Brief Communications

Astroglial Connexin43 Hemichannels Tune Basal Excitatory Synaptic Transmission

Oana Chever,* Chun-Yao Lee,* and Nathalie Rouach

Neuroglial Interactions in Cerebral Physiopathology, Center for Interdisciplinary Research in Biology, Collège de France, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7241, Institut National de la Santé et de la Recherche Médicale U1050, 75005 Paris, France

Fast exchange of extracellular signals between neurons and astrocytes is crucial for synaptic function. Over the last few decades, different pathways of astroglial release of neuroactive substances have been proposed to modulate neurotransmission. However, their involvement in physiological conditions is highly debated. Connexins, the gap junction forming proteins, are highly expressed in astrocytes and have recently been shown to scale synaptic transmission and plasticity. Interestingly, in addition to gap junction channels, the most abundant connexin (Cx) in astrocytes, Cx43, also forms hemichannels. While such channels are mostly active in pathological conditions, they have recently been shown to regulate cognitive function. However, whether astroglial Cx43 hemichannels are active in resting conditions and regulate basal synaptic transmission is unknown. Here we show that in basal conditions Cx43 forms functional hemichannels in astrocytes from mouse hippocampal slices. We furthermore demonstrate that the activity of astroglial Cx43 hemichannels in resting states regulates basal excitatory synaptic transmission of hippocampal CA1 pyramidal cells through ATP signaling. These data reveal Cx43 hemichannels as a novel astroglial release pathway at play in basal conditions, which tunes the moment-to-moment glutamatergic synaptic transmission.

Key words: astrocytes; basal synaptic transmission; connexin43; hemichannel; hippocampus; physiology

Introduction

Astrocytes are active partners of neurons. They regulate neuronal activity through various mechanisms, including the release of neuroactive substances (Nedergaard and Verkhratsky, 2012; Araque et al., 2014). The dialog that occurs in physiological conditions and the nature of the various underlying molecular mechanisms of astroglial gliotransmitter release are still unclear. Indeed, different molecular pathways mediate the release of gliotransmitters such as vesicular exocytosis, connexin (Cx) and pannexin (Px) hemichannels (HCs), P2X7, TREK-1, Best1, and swelling-activated anion channels. However, their involvement in basal conditions is a matter of intense debate and study (Ham-

ilton and Attwell, 2010; Panatier et al., 2011; Agulhon et al., 2012; Nedergaard and Verkhratsky, 2012).

Typically, astrocytes abundantly express the gap junction protein subunits Cx43 and Cx30, which mediate the formation of extensive astroglial networks (Pannasch and Rouach, 2013). Gap junction channels, formed by the apposition of two HCs belonging to adjacent cells, enable direct cytoplasmic exchange of numerous small molecules, such as energy metabolites, ions, or neurotransmitters (Pannasch and Rouach, 2013). However, in addition to the classical formation of gap junctions, hexamers of Cx43 are also inserted at astroglial membranes as large-pore single HCs, which mediate direct cytosolic exchange with the extracellular space (Giaume et al., 2013). Cx43 HCs can release various neuroactive substances such as potassium, ATP, or glutamate (Giaume et al., 2013), which are known to modulate neuronal activity. Until now, astroglial HCs have been shown to be active mostly in pathological conditions, such as inflammation (Giaume et al., 2013) or during high neuronal activity (Torres et al., 2012). Yet, astroglial Cx43 HC activity in resting conditions and its putative role in regulating basal synaptic transmission have never been investigated.

Astroglial Cx HCs are indeed usually assumed to be inactive in basal conditions, due to their low open probability at resting membrane potential and ionic concentrations (Contreras et al., 2003; Kang et al., 2008). Nonetheless, outside the brain, Cx HCs do open in physiological conditions, such as in the retina where they regulate neural progenitor proliferation (Pearson et al., 2005), or in the inner ear where they sustain long-range intercellular calcium signals (Anselmi et al., 2008), as shown in astrocytes (Giaume et al., 2013). Remarkably, it has also recently been
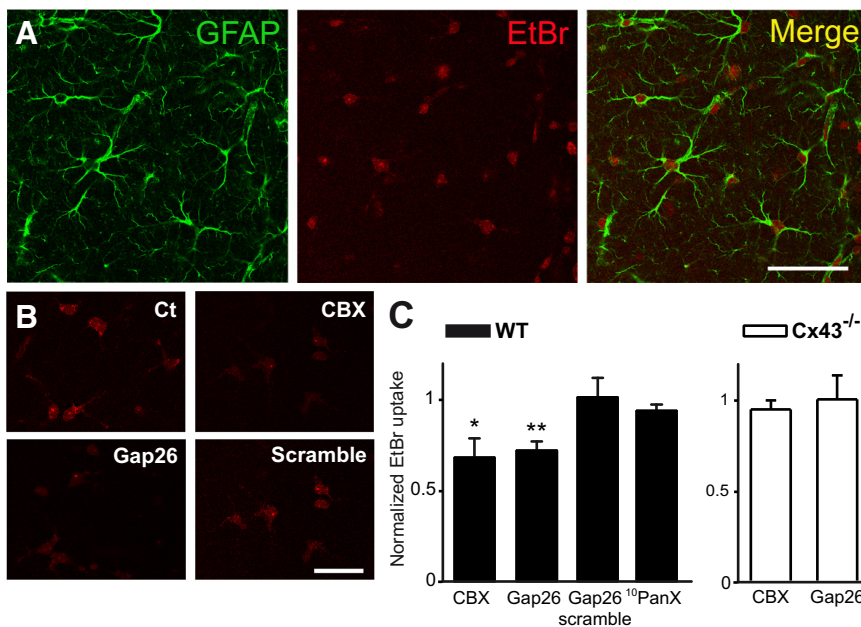
Figure 1. Astroglial Cx43 HCs are open in basal conditions. **A**, Representative basal EtBr uptake (red) in stratum radiatum astrocytes labeled with GFAP (green) in hippocampal slices. Scale bar, 50 μm. **B**, Higher magnification of astroglial EtBr uptake (red) in basal conditions (Ct), and in the presence of CBX (200 μM), Gap26 (100 μM), or Gap26 scramble peptides (100 μM), applied 15 min prior and during EtBr uptake assay. Scale bar, 25 μm. **C**, Astrocytic EtBr uptake normalized to control conditions in slices from wild-type mice (WT) treated with CBX (200 μM, $n = 7$), Gap26 (100 μM, $n = 7$), Gap26 scramble (100 μM, $n = 7$) and ^{10}Panx (400 μM, $n = 5$) peptides (left), and from astroglial conditional Cx43 knock-out mice (Cx43$^{-/-}$) treated with CBX (200 μM, $n = 4$) or Gap26 (100 μM, $n = 3$; right). Asterisks indicate statistical significance performed on raw data (*$p < 0.05$, **$p < 0.01$, Student's paired t test).

shown that astroglial Cx43 HCs regulate cognitive function, since blocking such channels in the amygdala impairs fear memory consolidation (Stehberg et al., 2012). In addition, we have recently shown that the deletion of astroglial Cxs alters synaptic transmission and plasticity (Pannasch et al., 2011). Altogether, these data suggest that Cx HCs regulate synaptic function. We here show that hippocampal astrocytic Cx43 HCs are open under resting conditions and modulate basal synaptic transmission through ATP signaling.

Materials and Methods

Animals. Experiments were performed according to the guidelines of European Community Council Directives of January 1, 2013 (2010/63/EU), and all efforts were made to minimize the number of animals used and their suffering. Experiments were performed on the hippocampus of wild-type mice and Cx43$^{fl/fl}$:hGFAP-Cre mice (Cx43$^{-/-}$), with conditional deletion of Cx43 in astrocytes (Theis et al., 2003). For all analyses, mice of both genders and their littermates were used (PN16-PN25).

Acute hippocampal slices. Acute transverse hippocampal slices (300–400 μm) were prepared as previously described (Pannasch et al., 2011). Slices were maintained at room temperature in a storage chamber containing a standard artificial CSF [ACSF (in mM): 119 NaCl, 2.5 KCl, 2.5 CaCl$_2$, 1.3 MgSO$_4$, 1 NaH$_2$PO$_4$, 26.2 NaHCO$_3$, and 11 glucose, saturated with 95% O$_2$ and 5% CO$_2$] for at least 1.5 h before the experiments. Basal HC activity and synaptic transmission were analyzed at a depth of 40–80 μm from the surface of acute slices and at a recovery time of at least 90 min after the slicing procedure, because in such conditions astrocytes exhibited no sign of reactivity, as assessed by GFAP and vimentin stainings. Indeed, in acute hippocampal slices fixed with 4% paraformaldehyde after a 90 min incubation period in oxygenated ACSF, we found that astrocytes did not show hallmarks of reactive gliosis, since they display: (1) no enhanced expression of GFAP, compared with sections from fixed brain following intracardiac perfusion of 4% paraformaldehyde (perfusion-fixed tissue; total GFAP staining area of individual astrocytes:

fixed acute slice, 197 ± 16.33 μm^2, $n = 41$; perfusion fixed tissue, 191 ± 8.64 μm^2, $n = 40$; $p > 0.05$, unpaired t test); and (2) no vimentin immunoreactivity (Pannasch et al., 2011). The recovery period after acute slice preparation is crucial for astroglial integrity because GFAP immunoreactivity was markedly reduced after a 90 min recovery period compared with a 10 min recovery period (relative astroglial GFAP intensity: 10 min recovery, 1.02 ± 0.07, $n = 63$ cells; 90 min recovery, 0.47 ± 0.03, $n = 82$ cells; $p < 0.001$, unpaired t test, Welch corrected).

Dye uptake by hemichannels. Living slices were distributed in small customized submerged chambers and incubated for 10 min in ACSF. To investigate the contribution of Cx and Px HCs to dye uptake, independent experiments were performed where slices were preincubated 15 min before and during the application of ethidium bromide (EtBr; 314 Da, 4 μM, 10 min), an HC-permeable fluorescent tracer, with carbenoxolone (CBX; 200 μM), a Cx/Px HC blocker, Gap26 (100 μM), a Cx43 HC blocking peptide, Gap26 scramble (100 μM), a scramble Gap26 peptide, or ^{10}Panx1 (400 μM), a Px1 HC blocking peptide. Slices were then rinsed for 15 min in ACSF, fixed for 2 h in 4% paraformaldehyde, immunostained for GFAP (monoclonal mouse anti-GFAP antibodies (1:500, clone GA-5; Sigma), with anti-mouse antibodies conjugated to Alexa Fluor 488 (1:4000; Invitrogen) and mounted in Fluoromount. Labeled cells were examined in a confocal laser-scanning microscope (TCS SP5, Leica). Stacks of consecutive confocal images with high-bit depth color (16 bit) were taken at 1 μm intervals and acquired in sequential mode with lasers (488 nm for GFAP and 561 nm for EtBr). Dye uptake analysis was performed in stratum radiatum CA1 astrocytes that were positive for GFAP. Fluorescence intensity was digitized in arbitrary units in 65,000 shades of gray with ImageJ software (National Institutes of Health). Dye uptake was evaluated and expressed as the difference between the fluorescence measured in astrocytes (20–40 cells per slice) and the background fluorescence measured in the same field where no labeled cells were detected.

Electrophysiology. Slices were transferred in a submerged recording chamber mounted on an Olympus BX51WI microscope equipped for infrared-differential interference contrast microscopy and were perfused with standard ACSF (2 ml/min, room temperature). All experiments were performed in CA1 stratum radiatum in the presence of picrotoxin (100 μM), and a cut was made between CA3 and CA1 to prevent epileptiform bursts. Field EPSPs (fEPSPs) were recorded with glass pipettes (2–5 MΩ) filled with ACSF. Whole-cell recordings were obtained from visually identified CA1 pyramidal cells using 5–8 MΩ glass pipettes filled with the following (in mM): 107.5 Cs-gluconate, 20 HEPES, 0.2 EGTA, 8 Na-gluconate, 8 TEA-Cl, 4 Mg-ATP, 0.3 Na$_3$-GTP, and 5 QX314, pH 7.4 (290 mOsm). Postsynaptic responses were evoked by Schaffer collateral single low stimulation (10–20 μA, 0.1 ms, 0.1 Hz) through a glass pipette (2–5 MΩ) filled with ACSF and located at a distance of 200 μm from the recorded area. Recordings were acquired with Axopatch-1D amplifiers (Molecular Devices), digitized at 10 kHz, filtered at 2 kHz, stored, and analyzed on computer using pClamp9 and Clampfit10 software (Molecular Devices).

Bioluminescence imaging. ATP release was measured from hippocampal slices maintained in 24-well plates containing oxygenated ACSF (three slices per well with 500 μl of ACSF) through the determination of extracellular ATP levels using the luciferin-luciferase assay (ATPLite kit, PerkinElmer) and a luminometer (Tristar LB941, Berthold). Each condition was run in triplicate, and absolute values were obtained from ATP standards. The release of extracellular ATP from hippocampal slices was

also dynamically and locally imaged by chemiluminescence emitted from the reaction of luciferase and luciferin, delivered locally and continuously through a 5 MΩ glass pipette connected to a micropump. Photon production was captured by a high-sensitivity EM-CCD camera (iXon 897, Andor) with a 20× water-immersion objective (numerical aperture 0.95; Olympus) at 1 Hz with 5 ms exposure time using Axon Imaging Workbench software (Molecular Devices).

Statistics. All data are expressed as the mean ± SEM, and *n* represents the number of independent experiments, unless otherwise stated. Statistical significance was determined on raw data by paired or unpaired *t* tests and one-way repeated-measures ANOVA followed by Dunnett or Bonferroni post-test.

Drugs. Gap26 (VCYDKSFPISHVR), Gap26 scramble (PSFDSRHCIVKYV), and [10]Panx1 (WRQAAFVDSY) were synthesized by Thermo Fisher Scientific (purity, >95%), and all others products were from Sigma.

Results

Astroglial Cx43 hemichannels are active in basal conditions

To investigate whether astroglial HCs are functional in basal conditions, we performed EtBr uptake assay in stratum radiatum astrocytes from acute hippocampal slices. The majority of GFAP-positive astrocytes exhibited a basal EtdBr labeling in control conditions (Fig. 1*A*), which is currently thought to reflect non-specific staining. However, we found that such basal astrocytic EtBr labeling is actually partly mediated by active uptake, because it was decreased by ~32% when HCs were acutely inhibited by CBX (200 μM, 15 min), a broad-spectrum blocker of both Cx and Px HCs ($-31.8 \pm 10.6\%$, $n = 7$, $p < 0.05$; Fig. 1*B,C*). HCs in astrocytes are formed by Cx43 and Px1 subunits (Giaume et al., 2013). Thus, to decipher the molecular identity of the HCs underlying basal uptake, we acutely and specifically blocked Cx43 or Px1 HCs using a short application (15 min) of extracellular loop mimetic peptides (Giaume et al., 2013). Gap26 (100 μM), a specific Cx43 HC blocking peptide, significantly decreased astrocytic basal EtBr uptake ($-28.5 \pm 4.8\%$, $n = 7$, $p < 0.01$; Fig. 1*B,C*) to a similar extent as CBX, whereas the Gap26 scramble peptide (100 μM) had no effect ($n = 7$, $p > 0.05$; Fig. 1*B,C*). To further test the specific involvement of Cx43 HCs, we used astroglial conditional Cx43 knock-out mice (Cx43$^{-/-}$), in which astrocytes do not express Cx43, and found that Gap26, as well as CBX, had no significant effect on basal EtBr uptake in Cx43$^{-/-}$ astrocytes (CBX, $n = 4$; Gap26, $n = 3$; $p > 0.05$; Fig. 1*C*). Finally, [10]Panx (400 μM), a specific Px1 HC mimetic peptide, also did not alter basal uptake in wild-type astrocytes ($n = 5$, $p > 0.05$, Fig. 1*C*). Altogether, these results indicate that Cx43 HCs, but not Px1 HCs, are functional in astrocytes during resting conditions.

Astroglial Cx43 hemichannels contribute to excitatory synaptic transmission through ATP signaling

Astroglial Cx43 HCs release numerous neuroactive molecules (Giaume et al., 2013), which can modulate neuronal excitatory activity. We thus investigated whether the basal activity of Cx43 HCs that we identified regulates basal glutamatergic synaptic transmission. To do so, we performed whole-cell patch-clamp recordings of excitatory synaptic activity induced by single and low stimulation of Schaffer collaterals in CA1 pyramidal cells. We found that acute application of the Cx43 HC blocker Gap26 (100 μM) rapidly induced (within <5 min) a ~33% decrease in EPSC amplitude ($-33.2 \pm 3.4\%$, $n = 9$, $p < 0.01$; Fig. 2*A,B*), while the Gap26 scramble peptide (100 μM, 10 min) had no significant effect ($n = 8$, $p > 0.05$; Fig. 2*C*). Furthermore, Gap26 (100 μM, 10 min) did not alter excitatory synaptic transmission in hippocampal slices from astroglial conditional Cx43 knock-out mice (Cx43$^{-/-}$, $n = 17$, $p > 0.05$; Fig. 2*D*), thus demonstrating the
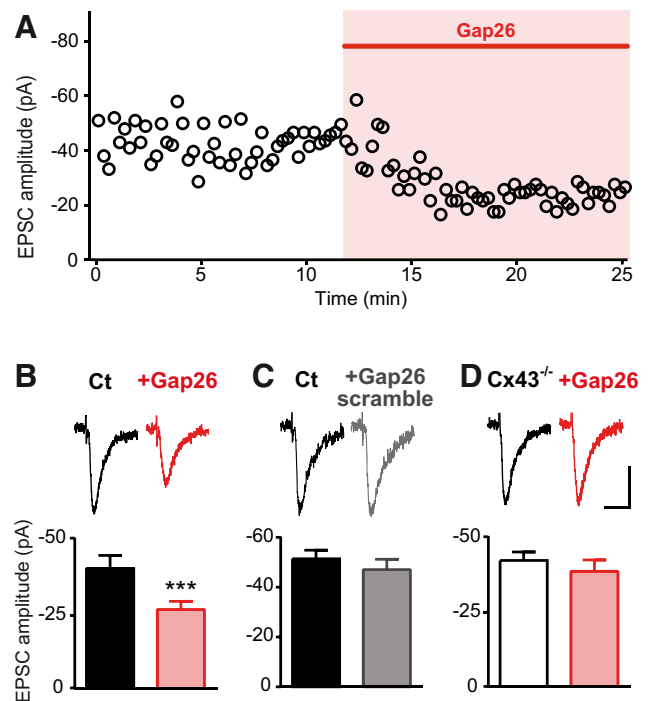

Figure 2. Astroglial Cx43 HCs modulate basal excitatory synaptic transmission. *A*, *B*, Astroglial Cx43 HCs potentiate synaptic transmission. *A*, Quantification over time of EPSC amplitudes recorded in a representative CA1 pyramidal cell before and during Gap26 application (red). *B*, Sample traces and quantification of evoked EPSCs recorded in CA1 pyramidal neurons before [control (Ct), black] and during Gap26 application (Gap26, red, $n = 9$). *C*, *D*, Specificity of the Gap26 mimetic peptide. *C*, Sample traces and quantification of evoked EPSCs recorded in CA1 pyramidal cells before (black) and during (gray) the application of the Gap26 scramble peptide ($n = 8$). *D*, Sample traces and quantification of evoked EPSCs recorded in CA1 pyramidal neurons from astroglial conditional Cx43 knock-out mice (Cx43 $^{-/-}$) before (black) and during (red) the application of Gap26 ($n = 17$). Gap26 and scramble peptides were applied at 100 μM, and their effects were quantified after 10 min. Calibration: 20 pA, 20 ms. Asterisks indicate statistical significance (***$p <$ 0.001, Student's paired t test).

specific effect of the Gap26-mediated astroglial Cx43 HC inhibition on synaptic activity. Altogether, these data indicate that astroglial Cx43 HCs strengthen basal excitatory synaptic transmission.

Because ATP is a gliotransmitter known to permeate Cx43 HCs (Kang et al., 2008) and to increase excitatory synaptic transmission through P2 receptor activation (Pankratov et al., 1998; Gordon et al., 2005), we investigated the involvement of ATP signaling in the Cx43 HC regulation of basal synaptic activity. Using a luciferin-luciferase luminescence assay, we quantitatively measured with a luminometer ATP release from hippocampal slices bathed in a defined volume of ACSF, and found an approximately fivefold decrease in basal extracellular ATP concentration in Gap26-treated slices ($n = 4$, $p < 0.001$; Fig. 3*A*). In addition, using real-time detection of local extracellular ATP variations in hippocampal slices, we found that Gap26 readily (within 2 min) decreased ATP levels ($n = 7$, $p < 0.01$; Fig. 3*B*). Altogether, these data show that ATP is tonically released through Cx43 HCs in resting conditions. To investigate whether basal extracellular ATP exerts a tonic facilitation of excitatory neurotransmission by the direct activation of ATP purinergic receptors, we acutely inhibited P2 receptors using the broad-spectrum antagonists RB2 and PPADS (30 μM, 20 min), and found that this decreased excitatory synaptic transmission by ~31% ($-31.1 \pm 3.8\%$, $n = 5$, $p < 0.01$; Fig. 4*A*), as observed with Gap26 inhibition of Cx43 HCs. In addition, in Gap26-treated slices (100 μM, 10 min), nor-
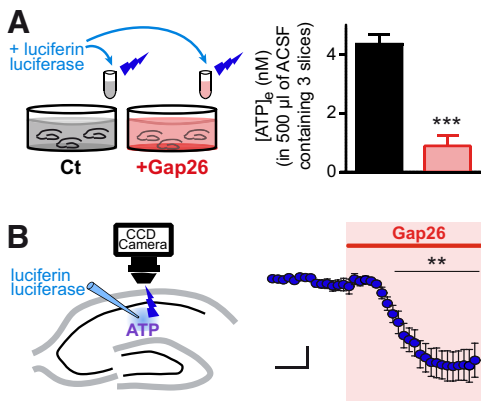
Figure 3. Astroglial Cx43 HCs release ATP in basal conditions. **A**, Left, Schematic representation of the experimental design to measure quantitatively ATP release from hippocampal slices maintained in wells with oxygenated ACSF. Right, Quantification of luminescence detection of ATP release measured in 500 μl of ACSF containing three hippocampal slices in control conditions (Ct; black) and after Gap26 application (30 min; red; $n = 4$). **B**, Left, Schematic representation of dynamic extracellular ATP imaging in a hippocampal slice, using local delivery of luciferin-luciferase through a pipette located in CA1 stratum radiatum. Right, Quantification over time of luminescence detection of extracellular ATP levels before and during Gap26 application (red, $n = 7$). Calibration: 1 arbitrary unit, 1 min. Asterisks indicate statistical significance [Student's paired t test (**A**) or one-way repeated-measures ANOVA and Dunnett's *post hoc* test (**B**): **$p < 0.01$, ***$p < 0.001$].

mal excitatory transmission was rescued by local application of ATP (1 μM, 3 min applied with a 250-μm-diameter barrel at the slice surface; fEPSP slope before and during the application of Gap26 alone or combined with ATP: control, -0.18 ± 0.01 mV/ms; Gap26, -0.14 ± 0.01 mV/ms; difference: $-24.6 \pm 1.1\%$ compared with control, $p < 0.001$; Gap26 plus ATP, -0.17 ± 0.01, $+24 \pm 7.1\%$ compared with Gap26, $n = 7$, $p < 0.01$, repeated-measures ANOVA with Bonferroni post-test). Finally, when hippocampal slices were pretreated with ATP P2 receptor antagonists (RB2 and PPADS, 30 μM, 20 min), acute inhibition of Cx43 HCs by Gap26 failed to induce the decrease in EPSC amplitude ($n = 6$, $p > 0.05$; Fig. 4B,C), which was previously observed under control conditions (Fig. 2A,B). Thus, the inhibition of ATP P2 receptors occludes the effect of Cx43 HC blockers on synaptic transmission.

Altogether, these data indicate that astroglial Cx43 HCs contribute to basal excitatory synaptic transmission via ATP signaling.

Discussion

The present results reveal astroglial Cx43 HCs as a novel pathway mediating signal exchanges between astrocytes and neurons in basal conditions. This result is remarkable since Cx or Px HCs have thus far been assumed to be active mostly in pathological conditions, such as ischemia, inflammation, or Alzheimer's disease (Giaume et al., 2013).

Acute slice preparation, although extensively used in neurophysiological investigations, is an experimental model that does not fully reflect the physiological conditions of an intact brain. In particular, early stages of reactive gliosis (Takano et al., 2014) and transient loss of glycogen granules (Fiala et al., 2003) have been reported in astrocytes from acute hippocampal slices, raising the possibility that traumatic injury during the slicing procedure might induce Cx43 HCs activation. We, however, found that at a depth of 40–80 μm from the acute slice surface, and at a recovery time of at least 90 min after the slicing procedure (i.e., the conditions used in our experiments), astrocytes exhibit no sign of reactivity (see Materials and Methods). In addition, the contri-
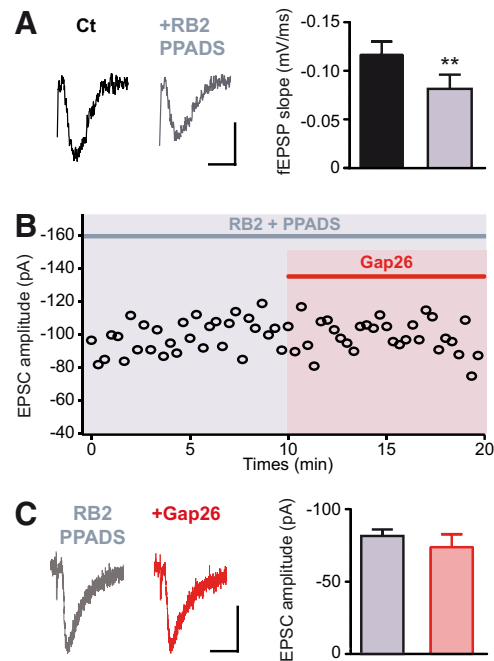

Figure 4. ATP signaling mediates astrocytic Cx43 HC regulation of synaptic transmission. **A**, Endogenous extracellular ATP increases basal excitatory synaptic transmission through P2 receptors. Sample traces and quantification of evoked CA1 fEPSPs recorded before (Ct; black) and during application of ATP P2 receptor antagonists (RB2+PPADS; gray, $n = 5$). Calibration: 0.2 mV, 10 ms. **B**, **C**, Blockade of ATP P2 receptors occludes the effect of Cx43 HC inhibition on synaptic transmission. **B**, Quantification over time of EPSC amplitudes recorded in a representative CA1 pyramidal cell in the presence of ATP P2 receptor antagonists (RB2+PPADS preincubation; gray) before and during Gap26 application (red). **C**, Corresponding sample traces and quantification of averaged EPSC amplitudes ($n = 6$). Calibration: 40 pA, 40 ms. The effect of Gap26 (100 μM) and a mix of RB2 and PPADS (30 μM) was quantified after a 10–20 min application. Asterisks indicate statistical significance (**$p < 0.01$, Student's paired t test).

bution of Cx HCs to physiological processes has recently been reported *in vivo* in the brain (Stehberg et al., 2012), suggesting that the Cx43 HC activity we report here under basal conditions in hippocampal slices may indeed occur in intact physiological conditions.

Notably, we found that HCs composed of Cx43, but not Px1, contribute specifically to the basal uptake of EtBr in astrocytes. To reveal in acute slices such significant Cx43-mediated HC uptake in basal conditions, we used confocal imaging of astrocytes with high-bit depth color (16 bit), enabling reliable detection with high sensitivity of variations in intracellular fluorescence intensity. Interestingly, although the direct opening of HCs in basal conditions has not been directly investigated in recent studies proposing an involvement of astroglial Cx43 HCs in physiological processes (Stehberg et al., 2012; Torres et al., 2012), the activation of Cx43 HCs, shown with dye uptake assays, has already been detected at resting membrane potential in HeLa-transfected cells (Contreras et al., 2003). One can hypothesize that synaptic activity triggers basal activation of astroglial Cx43 HCs. Indeed, glutamate decrease in extracellular calcium or astroglial depolarization, which occur during neuronal activity, dynamically modulate HC activation (Orellana et al., 2013). Because Cx43 HCs may be located in the vicinity of synapses, local changes in extracellular ion or neurotransmitter levels at the tripartite synapse may induce selective and prominent activation of Cx43 HCs from perisynaptic astroglial processes.

Furthermore, we demonstrate that astroglial Cx43 HC activity in the resting state boosts the moment-to-moment basal gluta-

matergic synaptic activity of CA1 pyramidal cells. Pharmacological inhibition of Cx43 HCs by mimetic peptides indeed acutely decreases the amplitude of EPSCs, while such blockers have no effect on astroglial conditional Cx43 knock-out mice. Our present findings nicely complement recent studies showing Cx43 HC contribution to neurophysiology. Indeed, a recent pioneering study has revealed a critical role for Cx43 HCs in physiological cognitive process by showing that intracerebral microinfusion of Cx43 HC blockers in the amygdala alters fear memory consolidation (Stehberg et al., 2012). Interestingly, in this study gliotransmitters, including ATP, were suggested to underlie such regulation. In addition, it has also recently been proposed that Cx43 HC activation by astroglial calcium waves, triggered by glutamate or calcium chelator uncaging, which mediate the decrease in extracellular calcium thought to occur during high neuronal activity, boosts interneuron excitability, and thereby inhibitory transmission, through ATP release targeting P2Y1 receptors on interneurons (Torres et al., 2012). This pathway has been suggested to provide a negative-feedback mechanism to tone down strong glutamatergic transmission and hyperexcitability of neuronal networks. Remarkably, in our study, the mechanism by which astroglial Cx43 HCs strengthen basal excitatory synaptic transmission also involves ATP release and activation of P2 purinergic receptors. However, we show here, using a short application of Cx43 HC blocking peptides, that Cx43 HCs acutely regulate basal neurotransmission mediated by excitatory synapses of CA1 pyramidal cells, while Torres et al. (2012), by using astroglial Cx43 transgenic mice, identified an effect on network activity involving interneurons during a high-activity regime. Because astroglial Cx43 HCs are not activated by extracellular ATP targeting P2 receptors (Iglesias et al., 2009; Orellana et al., 2013) and inhibitory synaptic activity was blocked by picrotoxin in our experiments to study specifically excitatory transmission, our data suggest that ATP released through astroglial Cx43 HCs directly activates P2 purinergic receptors on CA1 pyramidal cells, which are known to potentiate glutamatergic synaptic transmission (Pankratov et al., 1998; Gordon et al., 2005).

The regulation of basal excitatory synaptic transmission that we demonstrate here may well contribute to the effect of astroglial Cx43 HCs on memory consolidation (Stehberg et al., 2012). Additionally, one can hypothesize that the effects of astroglial Cx43 on physiological locomotor and exploratory activity in novel environments (Frisch et al., 2003; Theis et al., 2003), as well as on whisker-related sensory functions and plasticity (Han et al., 2014), may not only depend on Cx43-mediated gap junction coupling, but might also involve functional Cx43 HCs.

Thus, Cx43 HCs, a classic ATP release pathway in astrocytes that is usually thought to be at play in pathological situations, is actually also likely to be a key player in physiology by strengthening the moment-to-moment basal synaptic transmission.

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