy also beneficial (Członkowska 2009, Sofroniew and Vinters 2010). Thus, we should rather perceive the changes described in the present study as focal and not related to whole pool of glia.

Summarizing, our results confirm that during the course of EAE the expression of P2X$_7$R protein undergoes changes in brain of immunized rats. The overexpression observed in brain homogenates in the peak of the disease (10 d.p.i.) did not decline concomitantly with reversing of neurological symptoms (20 d.p.i.). In the symptomatic phase of the disease enhanced expression of P2X$_7$R protein was related to the synaptosomal fraction isolated from EAE rat brains. Overexpression of this receptor correlates well with the reported axonal degeneration in the onset of the disease (Wang et al. 2005). Indeed, P2X$_7$R-induced neuronal degeneration was described earlier after the ischemic insult (Le Feuvre et al. 2003).

However, in the recovery phase, P2X$_7$R overexpression was related mostly to glial fraction. Simultaneously, we observed enhanced expression of GFAP and activated astrocytes with features of hypertrophy. Knowing the fact that damaged cells release ATP, it seems likely that extracellular concentration of agonist during the symptomatic phase increases and spreads around the damaged axons further activating nearby astrocytic P2X$_7$ receptors. Thus, astroglial overexpression of this receptor in the recovery phase may be relevant for sustained astroglial activation observed after clinical recovery. Previous report describing reduced damage of axons and astrocyte activation in P2X$_7$R knockouts (Sharp et al. 2008) support this hypothesis.

CONCLUSION

Our observations suggest that P2X$_7$R-mediated signaling pathway is of importance in the pathology of EAE and may be not only related to the oligodendroglial cells death. However, astroglial contribution to the P2X$_7$R-related pathomechanisms operating during the course of EAE warrant further study.

ACKNOWLEDGMENTS

This study was supported by funds from the Polish Ministry of Science and Higher Education to the Mossakowski Medical Research Centre, Polish Academy of Sciences.

REFERENCES

Abbracchio MP, Burnstock G (1994) Purinoceptors: are there families of P2X and P2Y purinoceptors? Pharmacol Ther 64: 445–475.

Ayers MA, Hazelwood LJ, Catmull DV, Wang D, McKormack Q, Bernard CCA, Orian JM (2004) Early glial responses in murine models of multiple sclerosis. Neurochem Int 45: 409–419.

Bannerman P, Hahn A, Soulika A, Gallo V, Pleasure D (2007) Astrogliosis in EAE spinal cord: derivation from radial glia, and relationships to oligodendroglia. Glia 55: 57–64.

Białkowiec-Iskra E, Kurkowska-Jastrzębska I, Joniec I, Ciesielska A, Członkowska A, Członkowski A (2007) Dopamine, serotonin and noradrenaline changes in the striatum of C57BL mice following myelin oligodendrocyte glycoprotein (MOG) 35-55 and complete Freund

adjuvant (CFA) administration. Acta Neurobiol Exp (Wars) 67: 379–388.

Booth RFG, Clark JB (1978) A rapid method for the preparation of relatively pure metabolically competent synaptosomes from rat brain. Biochem J 176: 365–370.

Collo G, Neidhart S, Kawashima E, Kosco-Vilbois M, North RA, Buell G (1997) Tissue distribution of the P2X7 receptor. Neuropharmacol 36: 1277–1283.

Compston A, Coles A (2000) Multiple sclerosis. Lancet 359: 1221–1231.

Członkowska A (2009) Manipulating inflammation and gliosis in neurological diseases – a clinical perspective. Acta Neurobiol Exp (Wars) 69: abstract SIV.2

Daniels KK, Vickroy TW (1998) Simultaneous isolation of glial and neuronal fractions from rat brain homogenates: comparison of high-affinity L-glutamate transport properties. Neurochem Res 23: 103–113.

Di Virgilio F, Chiozzi P, Falzoni S Ferrari D, Sanz JM, Venketaraman V, Baricordi OR (1998) Cytolytic P2X purinoceptors. Cell Death Differ 5:191–199.

Franke H, Krügel U, Illes P (2006) P2 receptors and neuronal injury. Eur J Physiol 452: 622–644.

Hamilton N, Vayro S, Kirchhoff F, Verkhatsky A, Robbins J, Gorecki DC, Butt AM (2008) Mechanism of ATP- and glutamate-mediated calcium signaling in white matter asytocytes. Glia 56: 734–749.

Holley JE, Gveric D, Newcombe J, Cuzner ML, Gutowski NJ (2003) Astrocyte characterization in the multiple sclerosis scar. Neuropathol Appl Neurobiol 29: 434–444.

Duan S, Anderson CM, Keung EC, Chen Y, Swanson RA (2003) P2X7 receptor-mediated release of excitatory amino acids from astrocytes. J Neurosci 23: 1320–1328.

Kerschensteiner M, Stadelmann C, Buddeberg BS, Merkler D, Bareyre FM, Anthony DC, Linington C, Bruck W, Schwab ME (2004) Animal model –targeting experimental autoimmune encephalomyelitis lesions to a predetermined axonal tract system allows for refined behavioral testing in an animal model of multiple sclerosis. Am J Pathol 164: 1455–1469.

Kim JE, Ryu HJ, Yeo SI, KangTC (2010) P2X7 receptor differentially modulates astroglial apoptosis and clasmatodendrosis in the rat brain following status epilepticus. Hippocampus. [Epub ahead of print]

Kwiatkowska-Patzer B, Michalkiewicz J, Kubiszewska I, Zielińska J, Kasarello K, Kurzepa K, Lipkowski A (2009) Spinal cord hydrolysate ameliorates immunological reactions in experimental allergic encephalomyelitis. Acta Neurobiol Exp 69: 73–78.

Le Feuvre RA, Brough D, Touzani O, Rothwell NJ (2003) Role of P2X7 receptors in ischemic and excitotoxic brain injury in vivo. J Cereb Blood Flow Metab 23: 381–384.

Lister MF, Sharkey J, Sawatzky DA, Hodgkiss JP, Davidson DJ, Rossi AG, Finlayson K (2007) The role of the purinergic P2X7 receptor in inflammation. J Inflamm (Lond) 4: 5.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

Maglione M, Tress O, Haas B, Karram K, Trotter J, Willecke K, Kettenmann H. (2010) Oligodendrocytes in mouse corpus callosum are coupled via gap junction channels formed by connexin47 and connexin32. Glia 58: 1104–1117.

Matute C, Torre I, Perez-Cerda F, Perez-Samartin A, Alberdi E, Etxebarria E, Arranz AM, Ravid R, Rodrigez-Antigüedad A, Sanchez-Gomez MV, Domercq M (2007) P2X7 receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. J Neurosci 27: 9525–9533.

McLarnon JG, Ryu JK, Walker DG, Choi HB (2006) Upregulatedexpression of purinergic P2X(7) receptor in Alzheimer disease and amyloid-beta peptide-treated microglia and in peptide injected rat hippocampus. J Neuropathol Exp Neurol 65: 1090–1097.

Mingam R, De Smedt V, Amédée T, Bluthé RM, Kelley KW, Dantzer R, Layé S (2008) *In vitro* and *in vivo* evidence for a role of the $P2X_7$ receptor in the release of IL-1β in the murine brain. Brain Behav Immun 22: 234–244.

Morelli A, Ferrari D, Bolognesi G, Rizzuto R, Di Virgilio F (2001) Proapoptotic plasma membrane pore: P2X7 receptor. Drug Dev Res 52: 571–578.

Norton WT, Poduslo SE (1973) Myelination in rat brain: method of myelin isolation. J Neurochem 21: 749–757.

Pham H, ramp AA, Klonis N, Ng SW, Klopstein A, Ayers MM, Orian M (2009) The astrocytic response in early experimental autoimmune encephalomyelitis occurs across both the grey and white matter compartments. J Neuroimmunol 208: 30–39.

Pirko L, Lucchinetti CF, Subramainan S, Bakshi R (2007) Grey matter involvement in multiple sclerosis. Neurology 68: 634–642.

Sharp AJ, Polak PE, Simonini V, Lin SX, Richardson JC, Bongarzone ER, Feinstein DL (2008) P2X7 deficiency suppresses development of experimental autoimmune encephalomyelitis. J Neuroinflamm 5: 33.

Sim JA, Young MT, Sung HY, North RA, Surprenant A (2004) Reanalysis of P2X7 receptor expression in rodent brain. J Neurosci 24: 6307–6314.

Sofroniew MV, Vinters HV (2010) Astrocytes: bilogy and pathology. Acta Neuropathol 110: 7–35.

Strużyńska L (2000) The protective role of astroglia in the early period of experimental lead toxicity in the rat. Acta Neurobiol Exp (Wars) 60: 167–173.

Strużyńska L, Bubko I, Walski M, Rafałowska U (2001) Astroglial reaction during the early phases of acute lead toxicity in the adult rat brain. Toxicol 165: 121–131.

Suadicani SO, Brosnan CF, Scemes E (2006) P2X7 receptors mediate ATP release and amplification of astrocytic intercellular Ca2+ signaling. J Neurosci 26: 1378–1385.

Surprenant A, Rassendren F, Kawashima E. North RA, Buell G (1996) The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor P2X7. Science 272: 735–738.

Takenouchi T, Sekiyama K, Sekigawa A, Fujita M, Waragai M, Sugama S, Iwamaru H, Hashimoto M (2010) P2X7 receptor signaling pathway as a therapeutic target for neurodegenerative diseases. Arch Immunol Ther Exp 58: 91–96.

Tsumoda I, Fujinami RS (1996) Two models for multiple sclerosis: experimental allergic encephalomyelitis and Theiler's murine encephalomyelitis virus. J Neuropathol Exp Neurol 55: 673–686.

Wang D, Ayers MM, Catmuli DV, Hazelwood LJ, Bernard CCA, Orian JM (2005) Astrocyte-associated axonal damage in pre-onset stages of experimental autoimmune encephalomyelitis. Glia 51: 235–240.

Yanagisawa D, Kitamura Y, Takata K, Hide I, Nakata Y, Taniguchi T (2008) Possible involvement of P2X7 receptor activation in microglial neuroprotection against focal cerebral ischemia in rats. Biol Pharm Bull 31: 1121–1130.