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Expression of kininogen mRNAs and plasma kallikrein mRNA by cultured neurons, astrocytes and meningeal cells in the rat brain

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Abstract

Expression of kininogen mRNAs has been studied in cultures of three different types of cells in rat brain, including neurons and astrocytes from cerebral cortex and meningeal cells from the leptomeninges/choroid plexus. T-kininogen mRNA was expressed by meningeal cells, but not by neurons and astrocytes, and the expression in meningeal cells was enhanced by culture with prostaglandin E2 (PGE2) or dibutyryl cAMP (Bt2cAMP). Low-molecular-weight kininogen mRNA was not detected in these cultures of cells, even after treatment with PGE2. Although expression of high-molecular-weight kininogen mRNA was very low in these cultures of cells, PGE2 or Bt2cAMP markedly stimulated its expression in cultures of meningeal cells and slightly in neurons, but not in astrocytes. We also found that expression of plasma kallikrein mRNA was strong in cultures of meningeal cells and slight in astrocytes, but absent in neurons. These results suggest that cells in the leptomeninges/choroid plexus are major sources of kininogens in rat brain which may function as precursor proteins for kinins and/or potent cysteine proteinase inhibitors during cerebral inflammation.

Keywords: Kininogens; Plasma kallikrein; Meningeal cells; Neurons; Astrocytes

1. Introduction

The existence of kallikreins, kinins and B2-receptor in the central nervous system has been described in several studies (Walker et al., 1995), whereas few studies have reported the presence of kininogens in the brain. Mitra and Carraway (1988) have reported the presence of T-kininogen-like activity in rat brain. Damas et al. (1992) confirmed the presence of T-kininogen in rat brain by anti-T-kininogen antibody and by identification of T-kinin in brain homogenate after trypsin-treatment, and also found T-kininogen-like immunoreactivity in cultured cells of rat brain including dorsal root ganglia neurons, embryonic hippocampal neurons and astrocytes. Richoux et al. (1992) reported the presence of H- and T-kininogen in neurons of rat hypothalamus. In addition to these evidence on brain kininogens, mRNA encoding T-kininogen has been identified in rat brain (Mann and

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Lingrel, 1991). Although these results demonstrate that a local kininogen-supplying system is present in the brain, localization of this system has not been successful.

In the present study, we prepared three different cultures of cells, such as neurons, astrocytes and meningeal cells, from rat brain and studied which types of kininogen mRNAs were expressed in each culture.

2. Materials and methods

2.1. Cell cultures

The cerebral cortex and meninges were isolated from hemispheres of 1-day old newborn rats. The cerebral cortex was dissociated with 0.025% trypsin–0.025% DNase 1 at 37°C for 10 min, and passed through a 62 μm nylon mesh. The cells were washed and seeded on plates precoated with gelatin–polyornithine, and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). After a 2-h culture, the medium was replaced with FBS-free DMEM containing 1% N1 medium supplement (Sigma, CA, USA). After a 2-day culture in 5% CO_2–95% air, the medium was replaced with DMEM containing 10% FBS and 20 μM cytosine arabinoside. After additional culture for 2 days, more than 90% of the cells stained with a monoclonal antibody to neuron-specific microtubule associated protein-2 (MAP) of calf brain (Amersham, Tokyo, Japan).

Astrocytes were prepared from the cerebral cortex of 1-day old rats according to the procedure described by McCarthy and Vellis (1980). After primary culture for 9 days, the population of astrocytes were enriched by removing oligodendrocytes (McCarthy and Vellis, 1980). Cells were further grown for 2 weeks, and then used for experiments. More than 95% of the cells reacted positively to staining with a rabbit anti-human glial fibrillary acid protein (GFAP) (Advanced Immunochemical, USA).

Meningeal cells were prepared from the cerebrum of 1-day old newborn rats as described previously (Takano et al., 1995). Cells were negative for MAP and GFAP.

2.2. Detection of kininogen and plasma kallikrein mRNAs

Total RNA was extracted from cultured cells with acid guanidinium–phenol–chloroform. Total RNA (0.25 μg) was incubated at 42°C for 15 min with reverse transcriptase containing 3′-reverse primer. After incubation at 95°C for 5 min, the single-strand cDNA was amplified with 5′-forward primer and *Taq* DNA polymerase. Samples were denatured at 95°C for 9 min. A total of 40 cycles for high-molecular-weight (H-) kininogen and plasma kallikrein cDNAs or 25 cycles for T-kininogen and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNAs proceeded as follows: denaturation at 95°C for 1 min, annealing at 58°C for 2 min and extension at 72°C for 3 min. RT-PCR products (5 μl) were Southern blotted and autoradiographed using a Fuji Film Bio Imaging Analyzer BAS1000 (Fuji Film, Tokyo). The specific primers for T- or low-molecular-weight- (L-) kininogen annealed between exons 7 to 11 of T- or L-kininogen gene: the 5′-primer was 5′-ACATCACAGGTGGTTGCTGGA-3′ (nucleotide positions 918 to 938 of T-KGN cDNA and 921 to 941 of L-KGN cDNA), and the 3′-primer was 5′-TGAGAGTCTGCCCTTGTACT-3′ (1214 to 1234 of T-KGN cDNA and 1223 to 1243 of L-KGN cDNA). Primers for H-kininogen and GAPDH were described previously (Okamoto et al., 1998). The probes used in Southern hybridization of kininogen cDNAs were described previously (Okamoto et al., 1998). Primers for rat plasma kallikrein were designed based on the cDNA structure of rat plasma kallikrein (Seidah et al., 1989): the 5′-primer was 5′-CTTCCTTGCCGTGAGTCCAA-3′ (nucleotide position 210–230) and 3′-primer of 5′-GGGGTGACGACCTGGCTTAC-3′ (nucleotide position 673–693). The cDNA probe of Southern hybridization for plasma kallikrein was cloned from RT-PCR products of rat liver using TA Cloning Kit (Invitrogen, CA, USA).

2.3. Separation and detection of T- and L-kininogen mRNAs

To analyze RT-PCR products of T- and L-kininogen mRNAs, we used a fluorophore Cy5-labelled 5′-primer for RT-PCR, then amplified cDNA products were separated and detected using ALFred

DNA sequencer (Pharmacia), as described previously (Okamoto et al., 1998).

3. Results and discussion

3.1. Expression of T-/L-kininogen mRNA by neurons, astrocytes and meningeal cells of rat brain

RT-PCR was carried out to detect T- or L-kininogen mRNA in cultures of neurons, astrocytes and meningeal cells of rat brain using a set of primers common to both kininogen cDNAs (Fig. 1). Expression of T-/L-kininogen mRNA was strong in cultures of meningeal cells and slight in neurons, but undetectable in astrocytes under basal conditions. Addition of PGE2 at 10 μM or dibutyryl cAMP (Bt2cAMP) at 1 mM to cultures resulted in increased expression of T-/L-kininogen mRNA in cultures of meningeal cells, but not in those of neurons and astrocytes cultures. Since the structure of T-kininogen is highly homologous to that of L-kininogen, it was conceivable that RT-PCR products of meningeal cells were a mixture of these kininogen cDNAs. To determine which type of kininogen mRNA was expressed in meningeal cells, RT-PCR products were separated and detected by an ALFred DNA sequencer on the basis of a 6-bp difference between T- (315 bp) and L- (321 bp) kininogen cDNAs. As shown in Fig. 2, RT-PCR products from PGE2-treated meningeal cells exhibited a single peak corresponding to T-kininogen cDNA, while a signal from L-kininogen cDNA was not detected. A similar result was obtained in Bt2cAMP-treated meningeal cells (data not shown). In contrast, RT-PCR products from rat liver showed a large peak for T-kininogen cDNA and a small one for L-kininogen cDNA, confirming that rat liver supplies both T- and L-kininogens for plasma. Thus, it is likely that PGE2 stimulates the expression of T-kininogen gene, but not the L-kininogen gene via an elevation in intracellular cAMP levels, in cultures of meningeal cells.

T-kininogen is an acute-phase proteins and a cysteine proteinase inhibitor in rats, whose synthesis in the liver increases after induction of inflammation (Furuto-Kato et al., 1985; Greenbaum and Okamoto, 1988). Interleukin-6 has been identified to be a mediator of this hepatic response (Gauldie et al., 1987). In contrast to rat hepatocytes, expression of T-kininogen mRNA in meningeal cells was not affected by this cytokine in vitro (data not shown), suggesting that the expression of the T-kininogen gene in meningeal cells is regulated by a distinct mechanisms from that in hepatocytes. Since PGE2 is also a potent proinflammatory mediator, it is interesting to speculate that the leptomeninges/choroid plexus in rat brain supplies T-kininogen in response

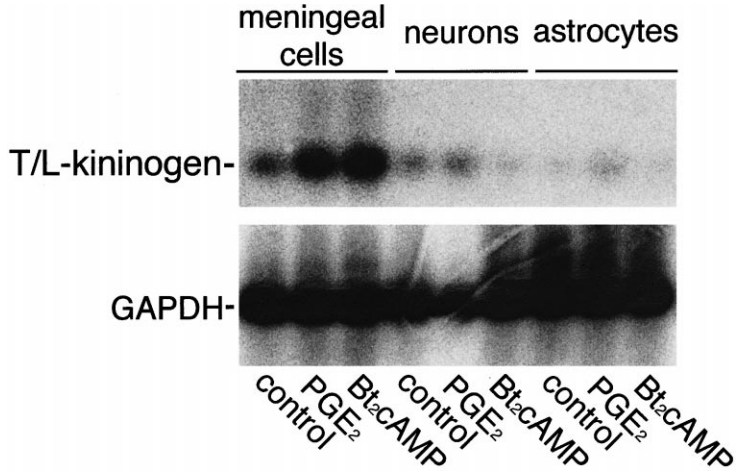

Fig. 1. Representative Southern blots of cDNA amplified from T- and L-kininogen mRNAs in cultures of meningeal cells, neurons and astrocytes of rat brain. Meningeal cells, neurons and astrocytes were prepared from newborn rats and cultured with or without 10 μM prostaglandin E2 (PGE2) or 1 mM dibutyryl cAMP (Bt2cAMP) for 24 h. Total RNA (0.25 μg) was analyzed by RT-PCR (25 cycles) using specific primers for T-/L-kininogen cDNAs or for GAPDH cDNA followed by Southern blotting using T-kininogen cDNA or GAPDH oligonucleotide as a probe, respectively. Experiments were carried out on four different cultures, and representative results are shown.

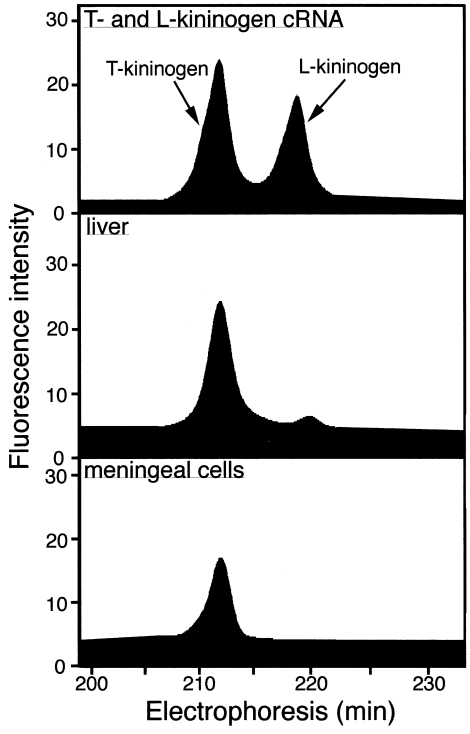

Fig. 2. Electrophoresis and detection of RT-PCR products of T- and L-kininogen mRNAs in PGE2-treated meningeal cells. RNA samples from normal rat liver (middle panel) and PGE2-treated meningeal cells for 24 h (lower panel) were amplified using Cy5-labelled 5′-primer and non-labelled 3′-primer specific for both T- and L-kininogen cDNAs, then the labelled products were resolved by polyacrylamide gel electrophoresis and analyzed using an ALFred DNA sequencer (Pharmacia). The retention time profiles for RT-PCR products from authentic T- and L-kininogen cRNAs are shown in the upper panel. Representative results of three independent experiments are shown.

to cerebral inflammation to various brain regions via a flow of cerebrospinal fluid through ventricles. Thus, T-kininogen may function as a cysteine proteinase inhibitor or precursor protein of kinins in cerebrospinal fluid during cerebral inflammation in rats, as the liver supplies this protein into the peripheral circulation during systemic inflammation.

3.2. Expression of H-kininogen and plasma kallikrein mRNA by neurons, astrocytes and meningeal cells of rat brain

To determine if rat brain cultured cells express H-kininogen mRNA, RNA samples from these cells were examined by RT-PCR followed by Southern blotting. Signal corresponding to H-kininogen mRNA was not detected in cultures of meningeal cells and astrocytes, while a weak signal was observed in neurons (Fig. 3). When these cells were treated with PGE2 (10 mM) or Bt2cAMP (1 μM) for 24 h, distinct levels of H-kininogen mRNA were found in cultures of meningeal cells and neurons, but not in astrocytes. H-kininogen mRNA expression in meningeal cells was detectable at 8 h and peaked at 24 h after PGE2 addition (data not shown). Although PGE2 also stimulated the expression of H-kininogen mRNA in cultures of neurons, it may be possible that a signal corresponding to H-kininogen mRNA in these cultures was derived from other types of cells, such as fibroblasts, glial cells or meningeal cells, rather than neurons, because about 10% of the population was non-neuronal cells in these cultures, even after enrichment of neurons by antimitotic treatment. In contrast to meningeal cells, PGE2 and Bt2cAMP did not influenced H-kininogen mRNA levels in rat hepatoma H-35 cells (data not shown), suggesting that expression of the H-kininogen gene and the T-kininogen gene in meningeal cells are regulated by different mechanisms from that in the liver.

H-kininogen is an endogenous substrate for plasma kallikrein whose synthesis has been thought to be restricted to the liver. In fact, no signals corresponding to plasma kallikrein mRNA have not been detected in rat tissues, except for the liver (Seidah et al., 1989). However, PGE2-induced expression of the H-kininogen gene in meningeal cells of rat brain led us to examine whether these cells express plasma kallikrein mRNA. As shown in Fig. 4, a 480-bp PT-PCR product was detected in cultures of untreated meningeal cells by hybridization with an internal probe. This is the size (483 bp) expected from the cDNA sequence of rat plasma kallikrein (Seidah et al., 1989). A weak signal was also observed in cultures of astrocytes, but not in neurons. Neither PGE2 nor Bt2cAMP affected levels of plasma kallikrein mRNA in these cultures. Thus, meningeal cells express not only H-kininogen mRNA in response to PGE2 stimulation but also plasma kallikrein mRNA, suggesting the existence of a local plasma kallikrein–H-kininogen system in rat brain.

The existence of tissue kallikrein in the choroid plexus epithelium in human brain and in ventricular

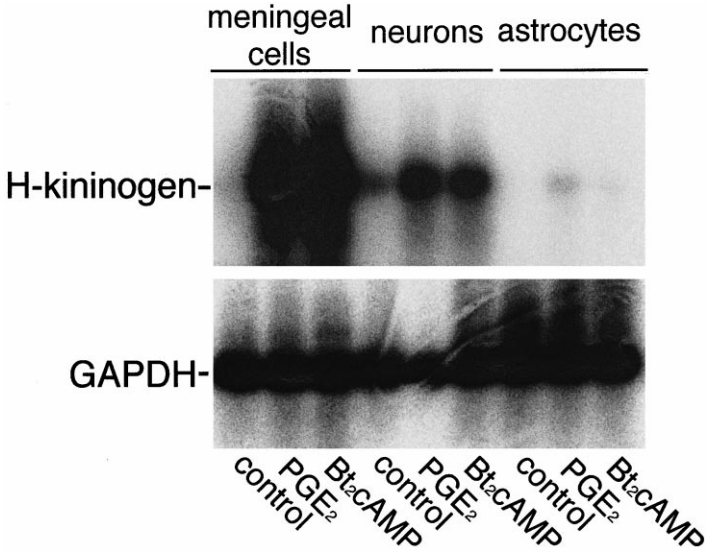

Fig. 3. Representative Southern blots of cDNA amplified from H-kininogen mRNA in cultures of meningeal cells, neurons and astrocytes of rat brain. Meningeal cells, neurons and astrocytes were prepared from newborn rats and cultured with or without 10 μM PGE2 or 1 mM Bt2cAMP for 24 h. Total RNA (0.25 μg) was analyzed by RT-PCR (40 cycles) using specific primers for H-kininogen cDNA followed by Southern blotting using H-kininogen cDNA as a probe. Experiments were carried out four different cultures, and representative results are shown.

meninges (ependymal cells) of rat brain has been demonstrated by immunohistochemistry (Simpson et al., 1985; Raidoo et al., 1996). Our present study provides evidence that, in addition to tissue kallikreins, the leptomeninges and choroid plexus synthesize and secrete plasma kallikrein in the brain.

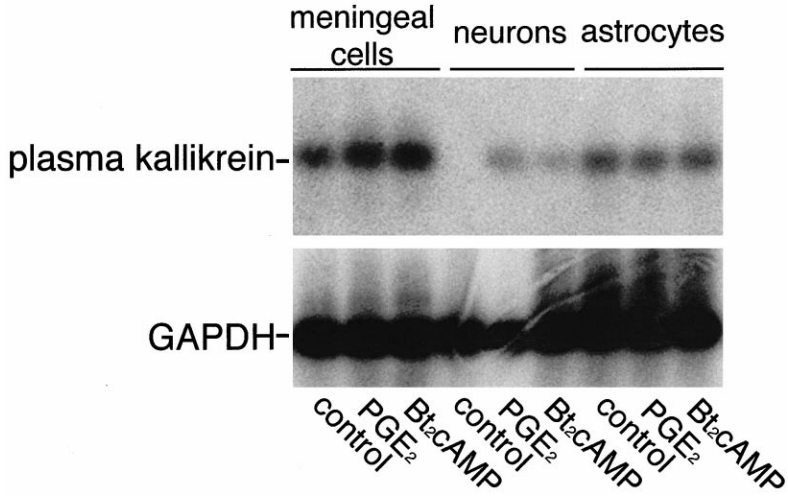

Fig. 4. Representative Southern blots of cDNA amplified from plasma kallikrein mRNA in cultures of meningeal cells, neurons and astrocytes of rat brain. Meningeal cells, neurons and astrocytes were prepared from newborn rats and cultured with or without 10 μM PGE2 or 1 mM Bt2cAMP for 24 h. Total RNA (0.25 μg) was analyzed by RT-PCR (40 cycles) using specific primers for rat plasma kallikrein cDNA followed by Southern blotting using rat plasma kallikrein cDNA as a probe. Experiments were carried out four different cultures, and representative results are shown.

Further studies will be needed to clarify the functions of H-kininogen and plasma kallikrein in these tissues.

Taken together, these findings suggest that cells in the leptomeninges and choroid plexus are major sources of kininogens in rat brain, and the evidence that PGE2 stimulates kininogen expression in cells from these tissue implies involvement of the kallikrein–kinin system in cerebral inflammation.

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