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BRAIN RESEARCH

Research Report

Cellular localization of P2X7 receptor mRNA in the rat brain

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ABSTRACT

P2X7 receptor is a member of the P2X family of ATP-gated ion channels. The cellular localization of P2X7 receptors in the central nervous system remains controversial because immunohistochemical staining patterns are inconsistent among antibodies. Here we examined the precise distribution of P2X7 mRNAs in the rat brain using isotopic in situ hybridization. P2X7-positive glial-like small cells were sporadically scattered in almost all areas of the brain. P2X7-positive glial-like small cells were also observed in nerve fiber tracts such as the anterior commissure, corpus callosum (CC), optic tract, and internal capsule. P2X7-positive neurons were found in the anterior olfactory nucleus, cerebral cortex, piriform cortex (Pir), lateral septal nucleus (LS), hippocampal pyramidal cell layers of CA1, CA3, CA4, pontine nuclei, external cuneate nucleus, and medial vestibular nucleus. P2X7 hybridization signals were also observed in the motor neurons of the trigeminal motor nucleus, facial nucleus, hypoglossal nucleus, and the anterior horn of the spinal cord. P2X7 mRNA was expressed in the ependymal cells around the olfactory ventricle, lateral ventricles (LV), third ventricle (3V), cerebral aqueduct (Aq), fourth ventricle (4V), and central canal. The P2X7 hybridization signal was also very strong in the area postrema (AP). The double staining experiments demonstrate that neurons, oligodendrocytes, and microglia expressed P2X7 receptor mRNAs. These findings suggest that P2X7 receptors may play a variety of roles in a wide range of cell types in the brain.

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Abbreviations: 2-AG, 2-arachidonoylglycerol; 3V, third ventricle; 4V, fourth ventricle; 5-HT, serotonin; AOD, anterior olfactory nucleus, dorsal part; AOL, anterior olfactory nucleus, lateral part; AOM, anterior olfactory nucleus, medial part; AOV, anterior olfactory nucleus, ventral part; AP, area postrema; Aq, aqueduct; CBF, cilia beat frequency; CC, corpus callosum; CNPase, 2′, 3′-cyclic-nucleotide-3′-phosphodiesterase; CREB, cyclic AMP response element binding protein; CSF, cerebrospinal fluid; CT, cerebral cortex; cRNA, complementary RNA; DAB, diaminobenzidine; DG, dentate gyrus; eCBs, endocannabinoid; GABA, glutamate and gamma-aminobutyric acid; GFAP, glial fibrillary acidic protein; H_2O_2, hydrogen peroxide; IL-1β, interleukin-1β; KO, knockout; LS, lateral septal nucleus; LSD, lateral septal nucleus, dorsal part; LV, lateral ventricle; NeuN, neuronal-specific protein; PB, phosphate buffer; Pir, piriform cortex; RT, reverse transcription

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1. Introduction

P2X receptors are ligand-gated ion channels that open in response to extracellular ATP. Seven different P2X receptor subunits P2X1–7 have been identified and are known to form homomeric and in some cases heteromeric assemblies (Sperlágh et al., 2002, 2006; Torres et al., 1999).

P2X7 receptors have been reported to be involved in neurodegenerative processes by regulating intracellular Ca^{2+} concentration, interleukin-1β(IL-1β) processing and release, multiple caspase activation, and glutamate release under pathological conditions such as inflammation, mechanical injury, ischemia, and stress (Chessell et al., 2005; Ferrari et al., 1996; Labasi et al., 2002; Le Feuvre et al., 2002; Solle et al., 2001; Sperlágh et al., 2006). P2X7 receptors were also found to mediate hydrogen peroxide (H_2O_2) production in microglia, which contributed to neurodegeneration in an Alzheimer's disease model (Parvathenani et al., 2003; McLarnon et al., 2006). On the other hand, P2X7 mediates 2-arachidonoylglycerol (2-AG) and endocannabinoid (eCBs) production in microglia and astrocytes, thereby contributing to neuroprotection (Witting et al., 2004, 2006). Stimulation of P2X7 receptors induces phosphorylation of cyclic AMP response element binding protein (CREB), resulting in an inhibitory effect on inflammation in microglia (Potucek et al., 2006). Although knockout (KO) of the P2X7 gene did not affect cell death following cerebral ischemia (Le Feuvre et al., 2002, 2003), many studies have shown that inhibition of P2X7 receptors improves recovery after neural injury in vivo and in vitro (Cavaliere et al., 2004, 2005; Melani et al., 2006; Wang et al., 2004).

P2X7 receptors are associated with not only pathological conditions such as neuronal degeneration but also neuronal or glial signal transduction. ATP facilitates glutamate and gamma-aminobutyric acid (GABA) release in nerve terminals in vivo and in vitro (Sperlágh et al., 2002, 2006) and in astrocytes in vitro (Duan et al., 2003; Pannicke et al., 2000; Sperlágh et al., 2006; Wirkner et al., 2005) by a mechanism involving P2X7 receptors. In cultured astrocytes, P2X7 receptors also mediate ATP release that amplifies astrocytic intercellular Ca^{2+} waves (Suadicani et al., 2006).

P2X7 receptors have been found to be localized to mast cells (Collo et al., 1997; Dahlquist and Diamant, 1974), transformed mouse fibroblasts (Rozengurt et al., 1977), macrophages (Steinberg et al., 1987), lymphocytes (Markwardt et al., 1997), microglia (Ferrari et al., 1996), astrocytes (Ballerini et al., 1996; Kukley et al., 2001), Schwann cells (Colomar and Amedee, 2001), and oligodendrocytes (James and Butt, 2002). Northern blot analyses revealed the expression of P2X7 mRNA in the thymus, spleen, live, heart, prostate, pancreas, leukocyte, testis, brain, skeletal muscle, lung, and placenta in rats and humans (Collo et al., 1997; Rassendren et al., 1997). In situ hybridization and immunohistochemistry demonstrated that expression of P2X7 in the ependymal layer of the third ventricular and activated microglia followed brain infarction (Collo et al., 1997; Melani et al., 2006). Recent immunohistochemical studies showed that the P2X7 protein was also expressed in the neurons of the adult rat retina or inner ear (Brandle et al., 1998, 1999), presynaptic terminals in the spinal cord, medulla oblongata, cortex, mossy fiber terminals of the hippocampus, and neuromuscular junction (Armstrong et al., 2002; Atkinson et al., 2002; Cavaliere et al., 2004; Deuchars et al., 2001; Franke et al., 2004; Lundy et al., 2002; Sperlágh et al., 2002). P2X7 was also found in the resting or activated microglia in intact condition or after pathologic injury (Rappold et al., 2006). However, other groups could not detect P2X7 receptors in resting microglia, neurons, or astrocytes (Collo et al., 1997; Kukley et al., 2004; Melani et al., 2006; Sim et al., 2004) by immunohistochemistry. Thus, the previous immunohistochemical studies showed inconsistent staining patterns.

Recent immunohistochemical experiments using two separate P2X7 KO mice strains have revealed that three available antibodies for P2X7 not only stained brain sections of wild-type mice but also brain sections of P2X7 KO mice (Anderson and Nedergaard, 2006), suggesting the specificity of these antibodies is not so reliable. However, most of the previous immunohistochemical studies were done using these antibodies. Therefore, in order to determine the

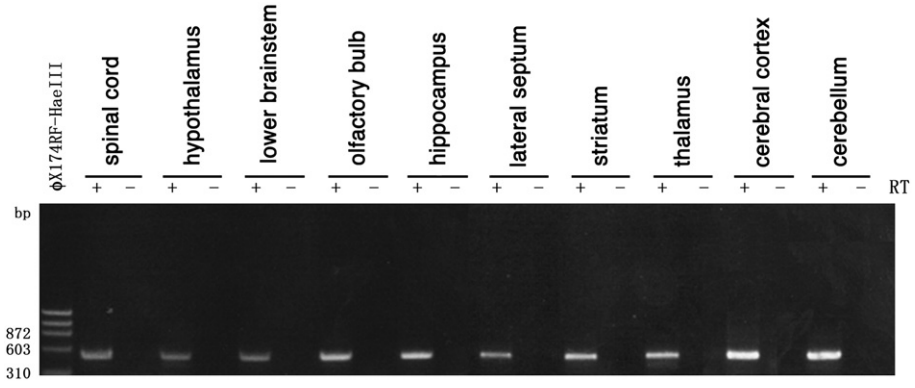

Fig. 1 – RT-PCR analyses of P2X7 expression in the adult rat brain including olfactory bulb, cerebral cortex, thalamus, hypothalamus, striatum, lateral septal area (LS), hippocampus, lower brainstem, cerebellum, and cervical spinal cord. All regions of the brain and spinal cord showed positive bands (+ lane), whereas no detectable signal was seen in each sample without reverse transcriptase (– lane). The size of the DNA standards is indicated on the left (fx174 RF-HaeIII). RT, reverse transcription.