ATP Released From Astrocytes During Swelling Activates Chloride Channels

Mark Darby, J. Brent Kuzmiski, William Panenka, Denise Feighan, and Brian A. MacVicar
Neuroscience Research Group, Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta T2N 4N1, Canada

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Darby, Mark, J. Brent Kuzmiski, William Panenka, Denise Feighan, and Brian A. MacVicar. ATP released from astrocytes during swelling activates chloride channels. J Neurophysiol 89: 1870–1877, 2003; 10.1152/jn.00510.2002. ATP release from astrocytes contributes to calcium ([Ca^{2+}]) wave propagation and may modulate neuronal excitability. In epithelial cells and hepatocytes, cell swelling causes ATP release, which leads to the activation of a volume-sensitive Cl^- current (I_{Cl,swell}) through an autocarpythone involving purinergic receptors. Astrocyte swelling is counterbalanced by a regulatory volume decrease, involving efflux of metabolites and activation of I_{Cl,swell} and K^+ currents. We used whole cell patch-clamp recordings in cultured astrocytes to investigate the autocrine role of ATP in the activation of I_{Cl,swell} by hypo-osmotic solution (HOS). Apyrase, an ATP/ADP nucleotidease, inhibited HOS-activated I_{Cl,swell}; whereas ATP and the P2Y agonists, ADP/PS and ADP, induced Cl^- currents similar to I_{Cl,swell}. Neither the P2U agonist, UTP nor the P2X agonist, α,β-methylene ATP, were effective. BzATP was less effective than ATP, suggesting that P2X receptors were not involved. P2 purinergic antagonists, suramin, RB2, and pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) reversibly inhibited activation of I_{Cl,swell}, suggesting that ATP-activated P2Y1 receptors. Thus ATP release mediates I_{Cl,swell} in astrocytes through the activation of P2Y1-like receptors. The multidrug resistance protein (MRP) transport inhibitors probenicid, indomethacin, and MK-571 all potently inhibited I_{Cl,swell}. ATP release from astrocytes in HOS was observed directly using luciferin-luciferase and MK-571. ATP release from astrocytes in spreading depression (Basarsky et al. 1999) is dependent on MAP kinase activation (Crepel et al. 1998; Lascola and Kraig 1996). Astrocytes known to swell in response to a number of stimuli including increased external K^+ ([K^+]_o) (MacVicar et al. 2002) and neurotransmitters (reviewed in Kimelberg 1995; Strange 1993). Multidrug resistance protein (MRP) and p-glycoprotein, the protein product of the multidrug resistance gene (MDR), are two ATP-binding cassette proteins that are expressed in astrocytes (Decleves et al. 2000). Finally, purinergic P2Y receptors are expressed on astrocytes both in cell culture (Centenemi et al. 1997; Cotrina et al. 1998a; Fam et al. 2000; Scemes et al. 2000) and in vivo (Franke et al. 2001; Zhu and Kimelberg 2001). Therefore all of the components that are involved in the swelling-induced release of ATP and activation of I_{Cl,swell} in hepatoma and epithelial cells (Roman et al. 1999; Schwiebert et al. 1995; Wang et al. 1996) are present in astrocytes.

The activation of I_{Cl,swell} is part of the cellular changes that occur in response to increased cell volume (Strange et al. 1996). Efflux of Cl^- and amino acids through the channel underlying I_{Cl,swell} in conjunction with efflux of K^+ through other channels is part of the active process to decrease volume, termed regulatory volume decrease (RVD) (Pasantes-Morales et al. 1994a,b). I_{Cl,swell} also allows the efflux of larger amino acids and can contribute to the non-[Ca^{2+}]-dependent release of glutamate during spreading depression (Basarsky et al. 1999).

The goal of this study was to determine whether ATP release from astrocytes contributes to the activation of Cl^- channels during cellular swelling. We tried several approaches. The first was to see if apyrase (an enzyme that degrades ATP) or purinergic receptor antagonists depressed the activation of I_{Cl,swell} during swelling. Second, we determined whether ATP (and other P2 agonists) evoked Cl^- currents and whether ATP-evoked Cl^- currents were sensitive to blockers of I_{Cl,swell}. Third, we examined whether inhibitors of either p-glycoprotein or MRP function could depress I_{Cl,swell}. Fourth, we directly measured ATP release from astrocytes and examined the sensitivity of ATP release to inhibitors of transport via MRP. Our results show that ATP release during hypo-osmotically induced swelling acts on P2 receptors to activate I_{Cl,swell} in astrocytes.

METHODS

Astrocyte primary cell cultures

Astrocyte primary cultures (McCarthy and de Vellis 1980) were obtained from Sprague Dawley rats (1 day postnatal). Cortical tissue
(with meninges and pia mater removed) was dissociated by mechanical trituration and transferred to tissue culture flasks (1 cortex/flask) containing glial media for 2–3 wk (Dulbecco’s modified Eagle medium (DMEM) with 58 mM NaHCO₃, 20 mM HEPES, 50 U/ml pen/strep, 10% fetal calf serum at 37°C and 5% CO₂).

Electrophysiological recordings

Astrocytes were plated onto poly-ornithine-coated glass coverslips ≥1 day prior to electrophysiological recordings. Coverslips with astrocytes were placed in a 200-μl recording chamber on an inverted microscope with phase-contrast optics (Axiovert, Zeiss) and superfused at 1–2 ml/min (20–22°C) with the following solution, which we used previously to isolate the Cl⁻ currents (Crepel et al. 1998), containing (in mM): 70 Trizma-Cl⁻, 100 sucrose, 1.5 CaCl₂, 10 HEPES, 10 glucose, 5 TEA-Cl, and 5 BaCl₂ adjusted to pH 7.3 with CsOH. The osmolarity of this solution was 290 mosM (Crepel et al. 1998), and the hypo-osmotic solution (HOS) was the standard extracellular solution without added sucrose. Patch-clamp pipettes had a resistance of 4–7 MΩ when filled with electrode solution containing (in mM) 60 Trizma-Cl⁻, 70 Trizma-base, 70 aspartic acid, 15 HEPES, 0.4 CaCl₂, 1 MgCl₂, 1 ATP, 0.5 GTP, and 1 EGTA, adjusted to pH 7.25 with CsOH. Membrane currents were recorded under voltage clamp (V₁ = −70 mV) using an Axopatch −1D amplifier (Axon Instruments, Foster City, CA). Cells with a stable holding current and access resistance (<20 MΩ; typical capacitance >30 pF) were recorded from for the subsequent experiments. These conditions were the same as we previously used to examine Cl⁻ currents in astrocytes (Crepel et al. 1998). We previously showed under these conditions that the liquid junction potential (LJP) was small (1 mV and varied only slightly when the intracellular conditions that the liquid junction potential (LJP) was small (1 mV and varied only slightly when the intracellular [Cl⁻] was changed (Crepel et al. 1998). Thus the membrane potential was not corrected for the LJP.

Data acquisition and analysis

We used two Digidata 1200 Interface boards (Axon Instruments) to simultaneously digitize membrane currents onto two separate computers using Clampex 7 or 8 (Axon Instruments). One computer measured a continuous gap-free recording of membrane current for each experiment (holding potential: −70 mV) and the other measured the current resulting from a 2-s voltage ramp from −120 to +60 mV applied every 30 s.

In all experiments with antagonists, we applied HOS twice to ensure reproducibility of the HOS-induced I_Cl,swell. The result in the experimental solution was compared statistically to the second HOS current normalized to the first HOS current. This is shown as the 0 concentration value in some graphs. Statistical analysis was done using ANOVA. post hoc multiple comparisons were performed used Tukey’s (P < 0.05). Values are presented as mean ± SE.

Luciferin-luciferase assays

ATP release was examined using luciferin-luciferase (Sigma, St. Louis, MO) that was added to the extracellular solution at 10–20 mg/ml. Measurements were made using a photomultiplier tube with a current-to-voltage converter (Hamamatsu, Hamamatsu, Japan). The output was low-pass filtered (100 Hz) and digitized using the same system as described in the preceding text for voltage-clamp recordings.

Materials

Culture reagents were obtained from Canadian Life Technologies (Burlington, Ontario), aspartic acid from Fisher (Edmonton, Alberta), and sucrose, glucose, BaCl₂, MgCl₂, and CaCl₂ were from VWR (Edmonton, Alberta). All other drugs including the grade I and grade VI apyrase were purchased from Sigma (Oakville, Ontario, Canada).

RESULTS

Hypo-osmotic induced current is blocked by enzyme to degrade ATP

Hypo-osmotic solutions (HOS) consistently induced a Cl⁻ current in astrocytes termed I_Cl,swell with properties identical to what we previously reported (Crepel et al. 1998). Figure 1 (A–C) shows the typical HOS activation of I_Cl,swell and the subtraction of the ramp currents that we used to quantify the magnitude of I_Cl,swell. We examined the actions of apyrase, which metabolizes ATP and ADP, on the magnitude of I_Cl,swell. A reduction of I_Cl,swell by apyrase would support a role for extracellular ATP and/or ADP in the activation of this current (e.g. Roman et al. 1999; Schwiebert et al. 1995; Wang et al. 1996). Two different forms of apyrase with different degrees of ATPase/ADPase ratio (grade I vs. grade VI; 5–20 units/ml) were used and both reversibly decreased the amplitude of the HOS-activated I_Cl,swell (Fig. 1, D–F). The preparation with the highest ATPase/ADPase ratio (G–VI) depressed I_Cl,swell with similar efficacy as the form with lower ATPase/ADPase ratio. This suggests that ATP and/or ADP and not a degradation product are the active factors released during swelling.

Purinergic receptor antagonists block I_Cl,swell

If ATP release is important in the response to swelling, then purinergic receptor activation should be necessary for the activation of I_Cl,swell by HOS. Therefore we examined the response of the HOS activated I_Cl,swell to antagonists of purinergic receptors (Fig. 2). In all experiments, we first tested the reproducibility and consistency of the activation of I_Cl,swell by HOS. To do this, we activated I_Cl,swell twice with HOS before receptor antagonists were applied. The second application of HOS consistently induced I_Cl,swell with a magnitude similar to the first. In Fig. 2, C and D, the second HOS-activated current was normalized to the first and was plotted as 0 concentration. Suramin, a wide-spectrum purinergic antagonist, reversibly depressed the HOS-activated I_Cl,swell (Fig. 2, A–C; maximum depression at 100 μM, 61 ± 2%, n = 4), RB2 (50 μM), a relatively selective P2Y antagonist, also blocked HOS activated I_Cl,swell to a similar extent as suramin (64 ± 5%, n = 4; Fig. 2E). Pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) (100 μM), an antagonist to P2X receptors but not P2U (Charlton et al. 1996; King et al. 1998), also depressed the HOS-activated I_Cl,swell (39.5 ± 6.2%, n = 3; Fig. 2E).

ATP activates a Cl⁻ current

The next step was to determine whether ATP itself and purinergic receptor agonists induced a Cl⁻ current with properties similar to the HOS-activated I_Cl,swell. We applied ATP to ensure that the putative ligand mimicked the actions of HOS in activating a Cl⁻ current. ATP activated a Cl⁻ current that reversed at potentials (1 mM ATP; reversal at −6.8 ± 5.4 mV; n = 4) not significantly different from the Cl⁻ equilibrium potential (−8.6 mV) and the I_Cl,swell reversal potential (−16 ± 8 mV, n = 4, P > 0.3; Fig. 3, A and B). 5-Nitro-2-(3-
phenylpropylamino) benzoic acid (NPPB, 1 mM; \(n = 3\)), which blocks \(I_{\text{Cl,swell}}\) (Crepel et al. 1998), also totally blocked the ATP-activated \(I_{\text{Cl,swell}}\). NPPB does not alter purinergic receptors directly (Feranchak et al. 2000; Mitchell et al. 1998).

There were differences in the magnitude of the \(I_{\text{Cl,swell}}\) current activated by ATP and the HOS-activated \(I_{\text{Cl,swell}}\). Figure 3C demonstrates that the current was maximally activated by 1 mM ATP; corresponding to current amplitude that was 53 ± 17% (\(n = 4\)) of the preceding HOS-activated current. Increasing the concentration of ATP to 5 mM did not activate a larger current. Therefore the maximum ATP-induced current was significantly less than the HOS-activated \(I_{\text{Cl,swell}}\). We then compared the percent depression induced by suramin, the wide spectrum purinergic antagonist. A supra-maximal concentration of suramin (500 \(\mu\)M) completely blocked the ATP-induced \(I_{\text{Cl,swell}}\) by ~50% (Fig. 3D). These results imply that another agent is released in addition to ATP to evoke HOS-activated \(I_{\text{Cl,swell}}\). However, we have not yet identified the nature of this other agent.

We examined the activation of the \(I_{\text{Cl,swell}}\) current by other purinergic receptor agonists to further define the receptor subtype involved (Fig. 3E). These experiments also addressed the possibility that the differences in the magnitude of the current amplitude indicated the involvement of a purinergic receptor that was activated more effectively by another agonist such as UTP. All agonists were tested and compared at 100 \(\mu\)M UTP, which activates P2Y\(_{2,3,4}\) and P2U receptors (Ralevic and Burnstock 1998), did not induce a \(I_{\text{Cl,swell}}\) current (\(n = 5\)) nor did \(\alpha,\beta\)-methylene ATP (\(\alpha,\beta\)-MeATP; \(n = 4\)), an agonist at P2X receptors. ADP and ADP\(_{\beta S}\) both evoked currents that reversed close to the \(\text{Cl}^-\) equilibrium potential (Fig. 3; ADP-induced current reversed at \(-9.6 \pm 2.0\) mV, \(n = 4\); ADP-\(\beta S\)-induced current reversed at \(-0.4 \pm 1.4\) mV, \(n = 5\); \(\text{Cl}^-\) equilibrium potential, \(-8.6\) mV). Both ADP and ADP\(_{\beta S}\) were slightly more efficacious at inducing a \(\text{Cl}^-\) current than was ATP (Fig. 3E). The P2X7 agonist BzATP induced a current at 100 \(\mu\)M that was, however, of lesser amplitude than that evoked by 100 \(\mu\)M ATP (16 ± 3%, \(n = 3\)). This indicates that ATP was not working through P2X7 receptors, which should be preferentially activated by BzATP (Panenka et al. 2001; Ralevic and Burnstock 1998). Our results with the agonists suggest that the purinergic receptor was the P2Y1 subtype (Ralevic and Burnstock 1998). This was consistent with the receptor antagonist profile that we described in the preceding text.

**Inhibitors of multidrug resistance protein but not \(p\)-glycoprotein blocked HOS-activated \(I_{\text{Cl,swell}}\) and ATP release**

We next examined the sensitivity of the HOS-activated \(I_{\text{Cl,swell}}\) to blockers of two transporters that are postulated to play a role in ATP release from other cell types. \(p\)-glycoprotein, the product of the MDR1 gene and MRP are both members of the ABC transport family that have been identified as potential modulators of \(I_{\text{Cl,swell}}\) in other cell types (Hainsworth

Probenicid blocks activity of the MRP transporter at relatively high concentrations (Courtois et al. 1999; Payen et al. 1999). We observed significant but reversible block of I_{Cl,swell} at 5–10 mM probenicid (Fig. 4, 103/11006 6% block at 10 mM, n = 4, Fig. 4C). In some cells, there appeared to be some block of a resting Cl^- current in addition to the block of I_{Cl,swell}.

Indomethacin, another inhibitor of MRP-mediated transport (Courtois et al. 1999; Payen et al. 1999), reversibly blocked HOS-activated I_{Cl,swell} at 500 µM (90 ± 11% depression, n = 5, Fig. 4C). Indomethacin only blocks MRP-mediated transport at this high concentration (Courtois et al. 1999; Payen et al. 1999). At lower concentrations (e.g., < 100 µM), indomethacin inhibits cyclo-oxygenase (COX). However, at 200 µM, the effects of indomethacin were substantially reduced. As an added control to ensure that the effect of indomethacin was due to blocking MRP and not due to COX inhibition, we tested the effect of acetylsalicylic acid (ASA), another COX inhibitor. ASA did not inhibit I_{Cl,swell} (100 µM, -4 ± 1%, n = 3), supporting our conclusion that the block of I_{Cl,swell} by indomethacin was independent of its effect on COX. In contrast to the potent inhibition by MRP inhibitors, verapamil, which blocks transport by p-glycoprotein, caused little reduction of I_{Cl,swell} at a supra-maximal concentration (1 mM, 19 ± 3%, n = 4, Fig. 4C).

To further substantiate MRP involvement in the activation of I_{Cl,swell}, we tested the effect of MK-571, another MRP transport inhibitor that potently and selectively blocks this transporter (Gekeler et al. 1995; Vernhet et al. 1999). MK-571 (100 µM) reduced HOS-activated I_{Cl,swell} by 96 ± 13% (n = 6) (Fig. 4C).

Finally we examined ATP efflux from astrocytes to determine if HOS, which induces cellular swelling, can induce the release of ATP. It is known that Ca^{2+} wave propagation is associated with ATP release from astrocytes, which has been measured in cell culture (Wang et al. 2000). We analyzed ATP release by measuring the photons produced by the luciferin-luciferase mediated degradation of ATP. Increased efflux of ATP will be associated with increased light output if ATP is released during HOS stimulation (e.g., Feranchak et al. 2000). Changing the extracellular solution from control to HOS increased the light output from astrocyte cultures indicating that ATP efflux was increased (Fig. 5). MK-571 totally blocked the HOS-mediated increase (n = 5 cultures). Although this technique did not allow the quantification of the local ATP concentration outside of the cellular membrane, it did provide

![Fig. 2](http://jn.physiology.org/)

**FIG. 2.** Suramin, RB2, and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) inhibited HOS-induced I_{Cl,swell}. A: membrane current evoked by 3 successive HOS stimulations (3 min in duration) illustrating the reversible block of I_{Cl,swell} by suramin (500 µM). B: HOS-induced I_{Cl,swell} (obtained by subtracting the current recorded before each HOS application from that obtained during each HOS application) in the presence and absence of suramin. C: dose response for the suramin block of I_{Cl,swell}. Maximal block was observed at 100 µM. The value at 0 concentration in this and the following graphs represents the 2nd HOS-induced current normalized with respect to the 1st application. D: RB2 also blocked I_{Cl,swell} as shown in the I-V relations of HOS-induced currents obtained before, during, and after the superfusion of 50 µM RB2. E: bar graph depicting the degree of inhibition of the HOS-induced current by 50 µM RB2 and by 100 µM PPADS. *, P < 0.05.
I have shown that the HOS-activated flux of ATP that was released from astrocytes during swelling. We measured the efflux of ATP induced the effect that this transport pathway was not involved. Finally, HOS was ineffective. The P2X7 agonist, BzATP did not as effective in activating receptors also depressed the HOS-activated antagonists (Mitchell et al. 1998; Roman et al. 1999; because the response to HOS can be depressed by purinergic receptors also depend on the activation of extracellular purinergic receptors.

DISCUSSION

The results of this study suggest that \( I_{\text{Cl,swell}} \) was activated by ATP that was released from astrocytes during swelling. We have shown that the HOS-activated \( I_{\text{Cl,swell}} \) was depressed by apyrase, an enzyme that degrades ATP and ADP. An NPPB-sensitive Cl\(^-\) current that was similar to \( I_{\text{Cl,swell}} \) was activated by application of ATP. Other agonists of the P2Y1 receptor (ADP and ADP\(\beta\)S) also induced a Cl\(^-\) current, whereas UTP, a P2U and P2Y\(_{2,3,4}\) agonist and \( \alpha,\beta\)-MeATP, a P2X agonist, were ineffective. The P2X7 agonist, BzATP was not as effective in activating \( I_{\text{Cl,swell}} \) indicating that P2X7 receptors were not involved. Both the nonspecific purinergic receptor antagonist, suramin and the selective P2Y agonist, RB2 blocked the HOS-activated \( I_{\text{Cl,swell}} \). PPADS that inhibits P2Y1 receptors also depressed the HOS-activated \( I_{\text{Cl,swell}} \). Supporting a role for P2Y1 receptor activation. Several pharmacological antagonists of the MRP transporter blocked the HOS-induced \( I_{\text{Cl,swell}} \). Verapamil, the potent blocker of p-glycoprotein transport had no effect on the HOS-activated \( I_{\text{Cl,swell}} \), suggesting that this transport pathway was not involved. Finally, HOS induced the efflux of ATP measured as an increase in light output using luciferin-luciferase reaction to assay ATP efflux. The HOS induced efflux of ATP was reversibly inhibited by MK-571, which was also effective in inhibiting the HOS-activated \( I_{\text{Cl,swell}} \). These results suggest that MRP causes the efflux of ATP during swelling which in turn acts in an autocrine manner to activate purinergic receptors and subsequently \( I_{\text{Cl,swell}} \).

In epithelial cells and hepatocytes, the activation of \( I_{\text{Cl,swell}} \) depends on the activation of extracellular purinergic receptors because the response to HOS can be depressed by purinergic receptor antagonists (Mitchell et al. 1998; Roman et al. 1999; Schwiebert et al. 1995; Wang et al. 1996). The present study demonstrates that astrocytes also regulate their volume through a similar mechanism. Our results provide evidence that ATP is one of the native messenger molecules that bind P2Y receptors and thereby activate \( I_{\text{Cl,swell}} \). The luciferin-luciferase experiment indicates that ATP itself is released. However, we cannot rule out a potential contribution of ATP release in addition to ATP, UTP, which may be involved in the response to HOS in other cells (Harden and Lazarowski 1999), was not involved in this process in astrocytes because we could not observe any Cl\(^-\) current activation by UTP. Exogenously applied ATP evoked a current with properties similar to \( I_{\text{Cl,swell}} \). Apyrase, the enzyme that degrades ATP, inhibited the HOS-activated \( I_{\text{Cl,swell}} \). We found that the P2 purinergic antagonists, suramin, RB2, and PPADS, inhibited \( I_{\text{Cl,swell}} \). Suramin is nonselective against P2Y and P2X, but RB2 is a specific P2Y receptor antagonist (Abbracchio and Burnstock 1994; Burnstock and Warland 1987; Najbar et al. 1996), thus implying P2Y purinergic regulation of \( I_{\text{Cl,swell}} \). PPADS inhibits P2Y1 receptors in addition to P2X receptors (Charlton et al. 1996; King et al. 1998), suggesting involvement of P2Y1 receptors. However, P2X receptors are not involved because we did not observe activation of Cl\(^-\) currents by \( \alpha,\beta\)-MeATP, a P2X agonist. It is likely that P2X7 receptors are not involved because the P2X7 agonist, BzATP was less effective than ATP in inducing \( I_{\text{Cl,swell}} \). Recently Neary et al. (1999) showed that signaling from P2Y receptors to Erk involved a \( \text{Ca}^{2+} \) independent isofrom of PKC. This pathway provides a mechanism by which the HOS-mediated activation of purinergic receptors could activate the Erk MAP kinase cascade in a \( \text{Ca}^{2+} \)-independent manner and thereby activate \( I_{\text{Cl,swell}} \) (Crepel et al. 1998).

A critical issue is the mechanism by which ATP is released into the extracellular space. In hepatocytes, the MDR1 gene product, p-glycoprotein functions as an ATP transporter (Abraham et al. 1993; Roman et al. 1997; Vanoye et al. 1999) and as an intimate regulator of \( I_{\text{Cl,swell}} \) and RVD (Hardy et al. 1995; Luckie et al. 1993; Warland 1987; Najbar et al. 1996) 

FIG. 3. ATP and P2Y1 agonists activated Cl\(^-\) currents in cultured astrocytes. A: recording of the mean of the current evoked by ATP (5 mM) showing substantial block by 1 mM 5-nitro-2-(3-phenylpropylylamino) benzoic acid (NPPB). B: individual non-subtracted I-V relations of the control current and of the currents activated by 5 mM ATP and 5 mM ATP with 1 mM NPPB. The ATP-activated currents reversed at the Cl\(^-\) equilibrium potential. C: bar graphs of the magnitude of the ATP-induced current compared with the HOS-induced \( I_{\text{Cl,swell}} \) recorded in the same cells. The maximal Cl\(^-\) current was observed at 1 mM ATP and was ~50% of the HOS current amplitude. D: bar graph showing the degree of inhibition evoked by 500 \( \mu \)M suramin on the HOS-induced current and on the 5 mM ATP-induced current. Approximately 50% of the HOS-evoked current was blocked by suramin whereas 100% of the ATP-evoked current was blocked. E: bar graph showing the relative amplitudes of the Cl\(^-\) current evoked by various purinergic receptor analogs (at 100 \( \mu \)M) as compared with the amplitude of the HOS-induced \( I_{\text{Cl,swell}} \). The P2Y1 agonists ADP and ADP\(\beta\)S evoked substantial currents, whereas the P2U agonist UTP and the P2X agonist \( \alpha,\beta\)-MeATP were ineffective. The P2X7 agonist, BzATP evoked a current of smaller amplitude than ATP at the same concentration (100 \( \mu \)M).
p-glycoprotein is expressed in the astrocyte endfoot processes of the brain microvasculature (Golden and Pardridge 1999) and both MRP1 and p-glycoprotein are expressed in cultured astrocytes (Decleves et al. 2000). In hepatocytes and fibroblasts, verapamil potently blocked p-glycoprotein activity and the activation of $I_{\text{Cl,swell}}$. Indomethacin did not block the current at lower concentrations (200 µM) which blocks cyclo-oxygenase enzyme activity. Also acetylsalicylic acid (ASA), which effectively blocks cyclo-oxygenase at 100 µM but has no effect on MRP activity, did not inhibit this current. Verapamil, which is a potent p-glycoprotein transport inhibitor, only slightly inhibited $I_{\text{Cl,swell}}$.

FIG. 4. Antagonists to the MRP transporter activity depressed HOS-induced $I_{\text{Cl,swell}}$. A: a prolonged application of HOS-activated $I_{\text{Cl,swell}}$ that was reversibly blocked by probenicid (10 mM). B: I-V relations of the currents induced by HOS alone and HOS with 10 mM probenicid showing profound and reversible block. C: graph summarizing the effects of the various drugs used to test for the mechanism of ATP release. Probenicid, indomethacin, and MK-571 all inhibit the MRP transporter and all were equally potent in their block of $I_{\text{Cl,swell}}$. Indomethacin did not block the current at lower concentrations (200 µM) which blocks cyclo-oxygenase enzyme activity. Also acetylsalicylic acid (ASA), which effectively blocks cyclo-oxygenase at 100 µM but has no effect on MRP activity, did not inhibit this current. Verapamil, which is a potent p-glycoprotein transport inhibitor, only slightly inhibited $I_{\text{Cl,swell}}$.

FIG. 5. ATP release from astrocytes, measured using luciferin-luciferase, showed increased efflux in HOS that was blocked by the MRP inhibitor, MK-571. A: the output from the photomultiplier system shows the level of light production in astrocyte cultures with luciferin-luciferase was increased when cultures were exposed to HOS solution. The traces represent the output from a photomultiplier tube that detected photons generated by the luciferase-catalyzed reaction of luciferin with ATP. ▲, a gap of ~40 s during the solution change. The light output is proportional to the ATP efflux and shows that after the switch to HOS solution, the efflux was significantly enhanced. In MK-571 (100 µM), the efflux of ATP in HOS was blocked and the inhibition by MK-571 was reversible after wash.
Although in the intact CNS it is difficult to rigorously delineate which cell type swells preferentially in response to activity, we have shown that in the optic nerve astrocytes swell in response to high external [K+] (MacVicar et al. 2002). The profound swelling that accompanies spreading depression is also associated with activation of Cl\textsuperscript{−} channels and the release of glutamate through NPPB-sensitive Cl\textsuperscript{−} channels (Basarsky et al. 1998, 1999). If the release of ATP from astrocytes occurs during swelling in vivo, this could provide a novel mechanism by which purinergic receptors could be activated to provide negative feedback by ATP itself at high concentrations (Armstrong et al. 2002) or through activation of inhibitory adenosine receptors by the metabolism of ATP (Dunwiddie and Masino 2001). Alternatively ATP release could contribute to the generation of calcium waves in astrocytes (Guthrie et al. 1999).

In summary, we conclude that the activation of I\textsubscript{Clswell} is dependent on the stimulation of purinergic receptors because this current was blocked by the purinergic antagonists suramin and RB2, inhibited by the nucleotidase, apyrase and mimicked by exogenously applied ATP. We propose that the ATP needed to stimulate the purinergic receptors is released via a transporter having a pharmacological sensitivity similar to that of the MRP transporter family.

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