Neurobiology of Disease 132 (2019) 104570

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Synaptic and memory dysfunction in a β-amyloid model of early Alzheimer's disease depends on increased formation of ATP-derived extracellular adenosine

Francisco Q. Gonçalves[a,1], João P. Lopes[a,1], Henrique B. Silva[a], Cristina Lemos[a], António C. Silva[a], Nélio Gonçalves[a], Ângelo R. Tomé[a], Samira G. Ferreira[a], Paula M. Canas[a], Daniel Rial[a], Paula Agostinho[a,b], Rodrigo A. Cunha[a,b,*]

[a] CNC-Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal
[b] FMUC-Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal

A B S T R A C T

Adenosine A_{2A} receptors ($A_{2A}R$) overfunction causes synaptic and memory dysfunction in early Alzheimer's disease (AD). In a β-amyloid ($A\beta_{1-42}$)-based model of early AD, we now unraveled that this involves an increased synaptic release of ATP coupled to an increased density and activity of ecto-5′-nucleotidase (CD73)-mediated formation of adenosine selectively activating $A_{2A}R$. Thus, CD73 inhibition with α,β-methylene-ADP impaired long-term potentiation (LTP) in mouse hippocampal slices, which is occluded upon previous superfusion with the $A_{2A}R$ antagonist SCH58261. Furthermore, α,β-methylene-ADP did not alter LTP amplitude in global $A_{2A}R$ knockout (KO) and in forebrain neuron-selective $A_{2A}R$-KO mice, but inhibited LTP amplitude in astrocyte-selective $A_{2A}R$-KO mice; this shows that CD73-derived adenosine solely acts on neuronal $A_{2A}R$. In agreement with the concept that ATP is a danger signal in the brain, ATP release from nerve terminals is increased after intracerebroventricular $A\beta_{1-42}$ administration, together with CD73 and $A_{2A}R$ upregulation in hippocampal synapses. Importantly, this increased CD73 activity is critically required for $A\beta_{1-42}$ to impair synaptic plasticity and memory since $A\beta_{1-42}$-induced synaptic and memory deficits were eliminated in CD73-KO mice. These observations establish a key regulatory role of CD73 activity over neuronal $A_{2A}R$ and imply CD73 as a novel target for modulation of early AD.

1. Introduction

ATP plays a pivotal role in extracellular signaling (Burnstock, 2014). In the brain, ATP can function as a neurotransmitter, co-transmitter, gliotransmitter, or even a neuromodulator at specific synapses (Burnstock, 2014; Rodrigues et al., 2015). These multiple effects of ATP result from a direct activation of different P2 receptors (Burnstock, 2014; Rodrigues et al., 2015) but also depend on indirect effects upon conversion of extracellular ATP into adenosine, through the action of ecto-nucleotidases including ecto-5′-nucleotidase or CD73 (Zimmermann et al., 2012). Adenosine is a prototypical neuromodulator (Fredholm et al., 2005; Borea et al., 2018) and its main role is to assist in defining the salience of information encoding in brain circuits, through a parallel control of basal excitatory synaptic transmission through inhibitory A_1 receptors (A_1R) and of synaptic plasticity through facilitatory A_{2A} receptors ($A_{2A}R$) (Cunha, 2008). The differential activation of A_1R and $A_{2A}R$ is proposed to involve a different source of adenosine depending on the type of triggering stimuli (Cunha, 2008): A_1R activation mainly results from astrocytic-derived ATP (Pascual et al., 2005) and post-synaptic adenosine outflow (Lovatt et al., 2012), whereas $A_{2A}R$ are activated by CD73-mediated adenosine (Augusto et al., 2013; Cunha et al., 1996a; Rebola et al., 2008; Carmo et al., 2019) resulting from a robust frequency-dependent synaptic release of ATP (Cunha et al., 1996b; Wieraszko et al., 1989).

Although $A_{2A}R$ are mostly expressed in the basal ganglia (Fredholm et al., 2005), there has been an increasing awareness to their role in other brain areas (Rebola et al., 2008; Cunha, 2016; Kerkhofs et al., 2018; Simões et al., 2016). Indeed, the interest in hippocampal $A_{2A}R$ function was bolstered by the recognition that they are the main target of caffeine in the brain (Fredholm et al., 2005; Lopes et al., 2019) and their overfunction is paramount to define the extent of neurodegeneration upon brain insults (Cunha, 2016) associated with disorders where caffeine has been described as having a protective effect against memory deficits (Cunha & Agostinho, 2010; Laurent et al., 2014). In particular, the overactivation of hippocampal $A_{2A}R$ is sufficient to trigger memory impairment (Pagnussat et al., 2015) and is critically necessary for the emergence of synaptic and memory deficits in different animal models of early Alzheimer's disease (AD) (Canas et al.,

* Corresponding author at: Center for Neuroscience of Coimbra, Institute of Biochemistry, Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal.
E-mail address: cunharod@gmail.com (R.A. Cunha).
[1] Contributed equally.

https://doi.org/10.1016/j.nbd.2019.104570
Received 12 June 2019; Received in revised form 9 July 2019; Accepted 2 August 2019
Available online 05 August 2019
0969-9961/

2009; Laurent et al., 2016; Viana da Silva et al., 2016). However, it is currently unclear if this $A_{2A}R$ overfunction only involves $A_{2A}R$ upregulation (Cunha, 2016) or also depends on an increased generation of extracellular adenosine. In this respect, the evidence that ATP is a danger signal in the brain (Rodrigues et al., 2015) and that brain insults trigger a parallel upregulation of $A_{2A}R$ and CD73 (Barros-Barbosa et al., 2016; Wang et al., 2015), posits the novel hypothesis that an increased CD73-mediated formation of extracellular adenosine from increased ATP release might be paramount to sustain the $A_{2A}R$ overfunctioning that is critically required to disrupt synaptic plasticity and memory function in early AD (Canas et al., 2009; Viana da Silva et al., 2016). This hypothesis is reinforced by the close physical and functional association between CD73 and $A_{2A}R$ observed in the striatum (Augusto et al., 2013; Carmo et al., 2019), which has not been tested in the hippocampus. Thus, we now tested how ATP-derived adenosine formation by CD73 regulates the modulation of synaptic plasticity by selective $A_{2A}R$ activation in both physiological and pathological conditions in the hippocampus.

2. Material and methods

2.1. Animals

Male C57bl\6j mice (8–12 weeks old) were obtained from Charles River (Barcelona, Spain). $A_{2A}R$-KO, CD73-KO, $FbA_{2A}R$-KO and GFAP-$A_{2A}R$-KO as well as each individual control genotype (generically named as wild type) were generated and cross-bred as previously described (Augusto et al., 2013; Matos et al., 2015) and males with 8–12 weeks old were used in the experiments.

2.2. Drugs and model of early AD

α,β-Methylene ADP (AOPCP) was from Sigma (St. Louis, USA) and SCH58261 was from Tocris (Bristol, UK). Both drugs were used in supramaximal but selective concentrations: 100 μM AOPCP (Cunha et al., 1996b; Cunha, 2001) and 50 nM SCH58261 (Lopes et al., 2004). The $A\beta_{1\text{-}42}$ peptide fragment was purchased from Bachem (Bubendorf, Germany). $A\beta_{1\text{-}42}$ was dissolved in water to obtain a solution mostly composed of Aβ oligomers (Canas et al., 2009; Resende et al., 2008) with a final concentration of 2.25 mg/mL. Mice received, over a period of 15 min, either a single dose of 2 nmol in 4 μL of oligomeric $A\beta_{1\text{-}42}$ or the same volume of water (vehicle, which caused no behavioral or neurochemical effects, similarly to the administration of scrambled $A\beta_{42\text{-}1}$, see (Canas et al., 2009)) intracerebroventricularly (icv) after anesthesia. It has previously been shown that this apparently high dose of $A\beta_{1\text{-}42}$ actually translates into 5–30 pmol levels of $A\beta_{1\text{-}42}$ within the hippocampus, causing synaptic alterations and dysfunction, without evidence of cellular damage (Canas et al., 2009). Behavioral analysis was performed 14 days after $A\beta_{1\text{-}42}$ or vehicle administration, at a time where spatial reference memory is selectively affected in this icv-$A\beta_{1\text{-}42}$ model (Canas et al., 2009, 2014). Although there are no faithful animal models of AD, the ability to recapitulate the two main features of early AD, namely the impairment of reference memory and of synaptic function (reviewed in (Walsh & Selkoe, 2004)), validates this icv-$A\beta_{1\text{–}42}$ model as a model of early AD, which has been previously used by different groups (Canas et al., 2009; Hong et al., 2016; Kim et al., 2016; Santos et al., 2017).

2.3. Evoked ATP release

The release of ATP was measured on-line using the luciferin-luciferase assay (Cunha et al., 1996a; Carmo et al., 2019). Briefly, mouse hippocampal synaptosomes were prepared as previously described (Canas et al., 2009; Kaster et al., 2015). A suspension containing synaptosomes, an ATP assay mix (with luciferin and luciferase; from Sigma) and Krebs-HEPES solution was equilibrated at 25 °C during 10 min to ensure the functional recovery of nerve terminals. The suspension was then transferred to a white 96-well plate and measurements were performed in a luminometer (Victor3). After 60 s to measure basal ATP outflow, the evoked release of ATP was triggered with 32 mM of KCl (isomolar substitution of NaCl in the Krebs-HEPES solution), a well-established neurochemical strategy to trigger optimal signal-to-noise calcium-dependent vesicular release from synaptosomes without damage to these artificial synaptic structures (Bradford et al., 1973; Blaustein, 1975; White, 1978; Ferreira et al., 2015). The evoked release of ATP was calculated by integration of the area of the peak upon subtraction of the estimated basal ATP outflow (Cunha et al., 1996a; Carmo et al., 2019).

2.4. Binding assay

The density of $A_{2A}R$ was estimated by radioligand binding using a supramaximal concentration of the $A_{2A}R$ antagonist [^{3}H]SCH58261 (6 nM; provided by E. Ongini, Schering-Plough, Milan, Italy), as described previously (Lopes et al., 2004, Kaster et al., 2015). Specific binding was determined by the subtraction of nonspecific binding measured in the presence of 3 μM xanthine (XAC), a mixed $A_1R/A_{2A}R$ antagonist.

2.5. Western blot

Western blot analyses of hippocampal synaptosomes were performed as described previously (Carmo et al., 2019; Canas et al., 2009; Kaster et al., 2015). CD73 levels were assessed using a rabbit polyclonal anti-CD73 antibody (1:300, Sigma-Aldrich), whereas mouse monoclonal anti-synaptophysin (1:6000, Sigma-Aldrich), anti-PSD95 (1:1000, Chemicon) and anti-SNAP25 (1:6000, Sigma-Aldrich) antibodies were used to quantify synaptic markers, after labeling with secondary antibodies goat anti-rabbit IgG and goat anti-mouse IgG conjugated with alkaline phosphatase (1:20,000) (GE Healthcare, Chicago, USA). The membranes were then stripped for re-probing with GAPDH (rabbit polyclonal, 1:3000, Abcam) or α-tubulin (mouse monoclonal, 1:20,000, Sigma-Aldrich), as loading controls. Immunoreactive bands were detected after incubation of membranes with ECF reagent (GE Healthcare), on a Bio-Rad ChemidocPlus imaging system.

2.6. Extracellular catabolism of AMP

The extracellular catabolism of AMP was evaluated in hippocampal synaptosomes as previously described (Carmo et al., 2019; Cunha, 2001; Cunha et al., 2000). Briefly, synaptosomes were added to incubation vials with Krebs-HEPES solution at 30.5 °C. After 10 min of incubation, 10 μM AMP was added, without or with AOPCP, and aliquots were collected from the reaction medium (60 μL) at 0, 2, 5, 10, 15 and 30 min. Aliquot were centrifuged at 14,000 × *g* (15 s at 4 °C), and the supernatant was stored at −20 °C for HPLC quantification of adenosine (Carmo et al., 2019; Cunha, 2001). The remaining synaptosomes were pelleted and homogenized in 2% (*v*/v) Triton X-100, to quantify protein with the BCA assay.

2.7. Behavioral analysis

Behavior was evaluated as previously described (Kaster et al., 2015). Locomotion was first monitored in an open-field apparatus. Recognition memory was measured with the object recognition test, using the same arena in a room (8 lx) with visual cues, where mice were first exposed to two identical objects for 3 min and 90 min later to a familiar and a novel object for 3 min. Hippocampal-dependent spatial reference memory was assessed using the object displacement test, carried out as the object recognition test, but where one of the objects switched positions in the second session. Hippocampal-dependent

spatial reference memory was further assessed using a modified version of the Y maze test where we scored the number of entries and the time spent in the novel arm as a measure of spatial memory in a second 8 min visit to the maze 90 min after a first 8 min visit where one of the arms was blocked.

2.8. Electrophysiological recordings

Recordings were performed as described previously (Kaster et al., 2015; Costenla et al., 2011). Briefly, a stimulating electrode was placed onto Schaffer fibers, and the evoked field excitatory postsynaptic potentials (fEPSP) were recorded through an extracellular microelectrode (4 M NaCl; 1–2 MΩ) placed in the CA1 *stratum radiatum*. The recordings were analyzed using WinLTP (Anderson & Collingridge, 2001). Long-term potentiation (LTP) was induced by high-frequency stimulation (one train of 100 pulses of 1 Hz for 1 s). LTP was quantified as the percentage change between two values: the average slope of the ten potentials taken between 50 and 60 min after LTP induction in relation to the average slope of the fEPSP measured during the 10 min that preceded LTP induction.

2.9. Statistics

In all experimental procedures, three or more animals were used for each parameter analyzed and the individual sample sizes (n = number of animals) is specified for each experiment. All data are presented as mean ± SEM, and significance was considered at $P < .05$ using Student's *t*-test for comparison between two groups and one-way ANOVA (followed by a Bonferroni's post hoc test) or two-way ANOVA (followed by a Newman-Keuls post hoc test) for comparison of multiple groups.

2.10. Study approval

Animal procedures followed the European Community guidelines (Directive 2010/63/EU) and were approved by the Ethical Committee of the Center for Neuroscience and Cell Biology of Coimbra (ORBEA-138-2016).

3. Results

3.1. CD73 activity is required for the control of $A_{2A}R$-dependent hippocampal long-term potentiation

We first confirmed that α,β-methylene ADP (AOPCP, 100 μM) effectively inhibited CD73 activity. Indeed, HPLC analysis showed that AOPCP blunted extracellular adenosine formation upon addition of AMP (10 μM) to mouse hippocampal synaptosomes (Fig. 1a). This AMP-derived adenosine formation in hippocampal synapses only involved the activity of CD73, since there was no formation of extracellular adenosine upon AMP addition to hippocampal synaptosomes from CD73 knockout (KO) mice (Fig. 1b). In hippocampal slices, AOPCP (100 μM) did not affect basal excitatory synaptic transmission (104.7 ± 4.729% modification of fEPSP; $n = 5$, $t_4 = 1.067$, $p = .346$ vs 100%, unpaired *t*-test) in Schaffer fiber-CA1 pyramid synapses (Fig. 1c), excluding an association of ATP-derived adenosine with tonic A_1R activation (Lopes et al., 2019; Costenla et al., 2011). In contrast, AOPCP decreased LTP amplitude (from 54.7 ± 4.9% over baseline without AOPCP to 29.8 ± 7.9% over baseline with AOPCP; $n = 5$, $t_{11} = 4.622$, $p = .0007$, unpaired *t*-test) in slices from wild type mice (WT) (Fig. 1d–f), whereas AOPCP was devoid of effects on LTP amplitude in hippocampal slices from CD73-KO mice (Fig. 1d,g–h). Importantly, we had previously shown that CD73-KO mice have similar densities of A_1R and $A_{2A}R$ (Augusto et al., 2013).

The $A_{2A}R$ antagonist SCH58261 (50 nM) phenocopied the effect of AOPCP on hippocampal LTP (65.8 ± 6.7% without, 33.5 ± 11.2% with SCH58261, $n = 5$, $F_{2,14} = 4.007$, $p = .042$, one-way ANOVA followed by Bonferroni's post hoc test; Fig. 2a,c). Most importantly, the effect of AOPCP on LTP was occluded (i.e. prevented) if $A_{2A}R$ were previously blocked with SCH58261 (Fig. 2b–c). Furthermore, AOPCP was devoid of effects in hippocampal slices from global $A_{2A}R$-KO mice (Fig. 2d–e) and the impact of SCH58261 on LTP was lost in hippocampal slices from CD73-KO mice (data not shown) suggesting that CD73-derived adenosine is required for the control of hippocampal LTP by $A_{2A}R$. It is worth noting that the similar impact on LTP of the pharmacological inhibition of CD73 and $A_{2A}R$ is not recapitulated in the transgenic CD73-KO and global $A_{2A}R$-KO mice, both of which do not display alterations of LTP amplitude, possibly because of the emergence of similar compensatory alterations upon elimination of either CD73 or $A_{2A}R$ since conception.

3.2. ATP-derived adenosine selectively activates neuronal $A_{2A}R$ to modulate long-term potentiation

Since both astrocytic (Matos et al., 2015; Orr et al., 2015) and neuronal $A_{2A}R$ (Viana da Silva et al., 2016; Li et al., 2015) control memory and synaptic plasticity, we used mice with $A_{2A}R$ deletions either in forebrain neurons (fb-$A_{2A}R$-KO) or in astrocytes (GFAP-$A_{2A}R$-KO) to test which $A_{2A}R$ population was targeted by ATP-derived adenosine. AOPCP did not affect LTP in hippocampal slices from fb-$A_{2A}R$-KO (44.4 ± 9.4% without, 40.0 ± 10.8% with AOPCP, $t_{17} = 0.296$, $p = .771$, unpaired *t*-test; Fig. 2f–g), similarly to what was recorded upon pharmacological inhibition with SCH58261 (Fig. 2b–c) and in global $A_{2A}R$-KO mice (Fig. 2d–e), but maintained its effect on LTP magnitude in GFAP-$A_{2A}R$-KO (58.7 ± 13.6% without, 18.1 ± 5.3% with AOPCP, $n = 6$, $t_6 = 2.774$, $p = .032$, unpaired *t*-test; Fig. 2h–i) indicating that CD73-derived adenosine selectively modulates LTP through the action of neuronal rather than astrocytic $A_{2A}R$.

3.3. Up-regulation of the ATP-CD73-$A_{2A}R$ pathway in a mouse model of early AD based on Aβ administration

Since $A_{2A}R$ overactivation triggers deficits of synaptic plasticity and memory in early AD (Canas et al., 2009; Laurent et al., 2016; Viana da Silva et al., 2016), we posited that this results from a combined increased formation of ATP-derived adenosine and $A_{2A}R$ upregulation. To model early AD conditions, we administered intracerebroventricularly (icv) oligomeric β-amyloid ($Aβ_{1–42}$), a synaptotoxic peptide proposed as a culprit of AD (Ferreira & Klein, 2011).

Hippocampal synaptosomes from $Aβ_{1-42}$ (2 nmol, icv)-treated mice displayed: i) larger ATP release upon K^+-depolarization (45.8 ± 5.1% over baseline after $Aβ_{1-42}$ vs. 32.0 ± 3.9% over baseline in control, $n = 12$, $t_{20} = 2.150$, $p = .044$, unpaired *t*-test; Fig. 3a); ii) increased CD73 density (244.2 ± 69.3% larger immunodensity in $Aβ_{1-42}$; $n = 3$, $t_5 = 3.525$, $p = .017$ vs. control, unpaired *t*-test; Fig. 3b); iii) increased CD73 activity (AMP extracellular catabolism of 1.054 ± 0.056 in $Aβ_{1-42}$ vs. 0.507 ± 0.018 nmol/min/mg protein in control, $n = 5$, $t_{10} = 9.361$, $p = .008$, unpaired *t*-test; Fig. 3c); and iv) increased $A_{2A}R$ density (specific ^{3}H-SCH58261 binding of 80.5 ± 4.8 in $Aβ_{1-42}$ vs. 46.7 ± 8.1 fmol/mg protein in control, $n = 3$, $t_4 = 3.591$, $p = .023$, unpaired *t*-test; Fig. 3d). This tentatively confirms our hypothesis that excessive formation of ATP-derived extracellular adenosine coupled to $A_{2A}R$ up-regulation might both be critical factors responsible for the onset of early AD alterations. It is worth mentioning that these modifications might be mostly confined to synapses: in fact, when assessing total hippocampal membranes, there was no significant difference of $A_{2A}R$ density between vehicle- and $Aβ_{1-42}$-treated mice (specific ^{3}H-SCH58261 binding of 21.6 ± 2.4 in $Aβ_{1-42}$ vs. 25.3 ± 1.8 fmol/mg protein in control, $n = 5$, $t_8 = 1.214$, $p = .259$, unpaired *t*-test).

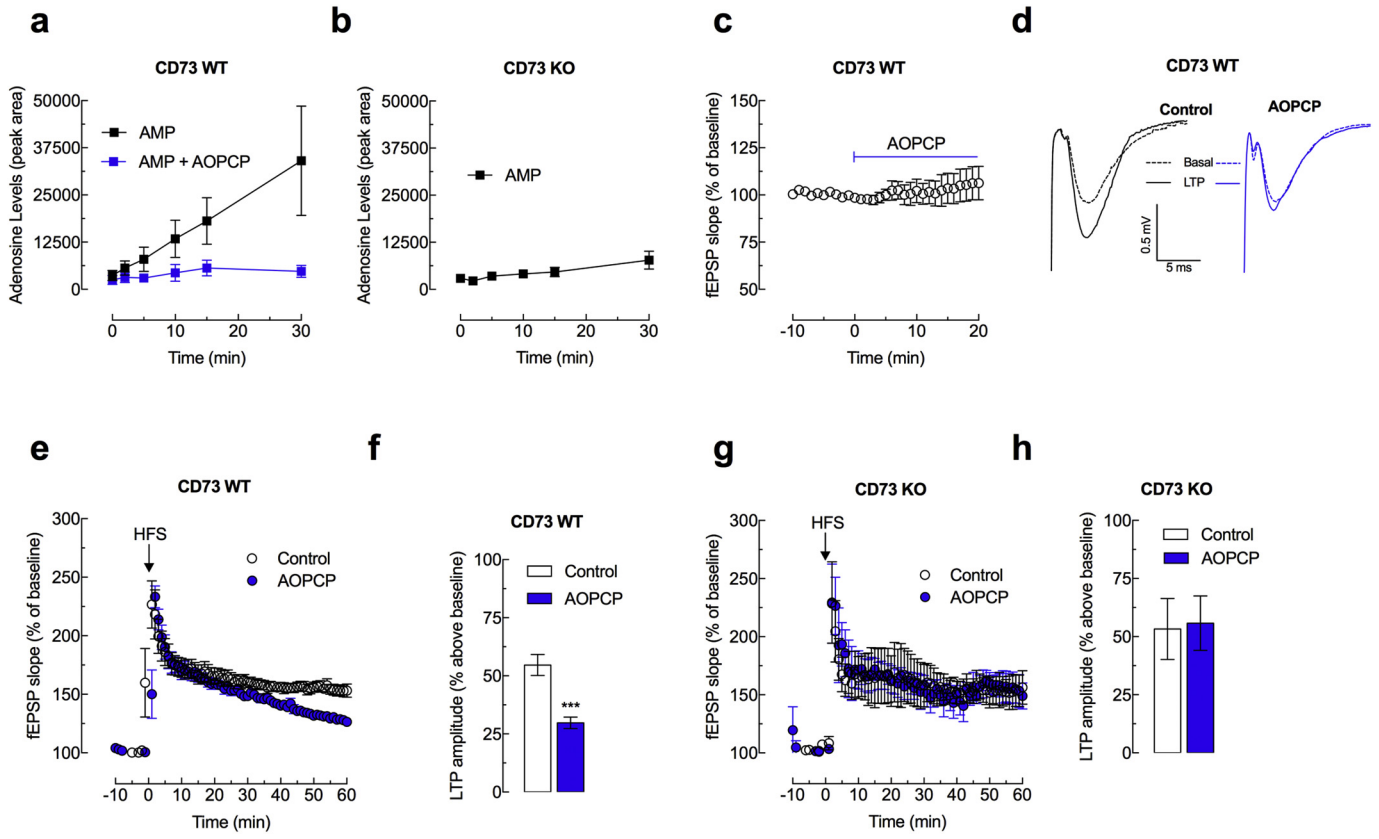


Fig. 1. ATP-derived extracellular adenosine formed by CD73 controls LTP in the mouse hippocampus.
(a) The formation of extracellular adenosine after addition of 10 μM AMP to mouse hippocampal synaptosomes was prevented by 100 μM AOPCP. (b) AOPCP selectively inhibits CD73 since in CD73-KO mice there was no extracellular adenosine formation upon addition of AMP and AOPCP is devoid of effects (not shown). (c) AOPCP (100 μM) did not affect basal excitatory synaptic transmission in Schaffer fiber-CA1 pyramid synapses in mouse hippocampal slices; (d) fEPSPs recorded in CA1 pyramids before (thinner lines) and 60 min after a high-frequency train (HFS) applied to afferent Schaffer fibers (thicker lines) in the absence (black traces) or presence (blue traces) of 100 μM AOPCP, revealed that AOPCP decreased LTP amplitude, (e) as illustrated in the time course of fEPSP recordings (d) and quantified in (f). The selectivity of AOPCP as a CD73 inhibitor was ensured by the lack of effect of AOPCP on LTP in CD73-KO mice (g, h). Data are mean ± S.E.M of 3 (a–c) and 5 (d–h) experiments (number of different animals tested). *** $p < .001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. CD73 deletion abolishes the behavioral and synaptic deficits in an early AD mouse model

The assumption that AD-related changes in the hippocampus result from a disproportionate formation of extracellular adenosine from released ATP, entails that CD73 elimination should blunt the triad of modifications characteristic of early AD, namely impaired reference memory, synaptic dysfunction and loss of synaptic markers, all recapitulated after $A\beta_{1\text{-}42}$ administration (Walsh & Selkoe, 2004; Ferreira & Klein, 2011). Accordingly, $A\beta_{1\text{-}42}$ administration to WT mice worsened performance in the object displacement test (localization index: 30.5 ± 5.8% vs. vehicle: 62.4 ± 6.8%, $n = 5$–7, $F_{1,19} = 13.1$, $p = .002$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4a), in the object recognition test (recognition index: 39.8 ± 5.3% vs. vehicle: 84.2 ± 3.8%, $n = 5$–7, $F_{1,20} = 9.739$, $p = .005$; Fig. 4b) and in the modified Y-maze test (time in the novel arm: 120.2 ± 13.1 s vs. vehicle: 166.3 ± 8.0 s, $n = 5$–6, $F_{1,19} = 4.414$, $p = .049$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4c) without locomotor alterations (Fig. 4d). By contrast, Aβ-treated CD73-KO mice preserved their localization index (53.0 ± 3.8% vs. vehicle: 62.4 ± 6.8%, $n = 5$–7, $F_{1,19} = 4.296$, $p = .7533$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4a), their recognition index (74.4 ± 7.6% vs. vehicle: 72.0 ± 7.7%, $n = 5$–7, $F_{1,20} = 0.752$, $p = .8698$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4b) and their time in the novel arm (161.8 ± 6.3 s vs. vehicle: 157.1 ± 14.8 s, $n = 5$–6, $F_{1,19} = 2.772$, $p = .9888$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4c) without locomotor alterations (Fig. 4d).

Synaptic dysfunction is at the core of initial changes in early AD (Walsh & Selkoe, 2004; Ferreira & Klein, 2011), as heralded by the decrease of synaptic markers two weeks after exposure to Aβ, in the absence of overt neuronal loss (Canas et al., 2009). Hippocampal synaptosomes from Aβ-treated WT mice had a lower density of SNAP25 (65.5 ± 7.3%, $n = 3$, $F_{1,8} = 20.08$, p = .002, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4e), synaptophysin (62.5 ± 4.3%, $n = 3$, $F_{1,8} = 13.39$, $p = .006$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4f) and PSD95 (data not shown). In contrast, CD73 genetic deletion prevented the loss of synaptic markers (SNAP25: 94.6 ± 2.9%, $n = 3$, $F_{1,8} = 10.73$, $p = .9615$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4e; synaptophysin: 102.3 ± 8.9%, $n = 3$, $F_{1,8} = 13.39$, $p = .9999$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4f). It is not only synaptotoxicity but also synaptic dysfunction that occurs in early AD (Walsh & Selkoe, 2004; Ferreira & Klein, 2011). Accordingly, hippocampal slices from Aβ-treated WT mice displayed a lower LTP amplitude (30.9 ± 6.2% vs. vehicle: 63.5 ± 5.1%, $n = 5$–7, $F_{1,21} = 6.284$, $p = .021$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4g–h), whereas this did not occur for Aβ-treated CD73-KO mice (LTP in Aβ-treated: 63.0 ± 9.4% vs. vehicle: 63.7 ± 7.7%, $n = 5$–7, $F_{1,21} = 9.925$, $p = .9613$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4g–h). Notably, the acute pharmacological inhibition during the slice experiment of either CD73

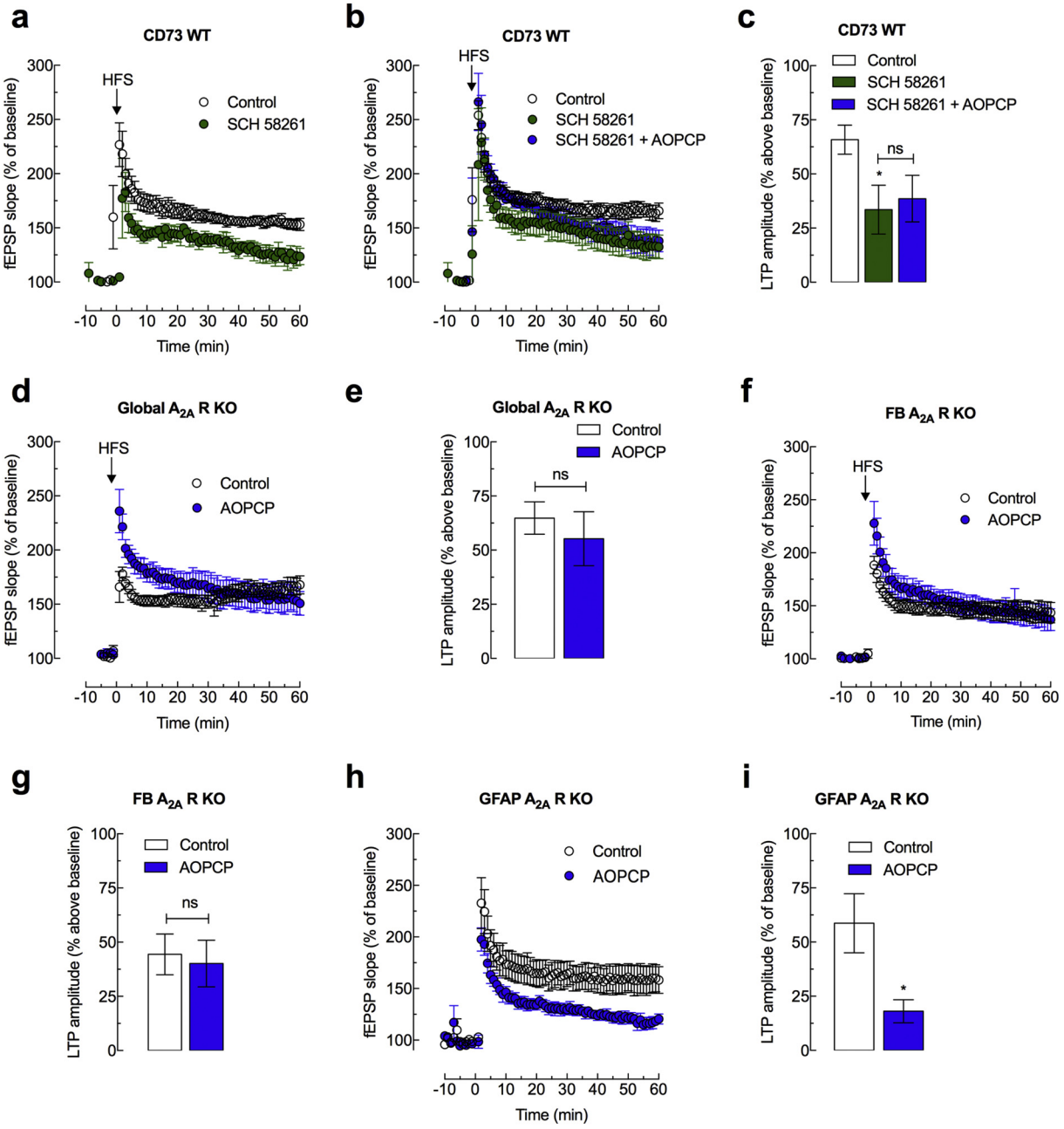


Fig. 2. ATP-derived extracellular adenosine formed by CD73 selectively activates neuronal $A_{2A}R$ to control LTP in the mouse hippocampus.
(a) fEPSPs recorded in Schaffer fiber-CA1 pyramid synapses from WT mouse hippocampus after a high-frequency train (HFS) indicate that the blockade of $A_{2A}R$ with 50 nM SCH58261 phenocopies the effects of AOPCP on hippocampal LTP. Furthermore, AOPCP is devoid of effects in the presence of SCH58261 (b, c) or in global $A_{2A}R$-KO mice (d, e). The effects of AOPCP are blunted in fb-$A_{2A}R$-KO mice (f, g) but preserved in GFAP-$A_{2A}R$-KO mice (h,i). Data are mean ± S.E.M of 6 experiments (number of different animals tested). * $p < .05$.

or $A_{2A}R$ reverted Aβ-induced LTP deficits (Fig. 4i–l): thus, hippocampal slices from Aβ-treated mice exposed to 100 μM AOPCP recovered their LTP (24.8 ± 6.7% without, 48.8 ± 7.6% with AOPCP, $n = 5$, $F_{1,15} = 17.69$, $p = .0008$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4i–j) to values similar to vehicle-treated mice (Fig. 4i–j). As previously reported in different hippocampal synapses (Viana da Silva et al., 2016), 50 nM SCH58261 also recovered LTP (24.8 ± 6.7% without, 51.9 ± 7.3% with SCH58261, $n = 5$, $F_{1,16} = 14.87$, $p = .001$, two-way ANOVA followed by Newman-Keuls post hoc test; Fig. 4k–l) to values similar to vehicle-treated mice (Fig. 4k–l).

These observations indicate that either genetic or pharmacological elimination of CD73, counteracts the behavioral and synaptic deficits that occur in early AD. This abrogation of CD73 activity phenocopies $A_{2A}R$ elimination, showing that preventing the formation of the endogenous ligand (CD73-derived adenosine) is equi-effective to directly blocking $A_{2A}R$, and reinforcing the association between CD73 and $A_{2A}R$ function in the hippocampus also in pathological conditions.

4. Discussion

The present study shows that CD73 regulates the extracellular catabolism of ATP into adenosine to control neuronal $A_{2A}R$ activation, impacting on hippocampal LTP and memory deterioration. We first established that CD73 is needed to trigger an adequate LTP in the hippocampus, since the blockade of CD73 activity by its inhibitor

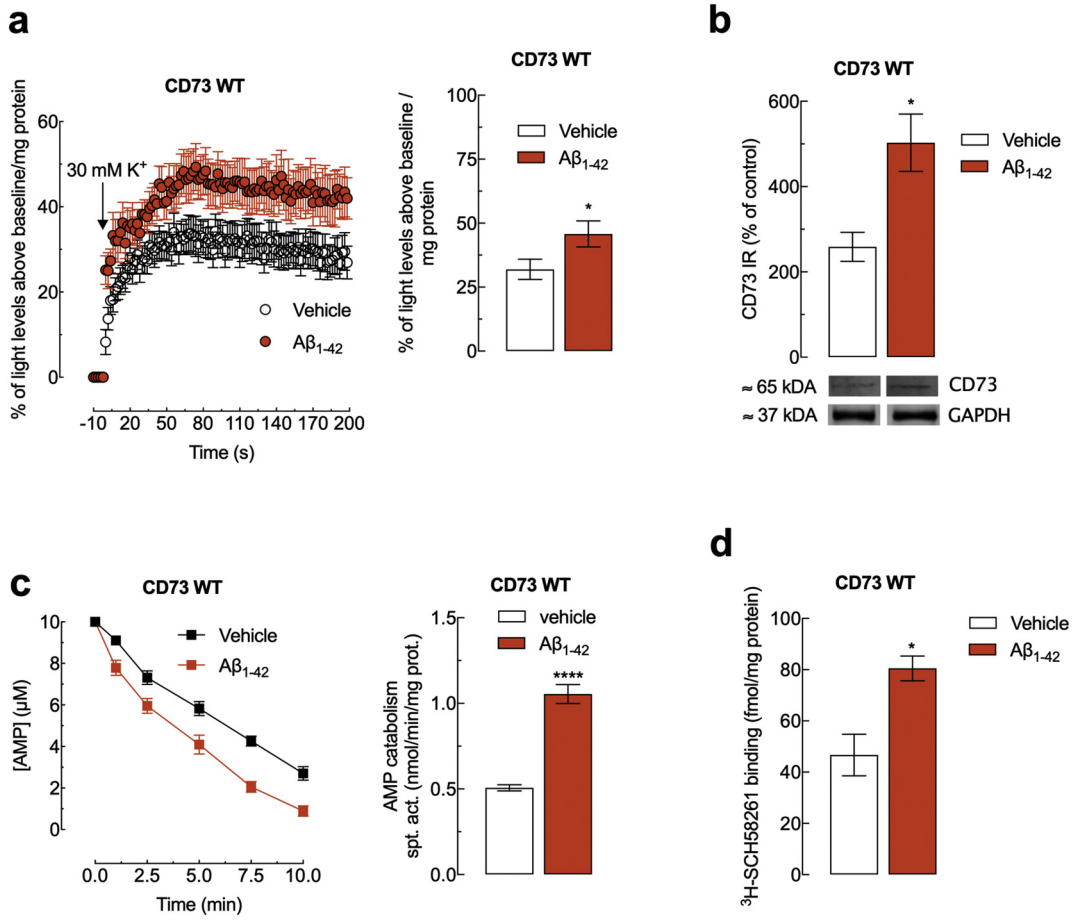
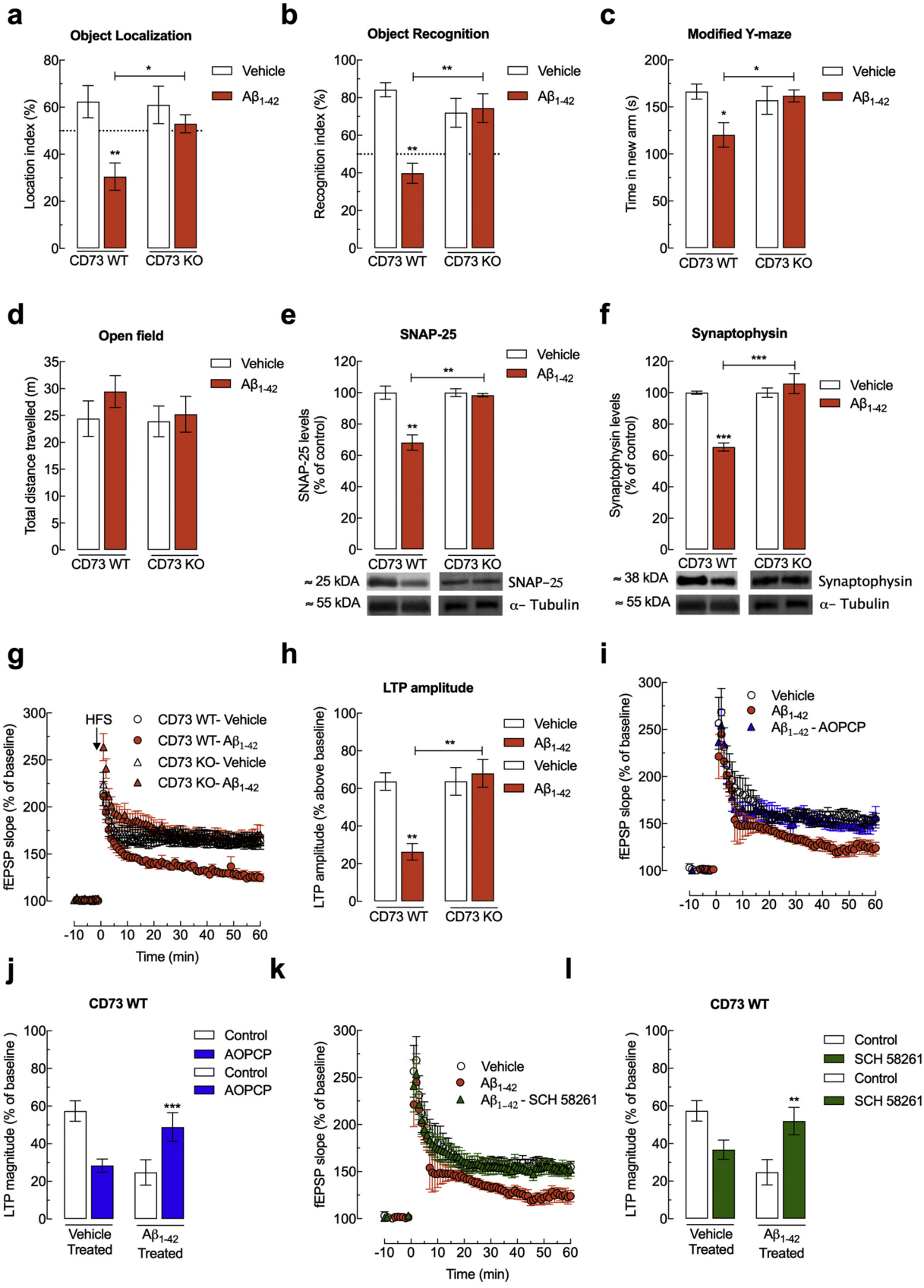


Fig. 3. Increased formation of ATP-derived extracellular adenosine and $A_{2A}R$ upregulation in the hippocampus of an early AD mouse model.
In a mouse model of early AD based on exposure to Aβ (2 nmol, icv, orange) compared to vehicle-treated (black), hippocampal synaptosomes display a luminometrically-detected higher release of ATP upon exposure to 30 mM K^+ (a), (b) a higher Western blot immunoreactivity of CD73, (c) a higher CD73 activity assessed by formation of extracellular adenosine after addition of 10 μM AMP, (d) and a higher $A_{2A}R$ density, assessed by binding density of 6 nM ^{3}H-SCH58261 to hippocampal synaptosomal membranes. Data are mean ± S.E.M of 12 (a), 3 (b), 5 (c) and 3 (d) experiments (number of different animals tested). * $p < .05$, **** $p < .0001$.

AOPCP dampened hippocampal LTP amplitude. The magnitude of this decrease was identical to that observed when $A_{2A}R$ function was abrogated by the antagonist SCH58261. Strikingly, this effect of SCH58261was blunted by the genetic deletion of CD73, indicating that the control of hippocampal LTP by CD73 depends on $A_{2A}R$ function. Indeed, CD73 operates AMP dephosphorylation, the final and rate-limiting step of the extracellular catabolism of ATP into adenosine (Zimmermann et al., 2012; Cunha, 2001), and both the genetic and the pharmacological abrogation of CD73 blunted the production of extracellular adenosine from adenine nucleotides in the hippocampus (Fig. 1; see also Lovatt et al., 2012, Cunha, 2001, Cunha et al., 2000, Kulesskaya et al., 2013). With the activities of other nucleotide-converting ecto-enzymes remaining almost intact in the brain of CD73-KO mice (Cunha, 2001; Kulesskaya et al., 2013), the absence of effects of AOPCP in these animals excludes the involvement of P2 receptors and further supports a tight association between CD73-mediated formation of ATP-derived extracellular adenosine and selective $A_{2A}R$ activation in the hippocampus to control LTP. This is in agreement with previous evidence in mouse striatum showing that CD73-mediated formation of extracellular adenosine is responsible for the activation of $A_{2A}R$ (Augusto et al., 2013; Carmo et al., 2019).

Basal synaptic transmission, a mechanism controlled by adenosine A_1 receptors (A_1R), was also assessed and was not modified upon pharmacological or genetic deletion of CD73 activity. This shows a lack of association of synaptic CD73 activity with A_1R function in physiological conditions (Lovatt et al., 2012; Rebola et al., 2008; Cunha et al., 1996b; Walsh & Selkoe, 2004) as well as upon brain damage (Carmo et al., 2019; Frenguelli et al., 2007; Zhang et al., 2012). In contrast, the pharmacological and genetic elimination of $A_{2A}R$ fully abrogated the effect of CD73 inhibition on LTP, demonstrating that the adenosine generated by the CD73 is fully directed to the control of synaptic plasticity by $A_{2A}R$ in the hippocampus (Fig. 2). Such observations reinforce the previous assumption that A_1R and $A_{2A}R$ activation is ensured by adenosine from different origins (Cunha, 2008). However, $A_{2A}R$ can be found in diverse cell types (Cunha, 2016) and both neuronal and astrocytic $A_{2A}R$ control memory-related functions (Viana da Silva et al., 2016; Matos et al., 2015; Orr et al., 2015; Li et al., 2015). Therefore, it is important to detail if CD73-derived adenosine was specifically targeted towards the activation of a cell-specific $A_{2A}R$ population. The comparison of changes in LTP amplitude in two mouse models where $A_{2A}R$ are selectively eliminated either in forebrain neurons (forebrain $A_{2A}R$-KO) or in astrocytes (GFAP-driven $A_{2A}R$-KO), uncovered that the effect of CD73 pharmacological inhibition was abrogated in forebrain $A_{2A}R$-KO, similarly to what was observed in the pharmacological blockade and full genetic deletion of $A_{2A}R$-KO (global $A_{2A}R$-KO), whereas in hippocampal slices from mice where astrocytic $A_{2A}R$ were eliminated, the effect of AOPCP was maintained. This provides the first evidence that extracellular adenosine generated from ATP catabolism mediated by CD73 is strictly directed towards the activation of neuronal $A_{2A}R$ to control synaptic plasticity.



(caption on next page)

Fig. 4. Genetic deletion of CD73 prevents memory and synaptic impairments in a mouse model of early AD.
CD73-KO mice and wild type (WT) littermates (10 weeks old) were injected icv with $A\beta_{1-42}$ (2 nmol). After 14 days, $A\beta_{1-42}$ decreased memory performance in the object displacement test (a), object recognition test (b) and modified Y-maze (c) in WT but not CD73-KO mice, (d) without locomotor alterations. In the first visit to the Y-maze, mice from all groups spent a similar time in the start arm (WT-vehicle: 243.8 ± 9.8 s; WT-Aβ: 234.2 ± 12.2 s; CD73-KO-vehicle: 242.0 ± 11.0 s; CD73-KO-Aβ: 233.1 ± 9.0 s; n = 5–6) and in the familiar arm (WT-vehicle: 236.2 ± 9.8 s; WT-Aβ: 245.8 ± 12.2 s; CD73-KO-vehicle: 238.0 ± 11.04 s; CD73-KO-Aβ: 246.9 ± 9.0 s; n = 5–6) and the velocity in the open field was also similar in all groups (WT-vehicle: 0.41 ± 0.05 m/s; WT-Aβ: 0.40 ± 0.05 m/s; CD73-KO-vehicle: 0.49 ± 0.05 m/s; CD73-KO-Aβ: 0.42 ± 0.06 m/s; n = 6–7). $A\beta_{1-42}$ also decreased the density of the synaptic proteins SNAP25 (e) and synaptophysin (f) and decreased LTP amplitude in WT but not CD73-KO mice (g,h). This lower LTP in $A\beta_{1-42}$-treated mice was reverted to control values by acutely applying 100 μM AOPCP (i,j) or 50 nM SCH58261 (k,l) directly to slices. Data are mean ± S.E.M of 5–7 (a), 5–7 (b), 5–6 (c), 6–7 (d), 3 (e–f), 5–7 (g–h), 4–5 (i–j) and 5 (k–l) experiments (number of different animals tested). $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

The tight relation between CD73 and $A_{2A}R$ activation in the hippocampus was extended to pathological conditions. This is of particular importance since $A_{2A}R$-KO mice, as now also reported for CD73-KO mice, have no major brain-related phenotype unless when subjected to brain insults (Augusto et al., 2013; Kulesskaya et al., 2013; Zhang et al., 2012). In fact, $A_{2A}R$ blockade affords a robust neuroprotection in different animal models of brain diseases such as Parkinson's or Alzheimer's diseases (AD), epilepsy or ischemia (reviewed in (Cunha, 2016)). As occurs for $A_{2A}R$ (Canas et al., 2009), we now report that the genetic elimination of CD73 prevented the behavioral deficits and synaptotoxicity triggered by the intracerebroventricular administration of $A\beta_{1-42}$ (Fig. 4a–h), a neurotoxic peptide deemed as a culprit of AD (Walsh & Selkoe, 2004; Ferreira et al., 2015). Thus, either blocking $A_{2A}R$ function directly or blocking the source of adenosine responsible for $A_{2A}R$ activation (i.e. CD73) affords similar protection against synaptic and memory dysfunction. This similar neuroprotective phenotype of CD73-KO and $A_{2A}R$-KO mice further strengthens the functional association between CD73 and $A_{2A}R$ in the hippocampus, extending the ability of CD73 to produce the extracellular adenosine pool selectively responsible for the activation of hippocampal $A_{2A}R$, to a pathological context of memory dysfunction. Notably, $A_{2A}R$ overactivation is sufficient to trigger memory deficits (Pagnussat et al., 2015; Li et al., 2015) and is critically necessary for the expression of memory deficits in early AD models (Canas et al., 2009; Laurent et al., 2016; Viana da Silva et al., 2016). $A_{2A}R$ overfunction is associated with increased $A_{2A}R$ density upon exposure to Aβ (Fig. 3d), first in neurons (Viana da Silva et al., 2016; Temido-Ferreira et al., 2018; Silva et al., 2018) and probably later in astrocytes (Orr et al., 2015; Faivre et al., 2018), as occurs in other AD models (Viana da Silva et al., 2016; Espinosa et al., 2013) as well as in cortical areas of AD patients (Temido-Ferreira et al., 2018; Ji et al., 1992; Albasanz et al., 2008). We now report a parallel up-regulation of CD73 and $A_{2A}R$ after Aβ exposure, as also occurs upon other brain insults (Carmo et al., 2019; Canas et al., 2009; Viana da Silva et al., 2016; Kaster et al., 2015; Temido-Ferreira et al., 2018; Braun et al., 1998; Fontella et al., 2004) or aging (Cunha et al., 2001), which reinforces the association of CD73 with sites of altered synaptic plasticity (Lie et al., 1999). However, the time course of the up-regulation of CD73 and $A_{2A}R$ in relation to the onset of neurodegeneration still remains to be established. Neurochemical studies showed that Aβ exposure triggered an enhancement of the evoked extracellular ATP levels (Fig. 3a), and an increased activity of CD73 (Fig. 3c). This provides a new comprehensive scenario to understand the critical role of $A_{2A}R$ in early AD (Canas et al., 2009; Laurent et al., 2016; Viana da Silva et al., 2016), which depends on a simultaneous increased of $A_{2A}R$ density and of ATP-derived extracellular adenosine levels to sustain $A_{2A}R$ overfunction. However, the mechanism operated by $A_{2A}R$ to control synaptic plasticity still awaits to be clarified; this may not be a trivial task since $A_{2A}R$ are pleiotropic receptors engaging numerous transducing systems (reviewed in (Cunha, 2016)). This complexicity is best heralded by the observation that $A_{2A}R$ blockade decreased LTP amplitude in physiological conditions, whereas it increased LTP amplitude after Aβ exposure as well as after different brain insults (Viana da Silva et al., 2016; Kaster et al., 2015; Ferreira et al., 2017; Canas et al., 2018), in accordance with the described shift of transducing system operated by $A_{2A}R$ upon their upregulation by noxious conditions: in fact, whereas hippocampal $A_{2A}R$ may essencially recruit cAMP-protein kinase A-CREB pathway under control conditions (Li et al., 2015; Rebola et al., 2002), this transducing pathway is not associated with $A_{2A}R$-mediated neuroprotection (Canas et al., 2009), probably as a consequence of the ability of increased glutamate levels characteristic of neurodegenerative conditions (Lipton & Rosenberg, 1994), to redirect $A_{2A}R$ signaling from the protein kinase A to the protein kinase C pathway (Dai et al., 2010).

Our results provide the first demonstration that a purported ancillary neuromodulation system operated by purines actually has a critical impact on adequate synaptic plasticity that translates into synaptic and memory dysfunction in a model of early AD. In fact, our results show that irrespective of changes in the morphology or glutamatergic setup of excitatory synapses and of the numerous proposed targets for Aβ (Ferreira & Klein, 2011) that may become relevant in the progression rather than onset of AD, it is the overfunctioning of the purinergic system, namely the increased formation of ATP-derived adenosine funneled into $A_{2A}R$ activation that critically disrupts synaptic and memory dysfunction in a mouse model of early AD. Consequently, our work suggests CD73 as a possible novel target for the regulation of $A_{2A}R$ function, with a potential therapeutic application in the prevention/reversion of brain disorders. The next challenge is to understand how $A_{2A}R$ shift their unknown transducing system(s) to bolster synaptic plasticity in naïve mice and disrupt LTP in injured synapses, a phenocopy of NMDA receptors, which are critical to enable physiological LTP, but are paramount to trigger neurodegeneration.

Funding and disclosure

Supported by Santa Casa da Misericórdia, Prémio Maratona da Saúde, and ERDF through Centro 2020 (projects CENTRO-01-0246-FEDER-000010 and CENTRO-01-0145-FEDER-000008:BrainHealth 2020) and through FCT (projects PTDC/NEU-NMC/4154 and PTDC/MED-NEU/31274/2017).

R.A.C. is a scientific advisor of the Institute for Scientific Information on Coffee (ISIC). All other authors declare no competing financial interests.

Acknowledgments

We thank Jiang-Fan Chen for providing CD73-KO and forebrain $A_{2A}R$-KO lines and Detlev Boison for supplying GFAP-driven $A_{2A}R$-KO mice.

References

Albasanz, J.L., Perez, S., Barrachina, M., Ferrer, I., Martín, M., 2008. Up-regulation of adenosine receptors in the frontal cortex in Alzheimer's disease. Brain Pathol. 18 (2), 211–219.

Anderson, W.W., Collingridge, G.L., 2001. The LTP program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. J. Neurosci. Methods 108, 71–83.

Augusto, E., Matos, M., Sévigny, J., El-Tayeb, A., Bynoe, M.S., Müller, C.E., et al., 2013. Ecto-5′-nucleotidase (CD73)-mediated formation of adenosine is critical for the striatal adenosine A_{2A} receptor functions. J. Neurosci. 33 (28), 11390–11399.

Barros-Barbosa, A.R., Ferreirinha, F., Oliveira, A., Mendes, M., Lobo, M.G., Santos, A., et al., 2016. Adenosine A_{2A} receptor and ecto-5′-nucleotidase/CD73 are upregulated

in hippocampal astrocytes of human patients with mesial temporal lobe epilepsy (MTLE). Purinergic Signal 12 (4), 719–734.
Blaustein, M.P., 1975. Effects of potassium, veratridine, and scorpion venom on calcium accumulation and transmitter release by nerve terminals in vitro. J. Physiol. 247 (3), 617–655.
Borea, P.A., Gessi, S., Merighi, S., Vincenzi, F., Varani, K., 2018. Pharmacology of adenosine receptors: the state of the art. Physiol. Rev. 98 (3), 1591–1625.
Bradford, H.F., Bennett, G.W., Thomas, A.J., 1973. Depolarizing stimuli and the release of physiologically active amino acids from suspensions of mammalian synaptosomes. J. Neurochem. 21 (3), 495–505.
Braun, N., Zhu, Y., Krieglstein, J., Culmsee, C., Zimmermann, H., 1998. Upregulation of the enzyme chain hydrolyzing extracellular ATP after transient forebrain ischemia in the rat. J. Neurosci. 18 (13), 4891–4900.
Burnstock, G., 2014. Purinergic signalling: from discovery to current developments. Exp. Physiol. 99 (1), 16–34.
Canas, P.M., Porciúncula, L.O., Cunha, G.M., Silva, C.G., Machado, N.J., Oliveira, J.M., et al., 2009. Adenosine A_{2A} receptor blockade prevents synaptotoxicity and memory dysfunction caused by β-amyloid peptides via p38 mitogen-activated protein kinase pathway. J. Neurosci. 29 (47), 14741–14751.
Canas, P.M., Simões, A.P., Rodrigues, R.J., Cunha, R.A., 2014. Predominant loss of glutamatergic terminal markers in a β-amyloid peptide model of Alzheimer's disease. Neuropharmacology. 76, 51–56.
Canas, P.M., Porciúncula, L.O., Simões, A.P., Augusto, E., Silva, H.B., Machado, N.J., et al., 2018. Neuronal adenosine A_{2A} receptors are critical mediators of neurodegeneration triggered by convulsions. *eNeuro*. 5 (6), ENEURO.0385-18.2018.
Carmo, M., Gonçalves, F.Q., Canas, P.M., Oses, J.P., Fernandes, F.D., Duarte, F.V., et al., 2019. Enhanced ATP release and CD73-mediated adenosine formation sustain adenosine A_{2A} receptor over-activation in a rat model of Parkinson's disease. Br. J. Pharmacol. https://doi.org/10.1111/bph.14771.
Costenla, A.R., Diógenes, M.J., Canas, P.M., Rodrigues, R.J., Nogueira, C., Maroco, J., et al., 2011. Enhanced role of adenosine A_{2A} receptors in the modulation of LTP in the rat hippocampus upon ageing. Eur. J. Neurosci. 34 (1), 12–21.
Cunha, R.A., 2001. Regulation of the ecto-nucleotidase pathway in rat hippocampal nerve terminals. Neurochem. Res. 26 (8–9), 979–991.
Cunha, R.A., 2008. Different cellular sources and different roles of adenosine: A_1 receptor-mediated inhibition through astrocytic-driven volume transmission and synapse-restricted A_{2A} receptor-mediated facilitation of plasticity. Neurochem. Int. 52 (1–2), 65–72.
Cunha, R.A., 2016. How does adenosine control neuronal dysfunction and neurodegeneration? J. Neurochem. 139 (6), 1019–1055.
Cunha, R.A., Agostinho, P.M., 2010. Chronic caffeine consumption prevents memory disturbance in different animal models of memory decline. J. Alzheimers Dis. 20, 95–116 Suppl.1.
Cunha, R.A., Vizi, E.S., Ribeiro, J.A., Sebastião, A.M., 1996a. 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 (5), 2180–2187.
Cunha, R.A., Correia-de-Sá, P., Sebastião, A.M., Ribeiro, J.A., 1996b. Preferential activation of excitatory adenosine receptors at rat hippocampal and neuromuscular synapses by adenosine formed from released adenine nucleotides. Br. J. Pharmacol. 119 (2), 253–260.
Cunha, R.A., Brendel, P., Zimmermann, H., Ribeiro, J.A., 2000. Immunologically distinct isoforms of ecto-5′-nucleotidase in nerve terminals of different areas of the rat hippocampus. J. Neurochem. 74 (1), 334–338.
Cunha, R.A., Almeida, T., Ribeiro, J.A., 2001. Parallel modification of adenosine extracellular metabolism and modulatory action in the hippocampus of aged rats. J. Neurochem. 76 (2), 372–382.
Dai, S.S., Zhou, Y.G., Li, W., An, J.H., Li, P., Yang, N., et al., 2010. Local glutamate level dictates adenosine A_{2A} receptor regulation of neuroinflammation and traumatic brain injury. J. Neurosci. 30 (16), 5802–5810.
Espinosa, J., Rocha, A., Nunes, F., Costa, M.S., Schein, V., Kazlauckas, V., et al., 2013. Caffeine consumption prevents memory impairment, neuronal damage, and adenosine A_{2A} receptors upregulation in the hippocampus of a rat model of sporadic dementia. J. Alzheimers Dis. 34 (2), 509–518.
Faivre, E., Coelho, J.E., Zornbach, K., Malik, E., Baqi, Y., Schneider, M., et al., 2018. Beneficial effect of a selective adenosine A_{2A} receptor antagonist in the APPswe/PS1dE9 mouse model of Alzheimer's disease. Front. Mol. Neurosci. 11, 235.
Ferreira, S.T., Klein, W.L., 2011. The Aβ oligomer hypothesis for synapse failure and memory loss in Alzheimer's disease. Neurobiol. Learn. Mem. 96 (4), 529–543.
Ferreira, S.G., Gonçalves, F.Q., Marques, J.M., Tomé, Â.R., Rodrigues, R.J., Nunes-Correia, I., et al., 2015. Presynaptic adenosine A_{2A} receptors dampen cannabinoid CB1 receptor-mediated inhibition of corticostriatal glutamatergic transmission. Br. J. Pharmacol. 172 (4), 1074–1086.
Ferreira, D.G., Batalha, V.L., Vicente Miranda, H., Coelho, J.E., Gomes, R., Gonçalves, F.Q., et al., 2017. Adenosine A_{2A} receptors modulate α-synuclein aggregation and toxicity. Cereb. Cortex 27 (1), 718–730.
Fontella, F.U., Bruno, A.N., Crema, L.M., Battastini, A.M., Sarkis, J.J., Netto, C.A., et al., 2004. Acute and chronic stress alter ecto-nucleotidase activities in synaptosomes from the rat hippocampus. Pharmacol. Biochem. Behav. 78 (2), 341–347.
Fredholm, B.B., Chen, J.F., Cunha, R.A., Svenningsson, P., Vaugeois, J.M., 2005. Adenosine and brain function. Int. Rev. Neurobiol. 63, 191–270.
Frenguelli, B.G., Wigmore, G., Llaudet, E., Dale, N., 2007. Temporal and mechanistic dissociation of ATP and adenosine release during ischaemia in the mammalian hippocampus. J. Neurochem. 101 (5), 1400–1413.
Hong, S., Beja-Glasser, V.F., Nfonoyim, B.M., Frouin, A., Li, S., Ramakrishnan, S., et al., 2016. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Science. 352 (6286), 712–716.
Ji, X.D., Stiles, G.L., van Galen, P.J., Jacobson, K.A., 1992. Characterization of human striatal A_2-adenosine receptors using radioligand binding and photoaffinity labeling. J. Recept. Res. 12 (2), 149–169.
Kaster, M.P., Machado, N.J., Silva, H.B., Nunes, A., Ardais, A.P., Santana, M., et al., 2015. Caffeine acts through neuronal adenosine A_{2A} receptors to prevent mood and memory dysfunction triggered by chronic stress. Proc. Natl. Acad. Sci. U. S. A. 112 (25), 7833–7838.
Kerkhofs, A., Canas, P.M., Timmerman, A.J., Heistek, T.S., Real, J.I., Xavier, C., et al., 2018. Adenosine A_{2A} receptors control glutamatergic synaptic plasticity in fast spiking interneurons of the prefrontal cortex. Front. Pharmacol. 9, 133.
Kim, H.Y., Lee, D.K., Chung, B.R., Kim, H.V., Kim, Y., 2016. Intracerebroventricular injection of amyloid-β peptides in normal mice to acutely induce alzheimer-like cognitive deficits. J. Vis. Exp.(109). https://doi.org/10.3791/53308.
Kulesskaya, N., Võikar, V., Peltola, M., Yegutkin, G.G., Salmi, M., Jalkanen, S., et al., 2013. CD73 is a major regulator of adenosinergic signalling in mouse brain. PLoS One 8 (6), e66896.
Laurent, C., Eddarkaoui, S., Derisbourg, M., Leboucher, A., Demeyer, D., Carrier, S., et al., 2014. Beneficial effects of caffeine in a transgenic model of Alzheimer's disease-like tau pathology. Neurobiol. Aging 35 (9), 2079–2090.
Laurent, C., Burnouf, S., Ferry, B., Batalha, V.L., Coelho, J.E., Baqi, Y., et al., 2016. A_{2A} adenosine receptor deletion is protective in a mouse model of Tauopathy. Mol. Psychiatry 21 (1), 97–107.
Li, P., Rial, D., Canas, P.M., Yoo, J.H., Li, W., Zhou, X., et al., 2015. Optogenetic activation of intracellular adenosine A_{2A} receptor signaling in hippocampus is sufficient to trigger CREB phosphorylation and impair memory. Mol. Psychiatry 20 (11), 1339–1349.
Lie, A.A., Blümcke, I., Beck, H., Wiestler, O.D., Elger, C.E., Schoen, S.W., et al., 1999. 5'-Nucleotidase activity indicates sites of synaptic plasticity and reactive synaptogenesis in the human brain. J. Neuropathol. Exp. Neurol. 58 (5), 451–458.
Lipton, S.A., Rosenberg, P.A., 1994. Excitatory amino acids as a final common pathway for neurologic disorders. N. Engl. J. Med. 330 (9), 613–622.
Lopes, L.V., Halldner, L., Rebola, N., Johansson, B., Ledent, C., Chen, J.F., et al., 2004. Binding of the prototypical adenosine A_{2A} receptor agonist CGS 21680 to the cerebral cortex of adenosine A_1 and A_{2A} receptor knockout mice. Br. J. Pharmacol. 141 (6), 1006–1014.
Lopes, J.P., Pliássova, A., Cunha, R.A., 2019. The physiological effects of caffeine on synaptic transmission and plasticity in the mouse hippocampus selectively depend on adenosine A_1 and A_{2A} receptors. Biochem. Pharmacol. 166, 313–321.
Lovatt, D., Xu, Q., Liu, W., Takano, T., Smith, N.A., Schnermann, J., et al., 2012. Neuronal adenosine release, and not astrocytic ATP release, mediates feedback inhibition of excitatory activity. Proc. Natl. Acad. Sci. U. S. A. 109 (16), 6265–6270.
Matos, M., Shen, H.Y., Augusto, E., Wang, Y., Wei, C.J., Wang, Y.T., et al., 2015. Deletion of adenosine A_{2A} receptors from astrocytes disrupts glutamate homeostasis leading to psychomotor and cognitive impairment: relevance to schizophrenia. Biol. Psychiatry 78 (11), 763–774.
Orr, A.G., Hsiao, E.C., Wang, M.M., Ho, K., Kim, D.H., Wang, X., et al., 2015. Astrocytic adenosine receptor A_{2A} and G_s-coupled signaling regulate memory. Nat. Neurosci. 18 (3), 423–434.
Pagnussat, N., Almeida, A.S., Marques, D.M., Nunes, F., Chenet, G.C., Botton, P.H., et al., 2015. Adenosine A_{2A} receptors are necessary and sufficient to trigger memory impairment in adult mice. Br. J. Pharmacol. 172 (15), 3831–3845.
Pascual, O., Casper, K.B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J.Y., et al., 2005. Astrocytic purinergic signaling coordinates synaptic networks. Science. 310 (5745), 113–116.
Rebola, N., Oliveira, C.R., Cunha, R.A., 2002. Transducing system operated by adenosine A_{2A} receptors to facilitate acetylcholine release in the rat hippocampus. Eur. J. Pharmacol. 454 (1), 31–38.
Rebola, N., Lujan, R., Cunha, R.A., Mulle, C., 2008. Adenosine A_{2A} receptors are essential for long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses. Neuron. 57 (1), 121–134.
Resende, R., Ferreiro, E., Pereira, C., Resende de Oliveira, C., 2008. Neurotoxic effect of oligomeric and fibrillar species of amyloid-beta peptide 1-42: involvement of endoplasmic reticulum calcium release in oligomer-induced cell death. Neuroscience. 155 (3), 725–737.
Rodrigues, R.J., Tomé, A.R., Cunha, R.A., 2015. ATP as a multi-target danger signal in the brain. Front. Neurosci. 9, 148.
Santos, V.V., Stark, R., Rial, D., Silva, H.B., Bayliss, J.A., Lemus, M.B., et al., 2017. Acyl ghrelin improves cognition, synaptic plasticity deficits and neuroinflammation following amyloid β ($A\beta_{1\text{-}40}$) administration in mice. J. Neuroendocrinol. 29 (5). https://doi.org/10.1111/jne.12476.
Silva, A.C., Lemos, C., Gonçalves, F.Q., Pliássova, A.V., Machado, N.J., Silva, H.B., et al., 2018. Blockade of adenosine A_{2A} receptors recovers early deficits of memory and plasticity in the triple transgenic mouse model of Alzheimer's disease. Neurobiol. Dis. 117, 72–81.
Simões, A.P., Machado, N.J., Gonçalves, N., Kaster, M.P., Simões, A.T., Nunes, A., et al., 2016. Adenosine A_{2A} receptors in the amygdala control synaptic plasticity and contextual fear memory. Neuropsychopharmacology. 41 (12), 2862–2871.
Temido-Ferreira, M., Ferreira, D.G., Batalha, V.L., Marques-Morgado, I., Coelho, J.E., Pereira, P., et al., 2018. Age-related shift in LTD is dependent on neuronal adenosine A_{2A} receptors interplay with mGluR5 and NMDA receptors. Mol. Psychiatry. https://doi.org/10.1038/s41380-018-0110-9.
Viana da Silva, S., Haberl, M.G., Zhang, P., Bethge, P., Lemos, C., Gonçalves, N., et al., 2016. Early synaptic deficits in the APP/PS1 mouse model of Alzheimer's disease involve neuronal adenosine A_{2A} receptors. Nat. Commun. 7, 11915.
Walsh, D.M., Selkoe, D.J., 2004. Deciphering the molecular basis of memory failure in Alzheimer's disease. Neuron. 44 (1), 181–193.

Wang, Q., Liu, Y., Zhou, J., 2015. Neuroinflammation in Parkinson's disease and its potential as therapeutic target. Transl. Neurodegener. 4, 19.
White, T.D., 1978. Release of ATP from a synaptosomal preparation by elevated extracellular K^+ and by veratridine. J. Neurochem. 30 (2), 329–336.
Wieraszko, A., Goldsmith, G., Seyfried, T.N., 1989. Stimulation-dependent release of adenosine triphosphate from hippocampal slices. Brain Res. 485 (2), 244–250.
Zhang, D., Xiong, W., Chu, S., Sun, C., Albensi, B.C., Parkinson, F.E., 2012. Inhibition of hippocampal synaptic activity by ATP, hypoxia or oxygen-glucose deprivation does not require CD73. PLoS One 7 (6), e39772.
Zimmermann, H., Zebisch, M., Strater, N., 2012. Cellular function and molecular structure of ecto-nucleotidases. Purinergic Signal 8 (3), 437–502.