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RESEARCH ARTICLE | *Cellular and Molecular Properties of Neurons*

Adenosine A_1 receptor-mediated protection of mouse hippocampal synaptic transmission against oxygen and/or glucose deprivation: a comparative study

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Kawamura M Jr, Ruskin DN, Masino SA. Adenosine A_1 receptor-mediated protection of mouse hippocampal synaptic transmission against oxygen and/or glucose deprivation: a comparative study. *J Neurophysiol* 122: 721–728, 2019. First published June 26, 2019; doi:10.1152/jn.00813.2018.—Adenosine receptors are widely expressed in the brain, and adenosine is a key bioactive substance for neuroprotection. In this article, we clarify systematically the role of adenosine A_1 receptors during a range of timescales and conditions when a significant amount of adenosine is released. Using acute hippocampal slices obtained from mice that were wild type or null mutant for the adenosine A_1 receptor, we quantified and characterized the impact of varying durations of experimental ischemia, hypoxia, and hypoglycemia on synaptic transmission in the CA1 subregion. In normal tissue, these three stressors rapidly and markedly reduced synaptic transmission, and only treatment of sufficient duration led to incomplete recovery. In contrast, inactivation of adenosine A_1 receptors delayed and/or lessened the reduction in synaptic transmission during all three stressors and reduced the magnitude of the recovery significantly. We reproduced the responses to hypoxia and hypoglycemia by applying an adenosine A_1 receptor antagonist, validating the clear effects of genetic receptor inactivation on synaptic transmission. We found activation of adenosine A_1 receptor inhibited hippocampal synaptic transmission during the acute phase of ischemia, hypoxia, or hypoglycemia and caused the recovery from synaptic impairment after these three stressors using genetic mutant. These studies quantify the neuroprotective role of the adenosine A_1 receptor during a variety of metabolic stresses within the same recording system.

NEW & NOTEWORTHY Deprivation of oxygen and/or glucose causes a rapid adenosine A_1 receptor-mediated decrease in synaptic transmission in mouse hippocampus. We quantified adenosine A_1 receptor-mediated inhibition during and synaptic recovery after ischemia, hypoxia, and hypoglycemia of varying durations using a genetic mutant and confirmed these findings using pharmacology. Overall, using the same recording conditions, we found the acute response and the neuroprotective ability of the adenosine A_1 receptor depended on the type and duration of deprivation event.

acute hippocampal slices; adenosine A_1 receptors; field recording; metabolic stress; synaptic transmission

INTRODUCTION

Adenosine is known as a neuromodulator (Dunwiddie and Masino 2001), regulating synaptic transmission and membrane potential in neurons (Dunwiddie et al. 1997; Masino et al. 2002) and calcium dynamics in glial cells (Kawamura and Kawamura 2011; Stevens et al. 2002). These acute effects of adenosine are caused by activation of cell surface receptors, and adenosine is known to exist in the extracellular space tonically (Dunwiddie and Hoffer 1980). Tonic levels are maintained by two pathways: breakdown from extracellular ATP (released by a variety of mechanisms; Kawamura and Ruskin 2012) and direct release from equilibrative nucleoside transporters in postsynaptic neurons (Lovatt et al. 2012) or presynaptic terminals (Cunha et al. 1996). It has recently been recognized that adenosine can also induce changes in DNA methylation (Williams-Karnesky et al. 2013). Therefore, beyond dynamic ongoing effects, major transformative functional effects of adenosine might result from its increased concentration.

A range of physiological/pathophysiological situations are known to increase extracellular adenosine (Masino et al. 2009), including ischemia (Fowler 1990; Frenguelli et al. 2007; Pearson et al. 2006), hypoxia (Dale et al. 2000; Fowler 1993), hypoglycemia (Fowler 1993; Zhu and Krnjević 1993), hypercapnia (Dulla et al. 2005), increased temperature (Masino et al. 2001), neuronal activity (Lovatt et al. 2012), electrical stimulation (Mitchell et al. 1993), and ketone body-based metabolism (Kawamura et al. 2014). Most of these situations represent shifts in metabolism or a metabolic stress; ketone body-based metabolism is often coupled with reduced glucose levels. As a general principle, altered brain metabolism is thought to be one of the modulators of extracellular adenosine (Latini and Pedata 2001).

Adenosine receptors have four subtypes expressed functionally in mammalian central nervous system (CNS): A_1, A_{2A}, A_{2B}, and A_3 (Dunwiddie and Masino 2001; Fredholm et al. 2000). Adenosine A_1 receptors (A_1Rs) in particular are distributed widely and known to inhibit synaptic transmission by suppressing influx into presynaptic voltage-dependent calcium channels in synaptic terminals (Gundlfinger et al. 2007; Wu and Saggau 1994), hyperpolarizing neuronal membrane potential by opening potassium channels (Haas and Greene 1984; Kawamura et al. 2010), and inhibiting *N*-methyl-D-aspartate (NMDA) receptors in postsynaptic neurons (de Mendonça et al. 1995). On the other hand, $A_{2A}Rs$ are reported to facilitate synaptic transmission (Lopes et al. 2002) by increasing calcium influx via presynaptic voltage-dependent calcium chan-

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nels (Gonçalves et al. 1997), depolarizing neuronal membrane potential (Chamberlain et al. 2013; Li and Henry 1998), and enhancing NMDA receptors in postsynaptic neurons (Rebola et al. 2008; Scianni et al. 2013; Mouro et al. 2018). Thus increased extracellular adenosine caused by metabolic changes can activate A_1Rs to suppress neuronal activity and A_{2A}Rs to facilitate neuronal activity. Activation of A_1R and blockade of A_{2A}R are neuroprotective against acute stresses such as stroke, hypoxic encephalopathy, and hypoglycemia (Cunha 2016, 2005; Fredholm 1997, 2007; Pedata et al. 2016).

In the present study, we used acute hippocampal slices from wild-type and adenosine A_1 receptor knockout (A_1KO) mice to examine systematically the role of the A_1R during metabolic stresses where large amounts of adenosine are known to be released. Previous work has shown that the A_1KO mouse exhibits a complete loss of A_1Rs in all brain regions, and synaptic transmission in the hippocampus does not respond to exogenous or elevated endogenous adenosine (Johansson et al. 2001). A_1KO mice have normal physiology in terms of body weight, heart rate, blood pressure, and body temperature but show thermal hyperalgesia, increased anxiety, and increased aggressiveness (Giménez-Llort et al. 2002; Johansson et al. 2001). A_1KO mice also have electrographic hippocampal seizures (Masino et al. 2011). During extracellular recordings from hippocampal slices obtained from mice that were wild type or null mutant for the A_1R, we quantified and characterized the consequences of varying periods of ischemia (low oxygen and low glucose), hypoxia (low oxygen), and hypoglycemia (low glucose) to elucidate systematically the protective effects afforded A_1Rs under these conditions.

MATERIALS AND METHODS

Slice preparation. All experiments were performed in accordance with Public Health Service Policy as defined in the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and approved by Trinity College, and with the Guidelines for the Proper Conduct of Animal Experiments of the Science Council of Japan (2006) and approved by the Institutional Animal Care and Use Committee of the Jikei University. C57BL/6 mice (wild type or lacking adenosine A_1 receptors; Johansson et al. 2001) aged 4–7 wk, of either sex, were anesthetized with isoflurane and decapitated.

Standard slice preparation and recording conditions were employed, similarly to our previous studies (Johansson et al. 2001; Masino et al. 2002). Briefly, three to six coronal slices of dorsal hippocampus of 400-μm thickness were made from each brain in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM) 126 NaCl, 3 KCl, 1.5 $MgCl_2$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 11 glucose, and 26 $NaHCO_3$ (osmolarity 320 mosM, pH 7.4 when saturated with 95% O_2 + 5% CO_2) with a vibrating slice cutter (series 1000, Vibratome, St. Louis, MO; or PRO 7, Dosaka, Kyoto, Japan). The slices were incubated in aCSF saturated with 95% O_2 + 5% CO_2 for at least 60 min at room temperature until the recording. The slice was placed on a nylon net in the recording chamber and submerged in and continuously superfused with aCSF at a flow rate of 2 ml/min at 32 ± 1°C. The temperature was controlled automatically at ~32°C and adjusted manually if there was any drift throughout recordings. If the temperature shifted over 1°C from 32°C, the recording was removed from data. Our nylon net chamber is able to superfuse both the upper and under sides of surfaces of the slices; a similar type of dual-superfusion chamber is known to enhance diffusional oxygen supply into the slices (Hájos et al. 2009; Ivanov and Zilberter 2011).

We applied four different experimental metabolic stress conditions: *1*) experimental ischemia (oxygen-glucose deprivation), achieved by removing 11 mM glucose in the aCSF and replacing it with 11 mM sucrose and also simultaneously changing from 95% O_2 + 5% CO_2 to 95% N_2 + 5% CO_2; *2*) experimental hypoxia, by changing to saturated aCSF with 95% N_2 + 5% CO_2; *3*) experimental hypoglycemia, by removing 11 mM glucose in the aCSF and adding the same concentration of sucrose; and *4*) reduced (3 mM) glucose with hypoxia, by reducing 11 mM glucose to 3 mM (while including 8 mM sucrose in the aCSF) and simultaneously changing to 95% N_2 + 5% CO_2. Removing oxygen from the aCSF greatly decreases oxygen supply but cannot cause complete anoxia in the recording slices because the dual-superfusion chamber is open to the atmosphere (Hájos et al. 2009). Therefore, we use the term "hypoxia" in the current report.

Extracellular recordings. Field excitatory postsynaptic potentials (fEPSP) were recorded similarly to our previous studies (Johansson et al. 2001; Masino et al. 2002). Briefly, medium wall (1.5 mm) capillary filament glass was pulled on a Sutter P-97 micropipette puller (Novato, CA), giving electrode resistances of 8–12 MΩ. The recording electrode filled with 3 M NaCl was placed in the stratum radiatum of CA1 region. A twisted bipolar insulated tungsten electrode was placed as stimulation electrode in the stratum radiatum; stimuli were delivered at 15- or 30-s intervals. Pulse duration was 100 μs, and the intensity was adjusted such that the amplitude of evoked fEPSP was half of the maximal response.

Drugs and their application. The adenosine receptor antagonist 8-cyclopentyltheophylline (CPT) was dissolved in aCSF at 100 times the desired final concentration and applied via syringe pump upstream in the superfusion line to reach final concentration before reaching the slice chamber (Masino et al. 1999). In all figures, the point indicated as the onset of changing condition is the calculated time when the solution first begins to mix into the volume of the slice chamber.

Data and statistical analysis. All electrophysiological responses were recorded via an alternating current amplifier (model 3000; A-M Systems, Carlsborg, WA) and filtered at 1 kHz. Data were digitized (16-channel analog-to digital board; National Instruments Japan, Tokyo, Japan) at a rate of 4 kHz and analyzed off-line using Igor Pro 5 (WaveMetrics, Lake Oswego, OR). All amplitude data are normalized by baseline (%baseline) and expressed as means ± SE, similarly to our previous study (Kawamura et al. 2014). Amplitudes of fEPSPs were compared with the use of unpaired t-test for two groups or one-way ANOVA with Bonferroni correction for three groups using GraphPad InStat 3.10 (GraphPad Software, La Jolla, CA). Probability (P) < 0.05 was considered significant. The number of experiments (n) in figures refers to the recorded slices.

RESULTS

We recorded fEPSPs using extracellular recordings in 117 hippocampal slices from wild-type (WT) mice and 66 hippocampal slices from A_1KO mice under four different conditions: *1*) ischemia, *2*) hypoxia, *3*) hypoglycemia, and *4*) reduced glucose with hypoxia.

Irreversible synaptic loss with experimental ischemia. We first tested the effects of experimental ischemia (duration ranging from 5 to 30 min) on excitatory synaptic transmission in WT hippocampal slices. Longer experimental ischemia (10–30 min) caused three phases of synaptic changes: *1*) rapid decrease in fEPSP amplitude, *2*) transient increase of fEPSP amplitude, and *3*) disappearance of fEPSP (Fig. 1*A*). The transient increase in fEPSP amplitude (Fig. 1*A*, arrowhead) is termed a "transient reappearance" and thought to be caused by synchronized neuronal hyperexcitability and spreading depression (Madry et al. 2010; Pugliese et al. 2003). After transient reappearance, long-term ischemia abolished the fEPSP sud-

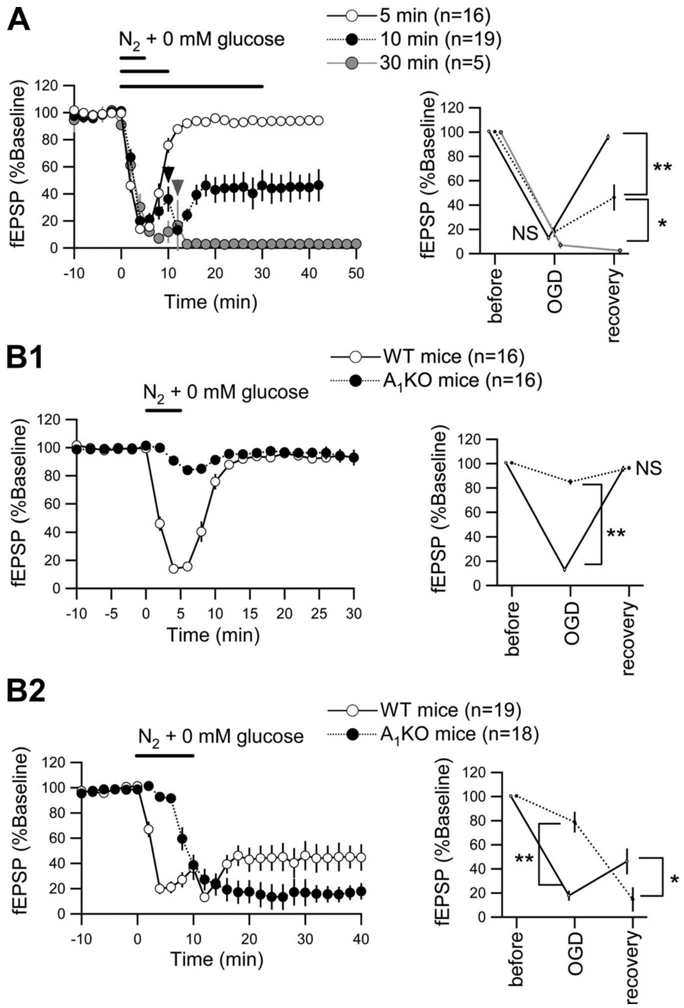

Fig. 1. Experimental ischemia-induced field excitatory postsynaptic potential (fEPSP) modulation. *A*, *left*: change in fEPSP amplitudes with 5-, 10-, or 30-min experimental ischemia [oxygen and glucose deprivation (OGD): N_2 + 0 mM glucose] in hippocampal slices from wild-type (WT) mice. Averages of fEPSP amplitudes are shown at each time point (2 min), and vertical bars are SE. Transient reappearances are shown at arrowheads in 10-min (black) and 30-min (gray) OGD. *Right*: summary of averages of fEPSP amplitudes at 1–2 min before ischemia (before), during ischemia (OGD), and 15–20 min after ischemia (recovery). NS, not significantly different; *$P < 0.05$; **$P < 0.01$ (1-way ANOVA with post hoc test). *B1*: effects of 5-min OGD (N_2 + 0 mM glucose) in slices from WT mice or adenosine A_1 receptor knockout (A_1KO) mice. Data shown from WT mice are the same as 5-min OGD in *A*. **$P < 0.01$ (unpaired *t*-test). *B2*: effects of 10-min OGD (N_2 + 0 mM glucose) in slices from WT or A_1KO mice. Data shown from WT mice are the same as 10-min OGD in *A*. *$P < 0.05$; **$P < 0.01$ (unpaired *t*-test).

denly and irreversibly, an effect thought to mimic a core region of brain impairment after stroke (Pugliese et al. 2003). This permanent and complete loss of synaptic transmission was only caused by long-term (30 min) experimental ischemia. Short-term ischemia (5 min) inhibited the amplitude of fEPSP rapidly, but this inhibition reversed completely after reperfusion of normal aCSF (Fig. 1*A*). A 10-min duration of ischemia caused a rapid inhibition of fEPSP and transient reappearance similar to that in 30-min ischemia, but the amplitude of fEPSP recovered partially and significantly after reperfusion of normal aCSF; there was not a complete and irreversible loss as was seen consistently with 30-min ischemia (Fig. 1*A*).

In slices obtained from A_1KO mice, the rapid inhibition of fEPSP with 5-min ischemia did not occur (Fig. 1*B1*), suggesting that this inhibition is reversible and caused by activation of A_1Rs. Similarly, with 10-min ischemia, the fEPSP was not rapidly inhibited in slices from A_1KO mice: it was suppressed with delayed onset and finally lost (Fig. 1*B2*). The significant partial recovery in the slices from WT mice after 10-min ischemia did not occur in the slices from A_1KO mice. Consistent with 10-min ischemia, more long-term ischemia (>20 min) in A_1KO mice also caused a delayed onset of fEPSP inhibition and irreversible loss of synaptic transmission (data not shown).

Together, these results indicate at least two things: first, the quick-onset fEPSP suppression during ischemia depends on A_1Rs, although fEPSP suppression after ischemia of a sufficient duration (>10 min) occurs even in the absence of A_1Rs. Second, activation of A_1Rs is essential for fEPSP recovery after ischemia. This A_1R -induced recovery is only effective within a limited duration of experimental ischemia: more prolonged ischemia (>10 min) caused irreversible synaptic loss even in the WT mice (Fig. 1*A*).

Modest synaptic loss of fEPSP with experimental hypoxia or hypoglycemia. We next recorded changes in fEPSP amplitude after experimental hypoxia (by removing oxygen) or experimental hypoglycemia (by removing glucose). All durations of hypoxia (5, 10, and 30 min) induced rapid and complete inhibition of fEPSP amplitude, but in all cases the inhibition was fully reversible (Fig. 2*A*), unlike when glucose and oxygen were both removed (Fig. 1*A*). The rapid inhibition with 10-min hypoxia was not seen in slices from A_1KO mice (Fig. 2*B1*), suggesting that hypoxia-induced reversible fEPSP inhibition is caused by activation of A_1Rs. Interestingly, a very long period of hypoxia (60 min) in the A_1KO mice induced a partial but significant suppression of fEPSP amplitude (Fig. 2*B2*) that was long lasting: it continued over 30 min after reperfusion of normal oxygenated aCSF (data not shown). This suppression was not caused by any nonspecific effect of the deletion of functional A_1Rs: a similar modest suppression was shown in the presence of the A_1R antagonist CPT (1 μM) in slices from WT mice (Fig. 2*B2*). These results argue strongly that long-lasting hypoxia causes modest loss of synaptic transmission but that activation of A_1Rs prevents any lasting impairment.

Similar results were quantified during and after experimental hypoglycemia. Durations of 5, 10, and 30 min of hypoglycemia caused rapid inhibition (partial inhibition with 5-min hypoglycemia, complete inhibition with longer durations) and a complete recovery of fEPSP amplitude in the hippocampal slices from WT mice. A lasting, modest loss of synaptic transmission was quantified in A_1KO mice or in the presence of CPT in WT mice for 30-min hypoglycemia (Fig. 3, *A* and *B*). These results indicate that, as with hypoxia, hypoglycemia causes slight irreversible suppression of synaptic transmission, but this impairment is typically prevented by the protective effect of A_1Rs.

Reduced glucose with hypoxia caused irreversible synaptic loss. We also tested changes in synaptic transmission while reducing glucose (from 11 to 3 mM) and removing oxygen. As shown above, 10 min of experimental ischemia caused partial but significant recovery after rapid inhibition of fEPSP amplitude in WT slices (Fig. 1*A*). However, after the same duration (10 min) of reduced glucose with hypoxia, the fEPSP ampli-

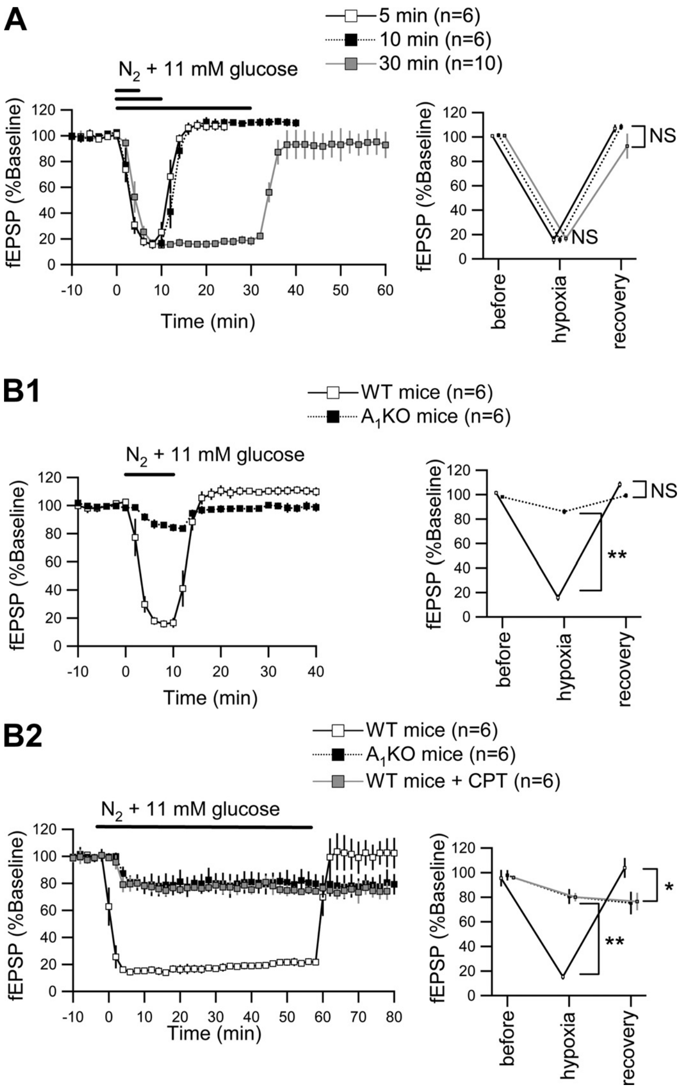

Fig. 2. Experimental hypoxia-induced field excitatory postsynaptic potential (fEPSP) modulation. *A*, *left*: change in fEPSP amplitudes with 5-, 10-, or 30-min experimental hypoxia (no oxygen: N_2 + 11 mM glucose) in hippocampal slices from wild-type (WT) mice. Averages and SE of fEPSP amplitudes are shown at each time point (2 min). *Right*: summary of averages of fEPSP amplitudes at 1–2 min before hypoxia (before), during hypoxia (hypoxia), and 15–20 min after hypoxia (recovery). NS, not significantly different (1-way ANOVA with post hoc test). *B1*: effects of 10-min hypoxia (N_2 + 11 mM glucose) in slices from WT mice or adenosine A_1 receptor knockout (A_1KO) mice. Data shown from WT mice are the same as 10-min hypoxia in *A*. $^{**}P < 0.01$ (unpaired *t*-test). *B2*: effects of 60-min hypoxia (N_2 + 11 mM glucose) in slices from WT mice, A_1KO mice, or in the presence of 1 μM 8-cyclopentyltheophylline (CPT) in WT mice (WT mice + CPT). A subset of data of WT mice and A_1KO mice are modified from a previous report (Johansson et al. 2001). $^{*}P < 0.05$; $^{**}P < 0.01$ (1-way ANOVA with post hoc test).

tude recovered completely (Fig. 4*A*; recovery of OGD, 46.2 ± 10.7%, $n = 19$; 3 mM glucose with hypoxia, 100.3 ± 0.3%, $n = 6$; $P < 0.05$; unpaired *t*-test), suggesting that even a reduced glucose level increases the recovery rate after a hypoxic event. Fifteen minutes of reduced glucose with hypoxia also resulted in recovery of the fEPSP, but the recovery amplitude was significantly smaller than after 10 min (Fig. 4*A*). Recovery was not observed in hippocampal slices from A_1KO mice, indicating that the recovery of fEPSP amplitude after reduced glucose with hypoxia is caused by activation of A_1Rs (Fig. 4*B*). A duration longer than 20 min of reduced glucose with hypoxia caused irreversible loss of synaptic transmission (Fig. 4*A*), similarly to experimental ischemia. Whereas glucose did extend the window of recovery after hypoxia, the acute protective effect of A_1Rs was overcome or disabled by a long-term (>15 min) exposure to reduced glucose with hypoxia.

DISCUSSION

In this study we quantified synaptic transmission during and after ischemia, hypoxia, or hypoglycemia of varying durations in acute mouse hippocampal slices. We also quantified the effects of genetic or pharmacological inactivation of A_1Rs during and after these metabolically stressful events. A_1R activation controlled the onset, rate, and magnitude of synaptic inhibition during the loss of oxygen and/or glucose and significantly influenced the magnitude of synaptic recovery.

Functional effects of A_1Rs during metabolic stress have been reported in several lines of experiments in hippocampal slices. Experimental ischemia with short duration (5–10 min) is

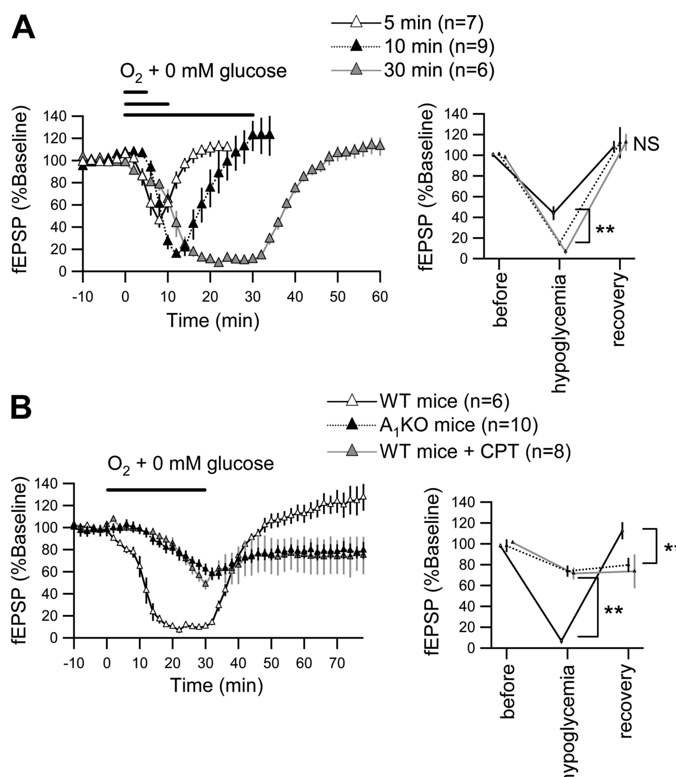

Fig. 3. Experimental hypoglycemia-induced field excitatory postsynaptic potential (fEPSP modulation. *A*, *left*: changes of fEPSP amplitudes with 5-, 10-, or 30-min experimental hypoglycemia (no glucose: 95% oxygen + 0 mM glucose) in hippocampal slices from wild-type (WT) mice. Averages and SE of fEPSP amplitudes are shown at each time point (2 min). *Right*: summary of averages of fEPSP amplitudes at 1–2 min before hypoglycemia (before), during hypoglycemia (hypoglycemia), and 15–20 min after hypoglycemia (recovery). Inhibition of fEPSP amplitude with 5-min hypoglycemia was significantly lower than with 10- and 30-min hypoglycemia. NS, not significantly different; $^{**}P < 0.01$ (1-way ANOVA with post hoc test). *B*: effects of 30-min hypoglycemia (O_2 + 0 mM glucose) in slices from WT mice, adenosine A_1 receptor knockout (A_1KO) mice, or in the presence of 1 μM 8-cyclopentyltheophylline (CPT) in WT mice (WT mice + CPT). Data shown from WT mice are the same as 30-min hypoglycemia in *A*. $^{**}P < 0.01$ (1-way ANOVA with post hoc test).

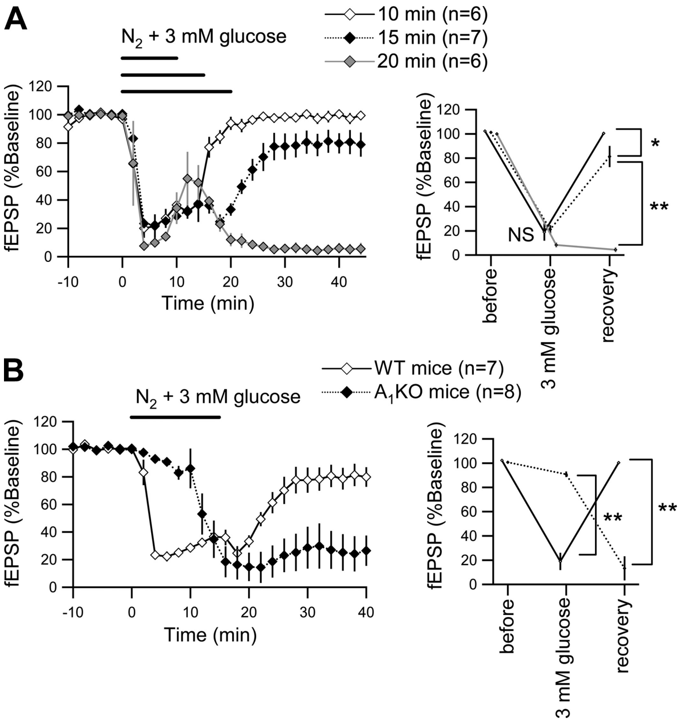

Fig. 4. Experimental reduced glucose with hypoxia-induced field excitatory postsynaptic potential (fEPSP) modulation. *A*, *left*: changes in fEPSP amplitudes with 10-, 15-, or 20-min reduced glucose with hypoxia (no oxygen: N_2 + 3 mM glucose) in hippocampal slices from wild-type (WT) mice. Averages and SE of fEPSP amplitudes are shown at each time point (2 min). *Right*: summary of averages of fEPSP amplitudes at 1–2 min before reduced glucose with hypoxia (before), during reduced glucose with hypoxia, and 15–20 min after reduced glucose with hypoxia (recovery). Recovery of fEPSP amplitude was lost gradually with an increased duration of reduced glucose with hypoxia. NS, not significantly different; $^*P < 0.05$; $^{**}P < 0.01$ (1-way ANOVA with post hoc test). *B*: effects of 15-min reduced glucose with hypoxia in slices from WT and adenosine A_1 receptor knockout (A_1KO) mice. Data shown from WT mice are the same as 15-min N_2 + 3 mM glucose in *A*. $^{**}P < 0.01$ (unpaired *t*-test).

reported to cause A_1R-induced reversible fEPSP inhibition (Fowler 1990; Frenguelli et al. 2007; Latini et al. 1999). A longer exposure to ischemia (30 min) evokes irreversible loss of synaptic activity even during extracellular adenosine release (Frenguelli et al. 2007). Short-term exposure to hypoxia (5 min) is known to cause fEPSP inhibition through activation of A_1R (Dale et al. 2000; Fowler 1989). It has been reported that >60 min of hypoxia induces a slight irreversible loss of the fEPSP when A_1Rs are inactivated (Johansson et al. 2001; Sebastião et al. 2001). Hypoglycemia has been reported to cause A_1R-induced reversible fEPSP inhibition in hippocampal slices (Zhu and Krnjević 1993). On the basis of these previous reports, we compared the effects of A_1R during these metabolic stresses using the same animal species and the same recording system, and compared genetic and pharmacological inactivation of A_1Rs. We found a particular duration of ischemia (10 min) that caused partial but significant recovery of fEPSP amplitude in the hippocampal slices (Fig. 1*A*). This recovery was mediated by activation of A_1R (Fig. 1*B2*). We also found that a rise in extracellular glucose concentration increased A_1R-mediated recovery rate (Fig. 4). Our new finding shows that long-term exposure to hypoglycemia evoked a small but significant irreversible depression of synaptic activity with blockade of A_1R (Fig. 3*B*), similarly to hypoxia (Fig. 3*B2*). Previous work investigated the effects of A_1R during metabolic stresses typically using pharmacology, with the exception of a long duration of hypoxia (Johansson et al. 2001). We examined all of these A_1R effects by using A_1KO mice in the current study and confirmed our findings using pharmacology.

Manipulating the duration of ischemia in hippocampal slices revealed sequential changes to synaptic transmission with varying levels of reversibility strongly influenced by A_1Rs: *1*) a rapid direct inhibition, *2*) a slower onset of A_1R-independent inhibition, *3*) a transient reappearance of activity, and *4*) an irreversible loss of transmission. The initial reversible inhibition is a well-known phenomenon caused by A_1Rs (Latini et al. 1999) that inhibit presynaptic voltage-dependent calcium channels (Gundlfinger et al. 2007; Wu and Saggau 1994) in glutamatergic nerve terminals (Rebola et al. 2005); the NOS-cGMP pathway also appears to be involved (Pinto et al. 2016).

We found delayed synaptic depression occurring in tissue lacking A_1R. Although the exact mechanism remains unknown, it has been reported that in vitro ischemia causes synaptic depression of fEPSPs by increasing calcium concentration in the presynaptic terminals (Jalini et al. 2016), which might cause excessive release of glutamate. The increase of glutamate release induces a transient reappearance of transmission during ischemia (Pugliese et al. 2003) and anoxic depolarization of pyramidal neurons (Madry et al. 2010). Because activation of A_1Rs inhibits calcium entry into axonal terminals, this may be a protective effect, delaying but not preventing the ischemia-induced synaptic impairment.

It has been reported that prolonged activation of A_1R with ischemia/hypoxia or applying a selective A_1R agonist causes synaptic depression of fEPSP in rat hippocampal slices (Chen et al. 2014; Stockwell et al. 2016). There is no A_1R-induced synaptic depression recorded in our experiments, suggesting that a species difference might be involved. In contrast, short-term exposure to ischemia is known to cause chemical long-term potentiation after recovery from rapid depression of synaptic activity (Ai and Baker 2006). It has been reported that the plasticity is caused by activation of A_{2A}R in rat hippocampus slices (Dias et al. 2013). In a few recordings, we observed an increase of fEPSP amplitude after recovery from short-term exposure to metabolic stresses. However, the amplitudes of fEPSP before and after events were not significantly different in all the tested types of short-term metabolic stresses (Figs. 1*A*, 2*A*, 3*A* and 4*A*).

Our method for experimental ischemia reproduced an acute phase of ischemic stroke (Yenari and Han 2012). In this condition, activation of A_1R produced protection of neuronal synaptic activity against 10-min ischemia (Fig. 1*B2*). However, long-term exposure to ischemia (30 min) caused irreversible synaptic loss even in slices from WT mice (Fig. 1*A*). Therefore, the effect of A_1R against the acute phase of ischemic stroke might be limited. Moreover, the effect of A_1R during the chronic phase of ischemic stroke is poor. The neuronal damage after global ischemic events is not different between WT mice and A_1KO mice in vivo (Olsson et al. 2004). The role of A_1R-induced neurosynaptic protection in the acute phase of ischemia should be investigated carefully in further studies.

Reduced glucose with hypoxia affected fEPSPs similarly to ischemia, but the magnitude of recovery after 10 min of treatment was increased significantly. This recovery was due to

A_1Rs, suggesting that the presence of an energy source in the aCSF enhances the neuroprotective effects of these receptors. Whereas 3 mM glucose is a physiological level in brain (Abi-Saab et al. 2002; Hu and Wilson 1997; Shram et al. 1997; Silver and Erecińska 1994), typically brain slices are recorded in a higher glucose level (11 mM). Enhanced responses due to higher glucose may be one reason why typical protocols use a supraphysiological glucose concentration; for example, glucose concentration is often at least 10 mM in solutions used for maintaining or recording from acute brain slices or slice cultures. However, normal synaptic physiology and long-term maintenance in vitro have been demonstrated with a physiological level of glucose using the present type of open, dual-superfusion chamber (Kawamura et al. 2014) or a closed, boxlike superfusion chamber (Tian and Baker 2002).

Both hypoxia and hypoglycemia caused rapid synaptic inhibition via A_1Rs but contrasted with ischemia in the later effects. Hypoglycemia caused a mild A_1R-independent depression with no transient reappearance and no loss of transmission even after 30 min of treatment; hypoxia caused a mild A_1R-independent depression with no transient reappearance and only a small-magnitude irreversible loss even after 60 min of treatment. However, an irreversible synaptic depression was masked by the presence of A_1R activation: the fEPSP did not completely recover after hypoxia or hypoglycemia in the hippocampal slices from A_1KO mice or in the presence of an A_1R antagonist in WT mice, clearly showing the involvement of this receptor in synaptic recovery. The mechanisms of A_1R antagonist-induced synaptic depression are still undetermined. It has been reported that inhibition of NMDA receptors causes hypoxia-induced fEPSP recovery (Sebastião et al. 2001), suggesting that A_1R-mediated NMDA receptor inhibition (de Mendonça et al. 1995) might be involved in the recovery.

The source of extracellular adenosine during experimental metabolic stress such as ischemia, hypoxia, or hypoglycemia is still undetermined (Kawamura and Ruskin 2012). It has been reported that knockout of CD73 does not prevent endogenous adenosine-induced synaptic inhibition, suggesting that breakdown from AMP to adenosine with ecto-5′-nucleotidase is not responsible for the increased extracellular adenosine and that ATP release is not the source (Zhang et al. 2012). A study using ATP and adenosine sensors showed ATP release during ischemia, but its concentration was markedly lower than that of adenosine (Frenguelli et al. 2007). Accordingly, it is thought that metabolic stress causes direct release of adenosine in the hippocampus, but the site of release remains unresolved. Certainly the equilibrative nucleoside transporter is thought to be one of the mechanisms to transfer adenosine from intracellular to extracellular space (Cunha et al. 1996; Lovatt et al. 2012). However, a previous study reported that inhibitors of equilibrative nucleoside transporters did not reduce endogenous adenosine-induced synaptic inhibition during ischemia or hypoxia (Zhang et al. 2012) and did not increase the concentration of extracellular adenosine in ischemia (Frenguelli et al. 2007). To elucidate the adenosine-releasing mechanism in the present study, additional experiments will be needed.

In sum, we report functional effects of A_1Rs across four experimental metabolic stresses. Activation of A_1Rs is essential to recover synaptic transmission after ischemia or after reduced glucose with hypoxia, and thus receptor activation might extend the time window for ischemia-induced irreversible synaptic loss thought to be caused by cell death. A_1Rs also prevented a moderate lasting synaptic depression caused by a longer duration of hypoxia or hypoglycemia. Selective genetic or pharmacological inactivation yielded similar functional effects. Altogether, activation of A_1Rs has a protective role against a range of metabolic stresses in brain, and receptor activity can serve to protect synaptic transmission entirely depending on the duration of a specific stress.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.K., D.N.R., and S.A.M. conceived and designed research; M.K. and S.A.M. performed experiments; M.K. analyzed data; M.K. prepared figures; M.K. drafted manuscript; D.N.R. and S.A.M. edited and revised manuscript; M.K., D.N.R., and S.A.M. approved final version of manuscript.

REFERENCES

Abi-Saab WM, Maggs DG, Jones T, Jacob R, Srihari V, Thompson J, Kerr D, Leone P, Krystal JH, Spencer DD, During MJ, Sherwin RS. Striking differences in glucose and lactate levels between brain extracellular fluid and plasma in conscious human subjects: effects of hyperglycemia and hypoglycemia. *J Cereb Blood Flow Metab* 22: 271–279, 2002. doi:10.1097/00004647-200203000-00004.

Ai J, Baker A. Long-term potentiation of evoked presynaptic response at CA3-CA1 synapses by transient oxygen-glucose deprivation in rat brain slices. *Exp Brain Res* 169: 126–129, 2006. doi:10.1007/s00221-005-0314-5.

Chamberlain SE, Sadowski JH, Teles-Grilo Ruivo LM, Atherton LA, Mellor JR. Long-term depression of synaptic kainate receptors reduces excitability by relieving inhibition of the slow afterhyperpolarization. *J Neurosci* 33: 9536–9545, 2013. doi:10.1523/JNEUROSCI.0034-13.2013.

Chen Z, Xiong C, Pancyr C, Stockwell J, Walz W, Cayabyab FS. Prolonged adenosine A1 receptor activation in hypoxia and pial vessel disruption focal cortical ischemia facilitates clathrin-mediated AMPA receptor endocytosis and long-lasting synaptic inhibition in rat hippocampal CA3-CA1 synapses: differential regulation of GluA2 and GluA1 subunits by p38 MAPK and JNK. *J Neurosci* 34: 9621–9643, 2014. doi:10.1523/JNEUROSCI.3991-13.2014.

Cunha RA. Neuroprotection by adenosine in the brain: from A_1 receptor activation to A_{2A} receptor blockade. *Purinergic Signal* 1: 111–134, 2005. doi:10.1007/s11302-005-0649-1.

Cunha RA. How does adenosine control neuronal dysfunction and neurodegeneration? *J Neurochem* 139: 1019–1055, 2016. doi:10.1111/jnc.13724.

Cunha RA, Vizi ES, Ribeiro JA, Sebastião AM. Preferential release of ATP and its extracellular catabolism as a source of adenosine upon high- but not low-frequency stimulation of rat hippocampal slices. *J Neurochem* 67: 2180–2187, 1996. doi:10.1046/j.1471-4159.1996.67052180.x.

Dale N, Pearson T, Frenguelli BG. Direct measurement of adenosine release during hypoxia in the CA1 region of the rat hippocampal slice. *J Physiol* 526: 143–155, 2000. doi:10.1111/j.1469-7793.2000.00143.x.

de Mendonça A, Sebastião AM, Ribeiro JA. Inhibition of NMDA receptor-mediated currents in isolated rat hippocampal neurones by adenosine A1 receptor activation. *Neuroreport* 6: 1097–1100, 1995. doi:10.1097/00001756-199505300-00006.

Dias RB, Rombo DM, Ribeiro JA, Sebastião AM. Ischemia-induced synaptic plasticity drives sustained expression of calcium-permeable AMPA receptors in the hippocampus. *Neuropharmacology* 65: 114–122, 2013. doi:10.1016/j.neuropharm.2012.09.016.

Dulla CG, Dobelis P, Pearson T, Frenguelli BG, Staley KJ, Masino SA. Adenosine and ATP link PCO2 to cortical excitability via pH. *Neuron* 48: 1011–1023, 2005. doi:10.1016/j.neuron.2005.11.009.

Dunwiddie TV, Diao L, Proctor WR. Adenine nucleotides undergo rapid, quantitative conversion to adenosine in the extracellular space in rat hippocampus. *J Neurosci* 17: 7673–7682, 1997. doi:10.1523/JNEUROSCI.17-20-07673.1997.

Dunwiddie TV, Hoffer BJ. Adenine nucleotides and synaptic transmission in the in vitro rat hippocampus. *Br J Pharmacol* 69: 59–68, 1980. doi:10.1111/j.1476-5381.1980.tb10883.x.

Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci* 24: 31–55, 2001. doi:10.1146/annurev.neuro.24.1.31.

Fowler JC. Adenosine antagonists delay hypoxia-induced depression of neuronal activity in hippocampal brain slice. *Brain Res* 490: 378–384, 1989. doi:10.1016/0006-8993(89)90258-8.

Fowler JC. Adenosine antagonists alter the synaptic response to in vitro ischemia in the rat hippocampus. *Brain Res* 509: 331–334, 1990. doi:10.1016/0006-8993(90)90560-X.

Fowler JC. Purine release and inhibition of synaptic transmission during hypoxia and hypoglycemia in rat hippocampal slices. *Neurosci Lett* 157: 83–86, 1993. doi:10.1016/0304-3940(93)90648-5.

Fredholm BB. Adenosine and neuroprotection. *Int Rev Neurobiol* 40: 259–280, 1997. doi:10.1016/S0074-7742(08)60723-0.

Fredholm BB. Adenosine, an endogenous distress signal, modulates tissue damage and repair. *Cell Death Differ* 14: 1315–1323, 2007. doi:10.1038/sj.cdd.4402132.

Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. Structure and function of adenosine receptors and their genes. *Naunyn Schmiedebergs Arch Pharmacol* 362: 364–374, 2000. doi:10.1007/s002100000313.

Frenguelli BG, Wigmore G, Llaudet E, Dale N. Temporal and mechanistic dissociation of ATP and adenosine release during ischaemia in the mammalian hippocampus. *J Neurochem* 101: 1400–1413, 2007. doi:10.1111/j.1471-4159.2006.04425.x.

Giménez-Llort L, Fernández-Teruel A, Escorihuela RM, Fredholm BB, Tobeña A, Pekny M, Johansson B. Mice lacking the adenosine A_1 receptor are anxious and aggressive, but are normal learners with reduced muscle strength and survival rate. *Eur J Neurosci* 16: 547–550, 2002. doi:10.1046/j.1460-9568.2002.02122.x.

Gonçalves ML, Cunha RA, Ribeiro JA. Adenosine A_{2A} receptors facilitate $^{45}Ca^{2+}$ uptake through class A calcium channels in rat hippocampal CA3 but not CA1 synaptosomes. *Neurosci Lett* 238: 73–77, 1997. doi:10.1016/S0304-3940(97)00803-3.

Gundlfinger A, Bischofberger J, Johenning FW, Torvinen M, Schmitz D, Breustedt J. Adenosine modulates transmission at the hippocampal mossy fibre synapse via direct inhibition of presynaptic calcium channels. *J Physiol* 582: 263–277, 2007. doi:10.1113/jphysiol.2007.132613.

Haas HL, Greene RW. Adenosine enhances afterhyperpolarization and accommodation in hippocampal pyramidal cells. *Pflugers Arch* 402: 244–247, 1984. doi:10.1007/BF00585506.

Hájos N, Ellender TJ, Zemankovics R, Mann EO, Exley R, Cragg SJ, Freund TF, Paulsen O. Maintaining network activity in submerged hippocampal slices: importance of oxygen supply. *Eur J Neurosci* 29: 319–327, 2009. doi:10.1111/j.1460-9568.2008.06577.x.

Hu Y, Wilson GS. Rapid changes in local extracellular rat brain glucose observed with an in vivo glucose sensor. *J Neurochem* 68: 1745–1752, 1997. doi:10.1046/j.1471-4159.1997.68041745.x.

Ivanov A, Zilberter Y. Critical state of energy metabolism in brain slices: the principal role of oxygen delivery and energy substrates in shaping neuronal activity. *Front Neuroenergetics* 3: 9, 2011. doi:10.3389/fnene.2011.00009.

Jalini S, Ye H, Tonkikh AA, Charlton MP, Carlen PL. Raised intracellular calcium contributes to ischemia-induced depression of evoked synaptic transmission. *PLoS One* 11: e0148110, 2016. doi:10.1371/journal.pone.0148110.

Johansson B, Halldner L, Dunwiddie TV, Masino SA, Poelchen W, Giménez-Llort L, Escorihuela RM, Fernández-Teruel A, Wiesenfeld-Hallin Z, Xu XJ, Hårdemark A, Betsholtz C, Herlenius E, Fredholm BB. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A_1 receptor. *Proc Natl Acad Sci USA* 98: 9407–9412, 2001. doi:10.1073/pnas.161292398.

Kawamura M Jr, Kawamura M. Long-term facilitation of spontaneous calcium oscillations in astrocytes with endogenous adenosine in hippocampal slice cultures. *Cell Calcium* 49: 249–258, 2011. doi:10.1016/j.ceca.2011.02.009.

Kawamura M Jr, Ruskin D. Adenosine and autocrine metabolic regulation of neuronal activity. In: *Adenosine*, edited by Masino S, Boison D. New York: Springer, 2012, p. 71–85.

Kawamura M Jr, Ruskin DN, Geiger JD, Boison D, Masino SA. Ketogenic diet sensitizes glucose control of hippocampal excitability. *J Lipid Res* 55: 2254–2260, 2014. doi:10.1194/jlr.M046755.

Kawamura M Jr, Ruskin DN, Masino SA. Metabolic autocrine regulation of neurons involves cooperation among pannexin hemichannels, adenosine receptors, and K_{ATP} channels. *J Neurosci* 30: 3886–3895, 2010. doi:10.1523/JNEUROSCI.0055-10.2010.

Latini S, Bordoni F, Pedata F, Corradetti R. Extracellular adenosine concentrations during in vitro ischaemia in rat hippocampal slices. *Br J Pharmacol* 127: 729–739, 1999. doi:10.1038/sj.bjp.0702591.

Latini S, Pedata F. Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem* 79: 463–484, 2001. doi:10.1046/j.1471-4159.2001.00607.x.

Li H, Henry JL. Adenosine A_2 receptor mediation of pre- and postsynaptic excitatory effects of adenosine in rat hippocampus in vitro. *Eur J Pharmacol* 347: 173–182, 1998. doi:10.1016/S0014-2999(98)00105-8.

Lopes LV, Cunha RA, Kull B, Fredholm BB, Ribeiro JA. Adenosine A_{2A} receptor facilitation of hippocampal synaptic transmission is dependent on tonic A_1 receptor inhibition. *Neuroscience* 112: 319–329, 2002. doi:10.1016/S0306-4522(02)00080-5.

Lovatt D, Xu Q, Liu W, Takano T, Smith NA, Schnermann J, Tieu K, Nedergaard M. Neuronal adenosine release, and not astrocytic ATP release, mediates feedback inhibition of excitatory activity. *Proc Natl Acad Sci USA* 109: 6265–6270, 2012. doi:10.1073/pnas.1120997109.

Madry C, Haglerød C, Attwell D. The role of pannexin hemichannels in the anoxic depolarization of hippocampal pyramidal cells. *Brain* 133: 3755–3763, 2010. doi:10.1093/brain/awq284.

Masino SA, Diao L, Illes P, Zahniser NR, Larson GA, Johansson B, Fredholm BB, Dunwiddie TV. Modulation of hippocampal glutamatergic transmission by ATP is dependent on adenosine A_1 receptors. *J Pharmacol Exp Ther* 303: 356–363, 2002. doi:10.1124/jpet.102.036731.

Masino SA, Kawamura M, Wasser CA, Pomeroy LT, Ruskin DN. Adenosine, ketogenic diet and epilepsy: the emerging therapeutic relationship between metabolism and brain activity. *Curr Neuropharmacol* 7: 257–268, 2009. doi:10.2174/157015909789152164.

Masino SA, Latini S, Bordoni F, Pedata F, Dunwiddie TV. Changes in hippocampal adenosine efflux, ATP levels, and synaptic transmission induced by increased temperature. *Synapse* 41: 58–64, 2001. doi:10.1002/syn.1060.

Masino SA, Li T, Theofilas P, Sandau US, Ruskin DN, Fredholm BB, Geiger JD, Aronica E, Boison D. A ketogenic diet suppresses seizures in mice through adenosine A_1 receptors. *J Clin Invest* 121: 2679–2683, 2011. doi:10.1172/JCI57813.

Masino SA, Mesches MH, Bickford PC, Dunwiddie TV. Acute peroxide treatment of rat hippocampal slices induces adenosine-mediated inhibition of excitatory transmission in area CA1. *Neurosci Lett* 274: 91–94, 1999. doi:10.1016/S0304-3940(99)00693-X.

Mitchell JB, Lupica CR, Dunwiddie TV. Activity-dependent release of endogenous adenosine modulates synaptic responses in the rat hippocampus. *J Neurosci* 13: 3439–3447, 1993. doi:10.1523/JNEUROSCI.13-08-03439.1993.

Mouro FM, Rombo DM, Dias RB, Ribeiro JA, Sebastião AM. Adenosine A_{2A} receptors facilitate synaptic NMDA currents in CA1 pyramidal neurons. *Br J Pharmacol* 175: 4386–4397, 2018. doi:10.1111/bph.14497.

Olsson T, Cronberg T, Rytter A, Asztély F, Fredholm BB, Smith ML, Wieloch T. Deletion of the adenosine A_1 receptor gene does not alter neuronal damage following ischaemia in vivo or in vitro. *Eur J Neurosci* 20: 1197–1204, 2004. doi:10.1111/j.1460-9568.2004.03564.x.

Pearson T, Damian K, Lynas RE, Frenguelli BG. Sustained elevation of extracellular adenosine and activation of A_1 receptors underlie the post-ischaemic inhibition of neuronal function in rat hippocampus in vitro. *J Neurochem* 97: 1357–1368, 2006. doi:10.1111/j.1471-4159.2006.03823.x.

Pedata F, Dettori I, Coppi E, Melani A, Fusco I, Corradetti R, Pugliese AM. Purinergic signalling in brain ischemia. *Neuropharmacology* 104: 105–130, 2016. doi:10.1016/j.neuropharm.2015.11.007.

Pinto I, Serpa A, Sebastião AM, Cascalheira JF. The role of cGMP on adenosine A_1 receptor-mediated inhibition of synaptic transmission at the hippocampus. *Front Pharmacol* 7: 103, 2016. doi:10.3389/fphar.2016.00103.

Pugliese AM, Latini S, Corradetti R, Pedata F. Brief, repeated, oxygen-glucose deprivation episodes protect neurotransmission from a longer ischemic episode in the in vitro hippocampus: role of adenosine receptors. *Br J Pharmacol* 140: 305–314, 2003. doi:10.1038/sj.bjp.0705442.

Rebola N, Lujan R, Cunha RA, Mulle C. Adenosine A_{2A} receptors are essential for long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses. *Neuron* 57: 121–134, 2008. doi:10.1016/j.neuron.2007.11.023.

Rebola N, Rodrigues RJ, Lopes LV, Richardson PJ, Oliveira CR, Cunha RA. Adenosine A_1 and A_{2A} receptors are co-expressed in pyramidal neurons and co-localized in glutamatergic nerve terminals of the rat hippocampus. *Neuroscience* 133: 79–83, 2005. doi:10.1016/j.neuroscience.2005.01.054.

Scianni M, Antonilli L, Chece G, Cristalli G, Di Castro MA, Limatola C, Maggi L. Fractalkine (CX_3CL1) enhances hippocampal *N*-methyl-D-aspartate receptor (NMDAR) function via D-serine and adenosine receptor type A2 ($A_{2A}R$) activity. *J Neuroinflammation* 10: 876, 2013. doi:10.1186/1742-2094-10-108.

Sebastião AM, de Mendonca A, Moreira T, Ribeiro JA. Activation of synaptic NMDA receptors by action potential-dependent release of transmitter during hypoxia impairs recovery of synaptic transmission on reoxygenation. *J Neurosci* 21: 8564–8571, 2001. doi:10.1523/JNEUROSCI.21-21-08564.2001.

Shram NF, Netchiporouk LI, Martelet C, Jaffrezic-Renault N, Cespuglio R. Brain glucose: voltammetric determination in normal and hyperglycaemic rats using a glucose microsensor. *Neuroreport* 8: 1109–1112, 1997. doi:10.1097/00001756-199703240-00009.

Silver IA, Erecińska M. Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. *J Neurosci* 14: 5068–5076, 1994. doi:10.1523/JNEUROSCI.14-08-05068.1994.

Stevens B, Porta S, Haak LL, Gallo V, Fields RD. Adenosine: a neuron-glial transmitter promoting myelination in the CNS in response to action potentials. *Neuron* 36: 855–868, 2002. doi:10.1016/S0896-6273(02)01067-X.

Stockwell J, Chen Z, Niazi M, Nosib S, Cayabyab FS. Protein phosphatase role in adenosine A_1 receptor-induced AMPA receptor trafficking and rat hippocampal neuronal damage in hypoxia/reperfusion injury. *Neuropharmacology* 102: 254–265, 2016. doi:10.1016/j.neuropharm.2015.11.018.

Tian GF, Baker AJ. Protective effect of high glucose against ischemia-induced synaptic transmission damage in rat hippocampal slices. *J Neurophysiol* 88: 236–248, 2002. doi:10.1152/jn.00572.2001.

Williams-Karnesky RL, Sandau US, Lusardi TA, Lytle NK, Farrell JM, Pritchard EM, Kaplan DL, Boison D. Epigenetic changes induced by adenosine augmentation therapy prevent epileptogenesis. *J Clin Invest* 123: 3552–3563, 2013. doi:10.1172/JCI65636.

Wu LG, Saggau P. Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus. *Neuron* 12: 1139–1148, 1994. doi:10.1016/0896-6273(94)90321-2.

Yenari MA, Han HS. Neuroprotective mechanisms of hypothermia in brain ischaemia. *Nat Rev Neurosci* 13: 267–278, 2012. doi:10.1038/nrn3174.

Zhang D, Xiong W, Chu S, Sun C, Albensi BC, Parkinson FE. Inhibition of hippocampal synaptic activity by ATP, hypoxia or oxygen-glucose deprivation does not require CD73. *PLoS One* 7: e39772, 2012. doi:10.1371/journal.pone.0039772.

Zhu PJ, Krnjević K. Adenosine release is a major cause of failure of synaptic transmission during hypoglycaemia in rat hippocampal slices. *Neurosci Lett* 155: 128–131, 1993. doi:10.1016/0304-3940(93)90689-I.