Cell Research (2008) 18:558-565.

www.nature.com/cr

ORIGINAL ARTICLE

Maxi-anion channel as a candidate pathway for osmosensitive ATP release from mouse astrocytes in primary culture

Hong-Tao Liu[1,2,3*], Abduqodir H Toychiev[1,4*], Nobuyuki Takahashi[1,4], Ravshan Z Sabirov[5], Yasunobu Okada[1,4]

[1]Department of Cell Physiology, National Institute for Physiological Sciences, Myodaiji-cho, Okazaki 444-8585, Japan; [2]Department of Anesthesiology, The First Affiliated Hospital, China Medical University, Shenyang 110001, China; [3]Japan Society for the Promotion of Science (JSPS), Okazaki 444-8585, Japan; [4]Department of Physiological Sciences, School of Life Science, The Graduate University for Advanced Studies (SOKENDAI), Okazaki 444-8585, Japan; [5]Department of Biophysics, National University, Tashkent 700174, Vuzgorodok, Uzbekistan

In the present study, we aimed to evaluate the pathways contributing to ATP release from mouse astrocytes during hypoosmotic stress. We first examined the expression of mRNAs for proteins constituting possible ATP-releasing pathways that have been suggested over the past several years. In RT-PCR analysis using both control and osmotically swollen astrocytes, amplification of cDNA fragments of expected size was seen for connexins (Cx32, Cx37, Cx43), pannexin 1 (Px1), the P2X7 receptor, MRP1 and MDR1, but not CFTR. Inhibitors of exocytotic vesicular release, gap junction hemi-channels, CFTR, MRP1, MDR1, the P2X7 receptor, and volume-sensitive outwardly rectifying chloride channels had no significant effects on the massive ATP release from astrocytes. In contrast, the hypotonicity-induced ATP release from astrocytes was most effectively inhibited by gadolinium (50 μM), an inhibitor of the maxi-anion channel, which has recently been shown to serve as a pathway for ATP release from several other cell types. Thus, we propose that the maxi-anion channel constitutes a major pathway for swelling-induced ATP release from cultured mouse astrocytes as well.

***Keywords*:** ATP release, swelling, anion channel, astrocyte

Cell Research (2008) **18**:558-565. doi: 10.1038/cr.2008.49; published online 15 April 2008

Introduction

In the brain, astrocytes play active roles in cell-to-cell signaling by releasing gliotransmitters, such as glutamate and ATP, and thus forming neuron-glia and glia-glia networks [1-5]. Astrocytic ATP release is involved in modulating a variety of neuronal activities, affecting excitability [6], synaptic transmission [7, 8], cell death [9], growth and survival [9, 10]. ATP released from astrocytes also modulates the activities of astrocytes [11, 12] and microglia [13-15] through the stimulation of purinergic receptors.

In a variety of cell types, the release of ATP has been reported to be mediated by a number of non-lytic membrane transport pathways including exocytotic vesicular transport, connexin or pannexin hemichannels, the P2X7 receptor, ABC transporters such as MDR1 and MRP, as well as anion channels such as CFTR, volume-sensitive outwardly rectifying (VSOR) anion channels and maxi-anion channels [17-19: for Reviews].

Astrocytes have been shown to release ATP in a non-lytic manner in response to osmotic swelling [11], mechanical stimulation [8, 20-23], deprivation of extracellular Ca^{2+} [22-24], and stimulation with glutamate [7], UTP [25, 26], noradrenaline [27] or NO [28]. However, the precise pathway for ATP release remains controversial. In the present study, we aimed to identify the pathway for swelling-induced ATP release from mouse astrocytes in primary culture by performing RT-PCR analyses and pharmacological studies to test candidate pathways hitherto reported.

*These two authors contributed equally to this work.

Correspondence: Yasunobu Okada
Tel: +81-564-55-7731; Fax: +81-564-55-7735
E-mail: okada@nips.ac.jp

Received 20 May 2007; revised 20 August 2007; accepted 7 November 2007; published online 15 April 2008

Results

Swelling-induced ATP release from mouse astrocytes

In normal Ringer solution, the basal release of ATP from mouse astrocytes was low and did not exceed 1.00 ± 0.13 nM over a 35-min incubation time (Figure 1, open squares). In contrast, when cell swelling was induced by exposure to a hypotonic solution (210 mosmol/kg-H_2O), the extracellular ATP concentration rapidly reached a maximum level of approx. 2.5-3 nM within 15-25 min (Figure 1, open circles).

When the cells were incubated in solutions of different osmolality (ranging from 290 to 130 mosmol/kg-H_2O) for 15 min, the release of ATP increased with decreasing medium osmolality (Figure 2). The osmolality dependence of ATP release had a sigmoidal shape with half-maximal ATP release observed at a medium osmolality of 228 ± 16 mosmol/kg-H_2O.

Gene expression of molecules comprising candidate pathways for ATP release in mouse astrocytes

A number of possible ATP-releasing pathways have been suggested over the past several years. To determine which candidate pathway may be important in mouse astrocytes, we assayed the expression of candidate pathway molecule mRNAs by RT-PCR analysis. Under normotonic conditions, as shown in Figure 3A and 3B, RT-PCR yielded amplified cDNA fragments of expected size for connexins (Cx32, Cx37, Cx43), pannexin 1 (Px1), MRP1, P2X7 receptors (P2X7), MDR1a, and MDR1b. However, CFTR mRNA was not detected in mouse astrocytes but could be detected in mouse lung homogenates (MLHs), which were used as a positive control (Figure 3C). This profile of gene expression was not affected by osmotic swelling of the astrocytes induced by exposure to hypotonic solution (210 mosmol/kg-H_2O) for 15 min (data not shown).

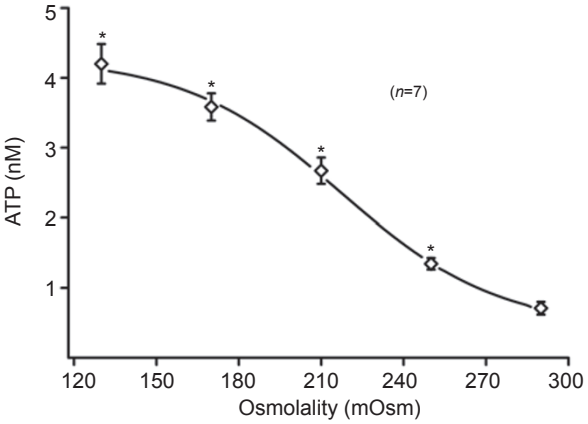

Figure 2 Osmolality dependence of ATP release from mouse astrocytes. The concentration of ATP released from mouse astrocytes cultured in 24-well plates was measured 15 min after application of isotonic or hypotonic solution. The extracellular ATP concentration is plotted as a function of the osmolality of the extracellular medium. Each symbol represents the mean ± SEM (vertical bar). *Significantly different from the isotonic control values at $P < 0.05$.

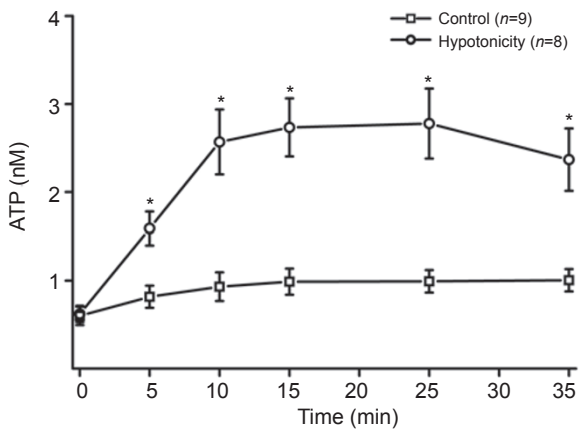

Figure 1 Time course of ATP release from mouse astrocytes in response to hypotonic stress. The concentration of ATP released from mouse astrocytes cultured in 12-well plates was measured by a luciferin-luciferase assay after application of control isotonic or hypotonic solution (290 or 210 mosmol/kg-H_2O). The extracellular ATP concentration is plotted as a function of the incubation time in control basal (open squares) and hypotonic (open circles) conditions. Each symbol represents the mean ± SEM (vertical bar). *Significantly different from the isotonic control values at given times at $P < 0.05$.

Pharmacological sensitivity of swelling-induced ATP release from mouse astrocytes

We next tested the sensitivity of ATP release from swollen astrocytes to blockers of not only the candidate pathways examined in the above RT-PCR analysis but also other possible pathways including exocytotic vesicular transport as well as the VSOR anion channel and the maxi-anion channel, which have not yet been molecularly identified. When mouse astrocytes were exposed to hypotonic solution of 210 mosmol/kg-H_2O for 15 min in the absence of any blockers, massive ATP release was observed as before (Figure 4A, first column). We then observed the effects of different blockers for candidate pathways by adding to the hypotonic solution at concentrations that were previously reported to be most effective. The data are summarized in Figure 4A (other columns). Swelling-induced ATP release was unaffected by the presence of glibenclamide (200 μM), a known blocker of the CFTR anion channel [29], a member of the ABC transporter superfamily. This is in accord

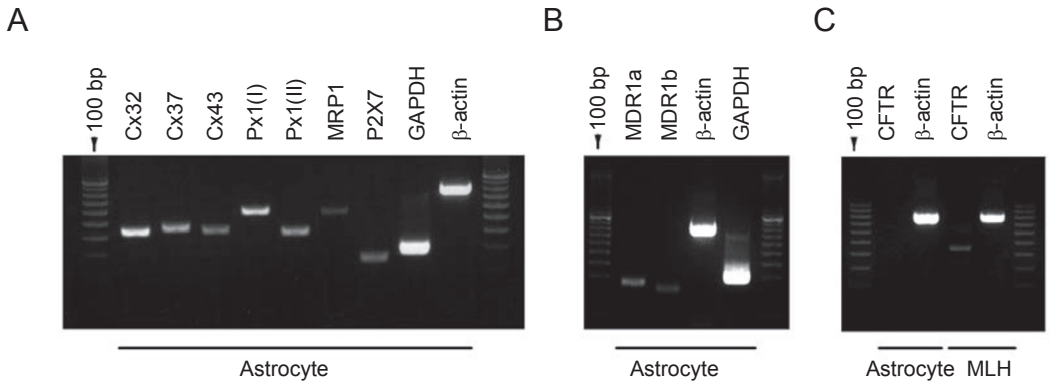

Figure 3 Expression of mRNAs of various candidates for ATP release pathways in cultured mouse astrocytes. **(A)** RT-PCR performed for Cx32 (lane 2), Cx37 (lane 3), Cx43 (lane 4), Px1 (lanes 5 and 6), MRP1 (lane 7), P2X7 (lane 8), GAPDH (lane 9), and β-actin (lane 10) in mouse astrocytes. **(B)** RT-PCR for MDR1a (lane 2), MDR1b (lane 3), β-actin (lane 4), and GAPDH (lane 5) in mouse astrocytes. **(C)** RT-PCR for CFTR (lanes 2 and 4), for β-actin (lanes 3 and 5) in mouse astrocytes (lanes 2 and 3) and mouse lung homogenates (MLH: lanes 4 and 5). The first and last lanes show size markers (100-bp ladders).

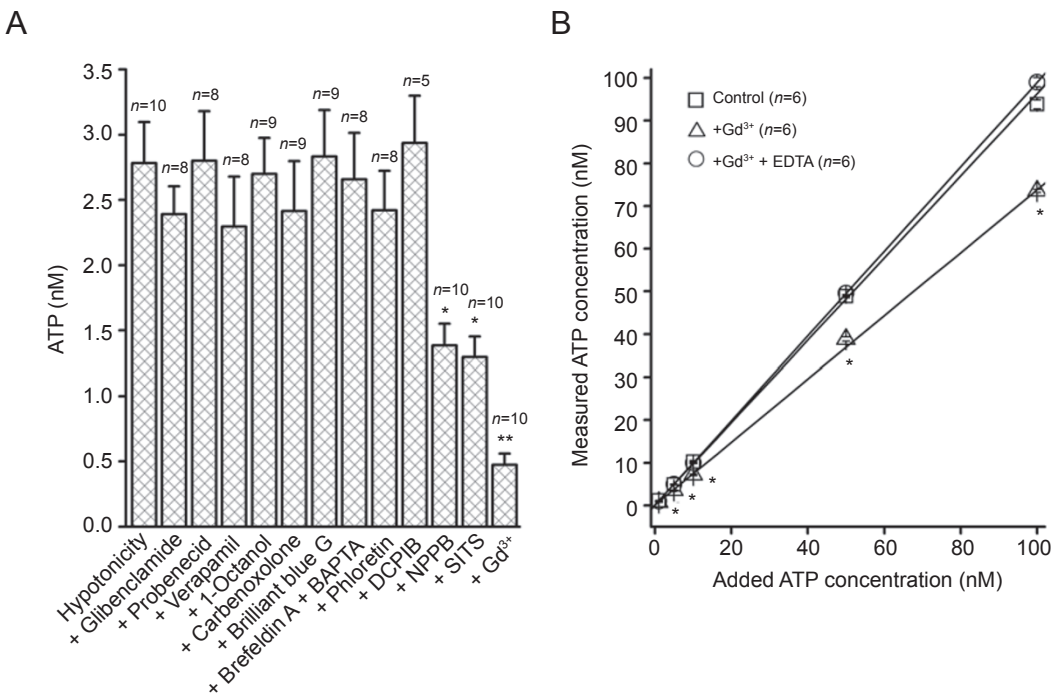

Figure 4 Effects of blockers of various candidate pathways for ATP release on the hypotonicity-induced ATP release from mouse astrocytes **(A)** and effects of Gd^{3+} and EDTA on the calibration for ATP measurements by the luciferin-luciferase reaction **(B)**. In **(A)**, the concentration of ATP released from mouse astrocytes cultured in 24-well plates was measured 15 min after application of hypotonic solution (210 mosmol/kg-H_2O). The concentrations of blockers are given in the text. Each symbol represents the mean ± SEM (vertical bar). *Significantly different from the control value (hypotonicity in the absence of drugs for **A** and Gd^{3+}- and EDTA-free Ringer solution for **B**) at $P < 0.05$.

with the RT-PCR data for CFTR (Figure 3C). Similarly, swelling-induced ATP release was not significantly affected by blockers, probenecid (1 mM) and verapamil (10 μM), which are known to block other ABC transporter proteins, MRP1 [30, 31] and MDR1 [32], respectively. Blockers of gap junction hemichannels also failed to affect swelling-induced ATP release; no significant effects were observed by adding 2 mM 1-octanol, a blocker of connexins [33], or 100 μM carbenoxolone, a blocker of both connexins and pannexins [34]. A blocker of the P2X7 receptor [24,

35], brilliant blue G (1 μM), was also ineffective. When exocytotic vesicular transport was suppressed by adding an intracellular Ca^{2+} chelator, BAPTA-AM (50 μM), together with an inhibitor of vesicular transport [23], brefeldin A (BFA; 5 μM), ATP release from swollen astrocytes was not significantly affected. We then observed the effects of anion channel blockers. The ATP release was suppressed to an intermediate degree by NPPB (100 μM) and SITS (100 μM), both of which are known to moderately inhibit maxi-anion channels, but more strongly block VSOR anion channels in mouse astrocytes [36]. In contrast, phloretin (100 μM), which is a blocker relatively specific to the VSOR anion channel [37], had no significant effect on swelling-induced ATP release. Also, DCPIB (10 μM), another blocker specific to the VSOR anion channel [38], failed to affect swelling-induced ATP release. We next examined the effect of Gd^{3+} (50 μM), which blocks, from the extracellular side, maxi-anion channels expressed in many cell types [17, 39, 40, 41] including mouse astrocytes [36]. As reported previously [42], Gd^{3+} itself suppressed the luciferin-luciferase reaction. As shown in Figure 4B, the slope of a calibration curve obtained by adding known amounts of ATP to normal Ringer solution in the presence of Gd^{3+} (triangles) was significantly smaller than that in the absence of Gd^{3+} (squares). However, the Gd^{3+} effect on the luciferin-luciferase reaction was completely eliminated by adding 600 μM EDTA to the luciferase solution (Figure 4B, circles). By supplementing the luciferin-luciferase reaction with EDTA, Gd^{3+} was found to be most effective in inhibiting hypotonicity-induced ATP release (Figure 4A, last column).

As shown in Figure 5, marked, moderate and little blocking effects of Gd^{3+}, SITS and DCPIB, respectively, were observed on swelling-induced ATP release during the whole time range (up to 35 min) of observations.

From the above results, it is concluded that swelling-induced astrocytic ATP release does not involve vesicular transport, ABC transporters such as CFTR, MDR1 and MRP1, connexin or pannexin hemichannels, or swelling-activated VSOR anion channels. Instead, the evidence suggests that the Gd^{3+}-sensitive maxi-anion channel serves as the pathway for ATP release from swollen mouse astrocytes.

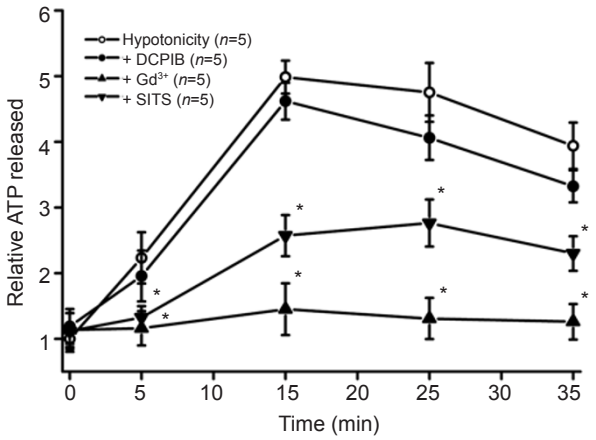

Figure 5 Effects of Gd^{3+} (50 μM), SITS (100 μM) and DCPIB (10 μM) on the time course of swelling-induced ATP release from mouse astrocytes. Each symbol represents the mean ± SEM (vertical bar). *Significantly different from the control hypotonic values in the absence of blockers.

Discussion

Under stimulation of receptors or exposure to stress, brain astrocytes affect neuronal brain functions by releasing a number of gliotransmitters, including glutamate and ATP. Ubiquitously expressed swelling-activated anion channels, called VSOR anion channels or volume-regulated anion channels (VRACs), are believed to serve as a pathway for swelling-induced release of the excitatory amino acids glutamate and aspartate from astrocytes [43, 44]. In fact, Abdullaev *et al*. [45] have recently shown that swelling-induced aspartate efflux is mediated by Gd^{3+}-insensitive VSOR anion channels in rat astrocytes. We have provided compelling evidence that not only the VSOR anion channel but also a Gd^{3+}-sensitive maxi-anion channel represent major conductive pathways for the release of glutamate from mouse astrocytes challenged by hypotonic or ischemic stress [36]. Recent pore sizing studies using a nonelectrolyte exclusion technique showed that the pore radius of the maxi-anion channel (~1.3 nm) [46], but not of the VSOR anion channel (~0.63 nm) [47], is large enough for the channel to be permeated not only by glutamate (cut-off radius ~0.35 nm) but also by the anionic forms of ATP (0.58~0.65 nm). Direct evidence has accumulated for the ATP conductivity of maxi-anion channels in a number of other cell types [39, 40, 41]. It is therefore highly likely that swelling-induced ATP release from mouse astrocytes is also mediated by Gd^{3+}-sensitive maxi-anion channels that are known to be activated by hypotonic and ischemic stresses in this cell type [36]. The present study showed, in fact, that ATP release from osmotically swollen astrocytes was most effectively blocked by Gd^{3+}.

Thus far, a large variety of molecules have been suggested to constitute the non-lytic and non-exocytotic pathways of ATP release. These include CFTR, MDR1 and MRP1 [31] for rat astrocytes, the connexins [22] and P2X7 receptors [24] for mouse astrocytes, as well as the pannexins [19] for other cell types. The present RT-PCR study demonstrated molecular expression of connexins (Cx32,

Cx37, Cx43), Px1, MDR1, MRP1 and the P2X7 receptor in both non-swollen and swollen mouse astrocytes. These data are in agreement with those from previous RT-PCR studies that examined MRP1 [30, 48] and the P2X7 receptor [49] in mouse astrocytes in normotonic conditions, and Px1 [50], MDR1 [31, 45, 51] and MRP1 [31, 48] in rat astrocytes, also in normotonic conditions. However, the present study failed to detect the CFTR transcript in mouse astrocytes, in contrast to a previous report [30]. Previous pharmacological studies suggested that ATP release involves ABC transporters in rat cortical astrocytes [11, 31] and the P2X7 receptor in mouse spinal cord astrocytes [24]. However, the present pharmacological study showed that swelling-induced ATP release from mouse cortical astrocytes was insensitive to blockers of ABC transporters (glibenclamide, probenecid, verapamil) and the P2X7 receptor (brilliant blue G). In addition, it was found that blockers of connexin and pannexin hemichannels (1-octanol, carbenoxolone) and VSOR anion channels (phloretin, DCPIB) did not significantly affect swelling-induced ATP release from mouse astrocytes.

It is evident that exocytotic release of secretory granules or vesicles is a non-lytic source of ATP in some secretory cell types [17]. A recent single-vesicle imaging study has provided evidence for astrocyte exocytosis [52]. On the basis of sensitivity to bafilomycin A_1, tetanus neurotoxin, botulinum neurotoxin C and Ca^{2+} chelators, it was suggested that mechanostress- or NO-induced ATP release from rat astrocytes is mediated by exocytosis [23, 28]. In the present study, however, intracellular Ca^{2+} chelation by pretreatment with a Ca^{2+} chelator, BAPTA-AM, and an inhibitor of vesicular transport, BFA, failed to inhibit swelling-induced ATP release from mouse astrocytes. Although the Ca^{2+} chelator and BFA may affect many processes, it appears that swelling-induced ATP release does not involve exocytosis which is an event essentially dependent on cytosolic Ca^{2+} and sensitive to BFA.

Since ATP exists in anionic forms at physiological pH, there is a possibility that some anion channel type (other than CFTR) may serve as the pathway for ATP release from mouse astrocytes. An involvement of the Ca^{2+}-dependent anion channel may be ruled out because of insensitivity of the ATP release to cytosolic Ca^{2+} chelation. Its insensitivity to phloretin and DCPIB may similarly rule out an involvement of the VSOR anion channel. The most effective blocking agent for the ATP release from swollen astrocytes was Gd^{3+}. Gd^{3+} is known to block not only maxi-anion channels [17, 36, 39, 40, 41] but also some cation channels such as mechano-gated cation channels [53] and several members of the TRP cation channel family [54] at similar concentrations, although it is not known how Gd^{3+} blocks both cation and anion channels. However, involvements of cation channels in release of anionic ATP would be implausible.

There is a possibility that the data shown in Figure 1 represent underestimates of the actual amount of ATP released from mouse astrocytes, because the activity of ecto-ATPases was not inhibited in the present experiments. To test this possibility, the effects of potent ecto-ATPase blockers, such as suramin and DIDS [55, 56] as well as ARL 67156 [57], could be examined. However, suramin was found to almost completely inhibit the luciferin-luciferase reaction (HT Liu and Y Okada, unpublished), and DIDS was reported to block the maxi-anion channel activity [58]. Thus, we made preliminary experiments by applying ARL 67156 (100 μM) and found that this ecto-ATPase blocker markedly increased the extracellular concentration of ATP released from mouse astrocytes in response to a hypotonic challenge (210 mosmol/kg-H_2O for 15 min) (HT Liu and Y Okada, unpublished). Even in the presence of ARL 67156, the hypotonicity-induced ATP release was found to be significantly suppressed by Gd^{3+} (HT Liu and Y Okada, unpublished). Although it is not known at present whether ARL 67156 affects any candidate pathways for ATP release, it appears that the Gd^{3+}-sensitive maxi-anion channel serves as a major pathway for swelling-induced ATP release from mouse astrocytes even under the conditions where ecto-ATPases were inhibited.

Taken together, our results suggest that swelling-induced release of anionic ATP from mouse cortical astrocytes is mediated by Gd^{3+}-sensitive maxi-anion channels. There is little information about the molecular nature of the maxi-anion channel. A mitochondrial porin (voltage-dependent anion channel (VDAC)) located in the plasma membrane has long been considered as the molecule underlying the maxi-anion channel activity [59-62], based upon similarities in the biophysical properties of these two channels and the purported presence of VDAC protein in the plasma membrane. In our recent study [63], we have deleted all the three genes encoding the VDAC isoforms and demonstrated that the maxi-anion channel activity in VDAC-deficient mouse fibroblasts was unaltered. The lack of correlation between VDAC protein expression and maxi-anion channel activity strongly argues against the long-held hypothesis of plasmalemmal VDAC being the maxi-anion channel. Molecular identification of the maxi-anion channel awaits further investigation.

Brain astrocytes are known to exhibit swelling in response to ischemic and traumatic brain injury [44]. Also, astrocytes are found to undergo swelling after stimulation with glutamate [64] as well as during hyperammonemia [65, 66] and lactacidosis [67]. Therefore, it is conceivable that ATP release from swollen astrocytes plays some role in such pathological conditions.

Materials and methods

Solutions and chemicals

The normal Ringer solution contained (mM): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 Na-HEPES, 6 HEPES, and 5 glucose (pH 7.4, 290 mosmol/kg-H_2O). Hypotonic solutions were prepared by mixing the normal Ringer solution with a HEPES-buffered solution containing (mM): 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 Na-HEPES, 6 HEPES, and 5 glucose (pH 7.4, 38 mosmol/kg-H_2O).

$GdCl_3$ was stored as a 50-mM stock solution in water and added directly to the hypotonic solution immediately before each experiment. NPPB, glibenclamide, SITS, phloretin, BFA, carbenoxolone, 1-octanol, probenecid and brilliant blue G were purchased from Sigma-Aldrich (St. Louis, MO). Verapamil and BAPTA/AM were obtained from Nacalai Tesque (Kyoto, Japan) and DOJINDO (Kumamoto, Japan), respectively. The drugs were added to hypotonic solution immediately before use from stock solutions in DMSO. DMSO did not have any effect, when added alone at a concentration less than 0.1%. Osmolality of all solutions was measured using a freezing-point depression osmometer (OM802: Vogel, Kevelaer, Germany).

Cell culture and tissue preparation

The experimental protocol was approved in advance by the Ethics Review Committee for Animal Experimentation of the National Institute for Physiological Sciences. Astrocytes were obtained from 2–3-day old pups of Slc:ddy mice (Japan SLC, Inc., Hamamatsu, Japan) using the method described previously [36]. The cells were cultured in 12- or 24-well plates, and, upon reaching confluency, were used for ATP measurements and RT-PCR assays. The cell number per well in 12- or 24-well plates was $(2.35 \pm 0.19) \times 10^5$ or $(9.49 \pm 1.03) \times 10^4$, respectively.

The MLHs were prepared from 100 mg tissue samples of the lung dissected from 90-day old mice after homogenizing with a glass/Teflon homogenizer (Iuchi, Osaka, Japan) in 1 ml Sepasol RNA I reagent.

Luciferin-luciferase ATP assay

The bulk extracellular ATP concentration was measured by the luciferin-luciferase assay, as described previously [36, 39], using astrocytes cultured in 12- or 24-well plates. After the culture medium was totally replaced with isotonic Ringer solution (1 000 and 425 μl for 12- and 24-well plates, respectively), cells were incubated at 37 °C for 60 min. As a control sample for background ATP release, an aliquot (100 μl) of the extracellular solution was collected. An osmotic challenge was then applied by gently removing most of the remaining extracellular solution (875 and 300 μl for 12- and 24-well plates, respectively), adding the hypotonic solution (1 000 and 400 μl for 12- and 24-well plates, respectively), and then placing the plates in an incubator at 37 °C. At specified time points, the plate was carefully rocked again to ensure homogeneity of the extracellular solution. For the luminometric ATP assay, samples (20 and 50 μl for 12- and 24-well plates, respectively) were collected from each well at specified time points. The ATP concentration was measured, under nearly normotonic conditions, by mixing 20 or 50 μl of sample solution with 530 or 500 μl normal Ringer solution, respectively, and 50 μl of luciferin-luciferase reagent. Ionic salt sensitivity of the luciferase reaction was negligible. When required, drugs were added to the hypotonic solution to give the final concentrations as indicated. We supplemented the luciferin-luciferase assay mixture with 600 μM EDTA when the samples contained Gd^{3+}, because Gd^{3+} interfered with the luciferin-luciferase reaction (see Figure 4B), as reported previously [42]. Other drugs employed in the present study had no significant effect on the luciferin-luciferase reaction.

RT-PCR analysis of mRNA expression

Total RNA was isolated from cultured mouse cortical astrocytes before and after 15-min exposure to hypotonic solution (210 mosmol/kg-H_2O) or from homogenates of the mouse lung using Sepasol RNA I reagent (Nacalai Tesque) according to the manufacturer's instructions. First-strand cDNA was synthesized from the isolated RNA using reverse transcriptase (Roche Diagnostics GmbH, Mannheim, Germany) and oligo-dT primers (Invitrogen Corp., Carlsbad, CA). The synthesis of cDNA was performed according to the manufacturer's protocol. The sequences of primers for P2X7, MRP1 and CFTR were the same as reported previously [30, 49]. All other gene-specific primers used for PCR were designed using Primer3 software (www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The sequences of primers for RT-PCR experiments are given in supplementary information, Table S1. PCR was carried out with Blend Taq (TOYOBO, Osaka, Japan) and a Gene Amp PCR System 9600 thermal cycler (Perkin-Elmer Life Sciences, Boston, MA). Cycling conditions were 4 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at the annealing temperature given for the individual primers (supplementary information, Table S1), and 1 min at 72 °C, and, finally, 10 min at 72 °C. The integrity of the isolated RNA and the reverse transcription reaction were examined using specific primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin. All PCR products were analyzed on a 2% agarose gel and had the sizes expected from the known cDNA sequences.

Data analysis

Data were analyzed with OriginPro 7.0 (MicroCal Software, Northampton, MA). Pooled data are given as means ± SEM of observations (n). Statistical differences of the data were evaluated by ANOVA and the paired or unpaired Student's t test where appropriate, and considered significant at $P < 0.05$.

Acknowledgments

This work was supported by The Grants-in-Aid for Scientific Research from JSPS (to H-TL and RZS) and from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to YO). We thank our colleagues EL Lee for manuscript reviewing, K Shigemoto and M Ohara for technical assistance, and T Okayasu for secretarial assistance.

References

1 Hansson E, Ronnback L. Glial neuronal signaling in the central nervous system. *FASEB J* 2003; **17**:341-348.
2 Araque A, Perea G. Glial modulation of synaptic transmission in culture. *Glia* 2004; **47**:241-248.
3 Fields RD, Burnstock G. Purinergic signalling in neuron-glia interactions. *Nat Rev Neurosci* 2006; **7**:423-436.
4 Haydon PG, Carmignoto G. Astrocyte control of synaptic

transmission and neurovascular coupling. *Physiol Rev* 2006; **86**:1009-1031.
5 Fellin T, Pascual O, Haydon PG. Astrocytes coordinate synaptic networks: balanced excitation and inhibition. *Physiology (Bethesda)* 2006: **21**:208-215.
6 Newman EA. Glial cell inhibition of neurons by release of ATP. *J Neurosci* 2003; **23**:1659-1666.
7 Zhang JM, Wang HK, Ye CQ, *et al*. ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron* 2003; **40**:971-982.
8 Koizumi S, Fujishita K, Tsuda M, Shigemoto-Mogami Y, Inoue K. Dynamic inhibition of excitatory synaptic transmission by astrocyte-derived ATP in hippocampal cultures. *Proc Natl Acad Sci USA* 2003; **100**:11023-11028.
9 Volonte C, Amadio S, Cavaliere F, D'Ambrosi N, Vacca F, Bernardi G. Extracellular ATP and neurodegeneration. *Curr Drug Targets CNS Neurol Disord* 2003; **2**:403-412.
10 Franke H, Illes P. Involvement of P2 receptors in the growth and survival of neurons in the CNS. *Pharmacol Ther* 2006; **109**:297-324.
11 Darby M, Kuzmiski JB, Panenka W, Feighan D, MacVicar BA. ATP released from astrocytes during swelling activates chloride channels. *J Neurophysiol* 2003; **89**:1870-1877.
12 Shinozaki Y, Koizumi S, Ishida S, Sawada J, Ohno Y, Inoue K. Cytoprotection against oxidative stress-induced damage of astrocytes by extracellular ATP via P2Y1 receptors. *Glia* 2005; **49**:288-300.
13 Inoue K. Microglial activation by purines and pyrimidines. *Glia* 2002; **40**:156-163.
14 McLarnon JG. Purinergic mediated changes in Ca^{2+} mobilization and functional responses in microglia: effects of low levels of ATP. *J Neurosci Res* 2005; **81**:349-356.
15 Davalos D, Grutzendler J, Yang G, *et al*. ATP mediates rapid microglial response to local brain injury *in vivo*. *Nat Neurosci* 2005; **8**:752-758.
16 Xiang Z, Chen M, Ping J, *et al*. Microglial morphology and its transformation after challenge by extracellular ATP *in vitro*. *J Neurosci Res* 2006; **83**:91-101.
17 Sabirov RZ, Okada Y. ATP release via anion channels. *Purinergic Signalling* 2005; **1**:311-328.
18 Schwiebert EM, Zsembery A. Extracellular ATP as a signaling molecule for epithelial cells. *Biochim Biophys Acta* 2003; **1615**:7-32.
19 Barbe MT, Monyer H, Bruzzone R. Cell-cell communication beyond connexins: the pannexin channels. *Physiology (Bethesda)* 2006; **21**:103-114.
20 Harden TK, Lazarowski ER. Release of ATP and UTP from astrocytoma cells. *Prog Brain Res* 1999; **120**:135-143.
21 Wang Z, Haydon PG, Yeung ES. Direct observation of calcium-independent intercellular ATP signaling in astrocytes. *Anal Chem* 2000; **72**:2001-2007.
22 Stout CE, Costantin JL, Naus CC, Charles AC. Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels. *J Biol Chem* 2002; **277**:10482-10488.
23 Coco S, Calegari F, Pravettoni E, *et al*. Storage and release of ATP from astrocytes in culture. *J Biol Chem* 2003; **278**:1354-1362.
24 Suadicani SO, Brosnan CF, Scemes E. P2X7 receptors mediate ATP release and amplification of astrocytic intercellular Ca^{2+} signaling. *J Neurosci* 2006; **2**:1378-1385.
25 Cotrina ML, Lin JH, Alves-Rodrigues A, *et al*. Connexins regulate calcium signaling by controlling ATP release. *Proc Natl Acad Sci USA* 1998; **95**:15735-15740.
26 Liu GJ, Werry EL, Bennett MR. Secretion of ATP from Schwann cells in response to uridine triphosphate. *Eur J Neurosci* 2005; **21**:151-160.
27 Gordon GR, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE, Bains JS. Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. *Nat Neurosci* 2005; **8**:1078-1086.
28 Bal-Price A, Moneer Z, Brown GC. Nitric oxide induces rapid, calcium-dependent release of vesicular glutamate and ATP from cultured rat astrocytes. *Glia* 2002; **40**:312-323.
29 Sheppard DN, Welsh MJ. Effect of ATP-sensitive K^+ channel regulators on cystic fibrosis transmembrane conductance regulator chloride currents. *J Gen Physiol* 1992; **100**:573-591.
30 Minich T, Riemer J, Schulz JB, Wielinga P, Wijnholds J, Dringen R. The multidrug resistance protein 1 (Mrp1), but not Mrp5, mediates export of glutathione and glutathione disulfide from brain astrocytes. *J Neurochem* 2006; **97**:373-384.
31 Ballerini P, Di Iorio P, Ciccarelli R, *et al*. Glial cells express multiple ATP binding cassette proteins which are involved in ATP release. *NeuroReport* 2002; **13**:1789-1792.
32 Roman RM, Lomri N, Braunstein G, *et al*. Evidence for multidrug resistance-1 P-glycoprotein-dependent regulation of cellular ATP permeability. *J Membr Biol* 2001; **183**:165-173.
33 Ye ZC, Wyeth MS, Baltan-Tekkok S, Ransom BR. Functional hemichannels in astrocytes: a novel mechanism of glutamate release. *J Neurosci* 2003; **23**:3588-3596.
34 Bruzzone R, Barbe MT, Jakob NJ, Monyer H. Pharmacological properties of homomeric and heteromeric pannexin hemichannels expressed in *Xenopus* oocytes. *J Neurochem* 2005; **92**:1033-1043.
35 Anderson CM, Nedergaard M. Emerging challenges of assigning P2X7 receptor function and immunoreactivity in neurons. *Trends Neurosci* 2006; **29**:257-262.
36 Liu HT, Tashmukhamedov BA, Inoue H, Okada Y, Sabirov RZ. Roles of two types of anion channels in glutamate release from mouse astrocytes under ischemic or osmotic stress. *Glia* 2006; **54**:343-357.
37 Fan HT, Morishima S, Kida H, Okada Y. Phloretin differentially inhibits volume-sensitive and cAMP-activated, but not Ca-activated, Cl- channels. *Br J Pharmacol* 2001; **133**:1096-1106.
38 Decher N, Lang HJ, Nilius B, Bruggemann A, Busch AE, Steinmeyer K. DCPIB is a novel selective blocker of $I_{Cl,swell}$ and prevents swelling-induced shortening of guinea-pig atrial action potential duration. *Br J Pharmacol* 2001; **134**:1467-1479.
39 Dutta AK, Sabirov RZ, Uramoto H, Okada Y. Role of ATP-conductive anion channel in ATP release from neonatal rat cardiomyocytes in ischaemic or hypoxic conditions. *J Physiol* 2004; **559**:799-812.
40 Sabirov RZ, Dutta AK, Okada Y. Volume-dependent ATP-conductive large-conductance anion channel as a pathway for swelling-induced ATP release. *J Gen Physiol* 2001; **118**:251-266.
41 Bell PD, Lapointe JY, Sabirov RZ, *et al*. Macula densa cell signaling involves ATP release through a maxi anion channel. *Proc Natl Acad Sci USA* 2003; **100**:4322-4327.
42 Boudreault F, Grygorcayk R. Cell swelling-induced ATP release and gadolinium sensitive channels. *Am J Physiol* 2002; **282**:

C219-C226.
43 Kimelberg HK. Water homeostasis in the brain: basic concepts. *Neuroscience* 2004; **129**:851-860.
44 Kimelberg HK. Astrocytic swelling in cerebral ischemia as a possible cause of injury and target for therapy. *Glia* 2005; **50**:389-397.
45 Abdullaev IF, Rudkouskaya A, Schools GP, Kimelberg HK, Mongin AA. Pharmacological comparison of swelling-activated excitatory amino acid release and Cl^- currents in cultured rat astrocytes. *J Physiol* 2006; **572**:677-689.
46 Sabirov RZ, Okada Y. Wide nanoscopic pore of maxi-anion channel suits its function as an ATP-conductive pathway. *Biophys J* 2004; **87**:1672-1685.
47 Ternovsky VI, Okada Y, Sabirov RZ. Sizing the pore of the volume-sensitive anion channel by differential polymer partitioning. *FEBS Lett* 2004; **576**:433-436.
48 Hirrlinger J, Moeller H, Kirchhoff F, Dringen R. Expression of multidrug resistance proteins (Mrps) in astrocytes of the mouse brain: a single cell RT-PCR study. *Neurochem Res* 2005; **30**:1237-1244.
49 Duan S, Anderson CM, Keung EC, Chen Y, Chen Y, Swanson RA. P2X7 receptor-mediated release of excitatory amino acids from astrocytes. *J Neurosci* 2003; **23**:1320-1328.
50 Lai CP, Bechberger JF, Thompson RJ, MacVicar BA, Bruzzone R, Naus CC. Tumor-suppressive effects of pannexin 1 in C6 glioma cells. *Cancer Res* 2007; **67**:1545-1554.
51 Decleves X, Regina A, Laplanche JL, *et al*. Functional expression of P-glycoprotein and multidrug resistance-associated protein (Mrp1) in primary cultures of rat astrocytes. *J Neurosci Res* 2000; **60**:594-601.
52 Bowser DN, Khakh BS. Two forms of single-vesicle astrocyte exocytosis imaged with total internal reflection fluorescence microscopy. *Proc Natl Acad Sci USA* 2007; **104**:4212-4217.
53 Hamill OP, McBride DW Jr. The pharmacology of mechanogated membrane ion channels. *Pharmacol Rev* 1996; **48**:231-252.
54 Moran MM, Xu H, Clapham DE. TRP ion channels in the nervous system. *Curr Opin Neurobiol* 2004; **14**:362-369.
55 Collopy-Junior I, Kneipp LF, da Silva FC, *et al.* Characterization of an ecto-ATPase activity in *Fonsecaea pedrosoi*. *Arch Microbiol* 2006; **185**:355-362.
56 Fonseca FV, Fonseca de Souza AL, Mariano AC, Entringer PF, Gondim KC, Meyer-Fernandes JR. *Trypanosoma rangeli*: characterization of a Mg-dependent ecto ATP-diphosphohydrolase activity. *Exp Parasitol* 2006; **112**:76-84.
57 Levesque SA, Lavoie EG, Lecka J, Bigonnesse F, Sevigny J. Specificity of the ecto-ATPase inhibitor ARL 67156 on human and mouse ectonucleotidases. *Br J Pharmacol* 2007; **152**:141-150.
58 Guibert B, Dermietzel R, Siemen S. Large conductance channel in plasma membranes of strocytic cells is functionally related to mitochondrial VDAC-channel. *Intern J Biochem Cell Biol* 1998; **30**:379-391.
59 Dermietzel R, Hwang TK, Buettner R, *et al*. Cloning and in situ localization of a brain-derived porin that constitutes a large-conductance anion channel in astrocytic plasma membranes. *Proc Natl Acad Sci USA* 1994; **91**:499-503.
60 Buettner R, Papoutsoglou G, Scemes E, Spray DC, Dermietzel R. Evidence for secretory pathway localization of a voltage-dependent anion channel isoform. *Proc Natl Acad Sci USA* 2000; **97**:3201-3206.
61 Bahamonde MI, Fernandez-Fernandez JM, Guix FX, Vazquez E, Valverde MA. Plasma membrane voltage-dependent anion channel mediates antiestrogen-activated maxi Cl^- currents in C1300 neuroblastoma cells. *J Biol Chem* 2003; **278**:33284-33289.
62 Elinder F, Akanda N, Tofighi R, *et al*. Opening of plasma membrane voltage-dependent anion channels (VDAC) precedes caspase activation in neuronal apoptosis induced by toxic stimuli. *Cell Death Differ* 2005; **12**:1134-1140.
63 Sabirov RZ, Sheiko T, Liu HT, Deng D, Okada Y, Craigen WJ. Genetic demonstration that the plasma membrane maxianion channel and voltage-dependent anion channels are unrelated proteins. *J Biol Chem* 2006; **281**:1897-1904.
64 Schneider GH, Baethmann A, Kempski O. Mechanisms of glial swelling induced by glutamate. *Can J Physiol Pharmacol* 1992; **70**:S334-S343.
65 Takahashi H, Koehler RC, Brusilow SW, Traystman RJ. Inhibition of brain glutamine accumulation prevents cerebral edema in hyperammonemic rats. *Am J Physiol Heart Circ Physiol* 1991; **261**:H825-H829.
66 Willard-Mack CL, Koehler RC, Hirata T, *et al*. Inhibition of glutamine synthetase reduces ammonia-induced astrocyte swelling in rat. *Neuroscience* 1996; **71**:589-599.
67 Izumi Y, Kirby-Sharkey CO, Benz AM, *et al*. Swelling of Muller cells induced by AP3 and glutamate transport substrates in rat retina. *Glia* 1996; **17**:285-293.

(**Supplementary Information** is linked to the online version of the paper on the Cell Research website.)