Adenosine A₁ receptor-mediated protection of mouse hippocampal synaptic transmission against oxygen and/or glucose deprivation: A comparative study

Masahito Kawamura
*The Jikei University School of Medicine*

David N. Ruskin
*Trinity College Hartford*

Susan A. Masino
*Trinity College Hartford*
Adenosine A1 receptor-mediated protection of mouse hippocampal synaptic transmission against oxygen and/or glucose deprivation: a comparative study

Masahito Kawamura, Jr.,1 David N. Ruskin,2 and Susan A. Masino2

1Department of Pharmacology, Jikei University School of Medicine, Tokyo, Japan; and 2Department of Psychology and Neuroscience Program, Trinity College, Hartford, Connecticut

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Kawamura M Jr, Ruskin DN, Masino SA. Adenosine A1 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 A1 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 A1 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 A1 receptor mediators 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 A1 receptor antagonist, validating the clear effects of genetic receptor inactivation on synaptic transmission. We found activation of adenosine A1 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 A1 receptor during a variety of metabolic stresses within the same recording system.

NEW & NOTEWORTHY Deprivation of oxygen and/or glucose causes a rapid adenosine A1 receptor-mediated decrease in synaptic transmission in mouse hippocampus. We quantified adenosine A1 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 A1 receptor depended on the type and duration of deprivation event.

acute hippocampal slices; adenosine A1 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); A1, A2A, A2B, and A3 (Dunwiddie and Masino 2001; Fredholm et al. 2000). Adenosine A1 receptors (A1Rs) in particular are distributed widely and known to inhibit synaptic transmission by suppressing influx into presynaptic voltage-dependent calcium channels in synaptic terminals (Gundlacher 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. 1994). On the other hand, A2A Rs are reported to facilitate synaptic transmission (Lopes et al. 2002) by increasing calcium influx via presynaptic voltage-dependent calcium chan-
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; Mourou et al. 2018). Thus increased extracellular adenosine caused by metabolic changes can activate $\alpha_1$Rs to suppress neuronal activity and $\alpha_2$Rs to facilitate neuronal activity. Activation of $\alpha_1$R and blockade of $\alpha_2$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 $\alpha_1$ receptor knockout (A1KO) mice to examine systematically the role of the $\alpha_1$R during metabolic stresses where large amounts of adenosine are known to be released. Previous work has shown that the A1KO mouse exhibits a complete loss of $\alpha_1$Rs in all brain regions, and synaptic transmission in the hippocampus does not respond to exogenous or elevated endogenous adenosine (Johansson et al. 2001). A1KO 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). $\alpha_1$KO 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 $\alpha_1$R, 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 $\alpha_1$Rs 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 $\alpha_1$ 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, similar 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$_2$PO$_4$, 11 glucose, and 26 NaHCO$_3$ (osmolarity 320 mosM, pH 7.4 when saturated with 95% O$_2$ and 5% CO$_2$) with a vibrating slice cutter (series 1000, Vibrotome, 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 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 (fEPSPs) 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 A1KO 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. 1A). The transient increase in fEPSP amplitude (Fig. 1A, 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-
In slices obtained from A1KO mice, the rapid inhibition of fEPSP with 5-min ischemia did not occur (Fig. 1B1), suggesting that this inhibition is reversible and caused by activation of A1Rs. Similarly, with 10-min ischemia, the fEPSP was not rapidly inhibited in slices from A1KO mice: it was suppressed with delayed onset and finally lost (Fig. 1B2). The significant partial recovery in the slices from WT mice after 10-min ischemia did not occur in the slices from A1KO mice. Consistent with 10-min ischemia, more long-term ischemia (>20 min) in A1KO 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 A1Rs, although fEPSP suppression after ischemia of a sufficient duration (>10 min) occurs even in the absence of A1Rs. Second, activation of A1Rs is essential for fEPSP recovery after ischemia. This A1R-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. 1A).

**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. 2A), unlike when glucose and oxygen were both removed (Fig. 1A). The rapid inhibition with 10-min hypoxia was not seen in slices from A1KO mice (Fig. 2B1), suggesting that hypoxia-induced reversible fEPSP inhibition is caused by activation of A1Rs. Interestingly, a very long period of hypoxia (60 min) in the A1KO mice induced a partial but significant suppression of fEPSP amplitude (Fig. 2B2) 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 A1Rs: a similar modest suppression was shown in the presence of the A1R antagonist CPT (1 μM) in slices from WT mice (Fig. 2B1). These results argue strongly that long-lasting hypoxia causes modest loss of synaptic transmission but that activation of A1Rs prevents any lasting impairment.

Similarly, 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 A1KO 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 A1Rs.

**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. 1A). However, after the same duration (10 min) of reduced glucose with hypoxia, the fEPSP amplit-
tude recovered completely (Fig. 4A); 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. 4A).

Recovery was not observed in hippocampal slices from A1KO mice, indicating that the recovery of fEPSP amplitude after reduced glucose with hypoxia is caused by activation of A1Rs (Fig. 4B). A duration longer than 20 min of reduced glucose with hypoxia caused irreversible loss of synaptic transmission (Fig. 4A), similarly to experimental ischemia. Whereas glucose did extend the window of recovery after hypoxia, the acute protective effect of A1Rs 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 A1Rs during and after these metabolically stressful events. A1R 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 A1Rs during metabolic stress have been reported in several lines of experiments in hippocampal slices. Experimental ischemia with short duration (5–10 min) is
ADENOSINE-INDUCED NEUROPROTECTION AGAINST METABOLIC STRESS

MANIPULATING THE DURATION OF ISCHEMIA TO PROTECT HIPPOCAMMAL NEURONS FROM METABOLIC STRESS

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: N₂ + 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, 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 A₁ receptor knockout (A₁KO) mice. Data shown from WT mice are the same as 15-min N₂ + 3 mM glucose in A. **P < 0.01 (unpaired t-test).

reported to cause A₁R-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 A₁R (Dale et al. 2000; Fowler 1989). It has been reported that >60 min of hypoxia induces a slight irreversible loss of the fEPSP when A₁Rs are inactivated (Johansson et al. 2001; Sebastião et al. 2001). Hypoglycemia has been reported to cause A₁R-induced reversible fEPSP inhibition in hippocampal slices (Zhu and Krnjevic’ 1993). On the basis of these previous reports, we compared the effects of A₁R during these metabolic stresses using the same animal species and the same recording system, and compared genetic and pharmacological inactivation of A₁Rs. We found a particular duration of ischemia (10 min) that caused partial but significant recovery of fEPSP amplitude in the hippocampal slices (Fig. 1A). This recovery was mediated by activation of A₁R (Fig. 1B). We also found that a rise in extracellular glucose concentration increased A₁R-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 A₁R (Fig. 3B), similarly to hypoxia (Fig. 3B2). Previous work investigated the effects of A₁R during metabolic stresses typically using pharmacology, with the exception of a long duration of hypoxia (Johansson et al. 2001). We examined all of these A₁R effects by using A₁KO 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 A₁Rs: 1) a rapid direct inhibition, 2) a slower onset of A₁R-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 A₁Rs (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 A₁R. 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 A₁Rs 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 A₁R with ischemia/hypoxia or applying a selective A₁R agonist causes synaptic depression of fEPSP in rat hippocampal slices (Chen et al. 2014; Stockwell et al. 2016). There is no A₁R-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 A₉₂R 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. 1A, 2A, 3A and 4A).

Our method for experimental ischemia reproduced an acute phase of ischemic stroke (Yenari and Han 2012). In this condition, activation of A₁R produced protection of neuronal synaptic activity against 10-min ischemia (Fig. 1B2). However, long-term exposure to ischemia (30 min) caused irreversible synaptic loss even in slices from WT mice (Fig. 1A). Therefore, the effect of A₁R against the acute phase of ischemic stroke might be limited. Moreover, the effect of A₁R during the chronic phase of ischemic stroke is poor. The neuronal damage after global ischemic events is not different between WT mice and A₁KO mice in vivo (Olsson et al. 2004). The role of A₁R-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
A1Rs, 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 A1Rs but contrasted with ischemia in the later effects. Hypoglycemia caused a mild A1R-independent depression with no transient reappearance and no loss of transmission even after 30 min of treatment; hypoxia caused a mild A1R-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 A1R activation: the fEPSP did not completely recover after hypoxia or hypoglycemia in the hippocampal slices from A1KO mice or in the presence of an A1R antagonist in WT mice, clearly showing the involvement of this receptor in synaptic recovery. The mechanisms of A1R 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 A1R-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 knock-out 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 (Fregnueli 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 (Fregnueli 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 A1Rs across four experimental metabolic stresses. Activation of A1Rs 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. A1Rs 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 A1Rs 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.

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