Zinc enhances long-term potentiation through P2X receptor modulation in the hippocampal CA1 region

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Abstract

Zn^{2+} is an essential ion that is stored in and co-released from glutamatergic synapses and it modulates neurotransmitter receptors involved in long-term potentiation (LTP). However, the mechanism(s) underlying Zn^{2+}-induced modulation of LTP remain(s) unclear. As the purinergic P2X receptors are relevant targets for Zn^{2+} action, we have studied their role in LTP modulation by Zn^{2+} in the CA1 region of rat hippocampal slices. Induction of LTP in the presence of Zn^{2+} revealed a biphasic effect – 5–50 μ_M enhanced LTP induction, whereas 100–300 μ_M Zn^{2+} inhibited LTP. The involvement of a purinergic mechanism is supported by the fact that application of the P2X receptor antagonists 2′,3′-O-(2,4,6-trinitrophenyl) ATP (TNP-ATP) and periodate-oxidized ATP fully abolished the facilitatory effect of Zn^{2+}. Notably, application of the $P2X_7$ receptor-specific antagonist Brilliant Blue G did not modify the Zn^{2+}-dependent facilitation of LTP. Exogenous ATP also produced a biphasic effect – 0.1–1 μ_M ATP facilitated LTP, whereas 5–10 μ_M inhibited LTP. The facilitatory effect of ATP was abolished by the application of TNP-ATP and was modified in the presence of 5 μ_M Zn^{2+}, suggesting that P2X receptors are involved in LTP induction and that Zn^{2+} leads to an increase in the affinity of P2X receptors for ATP. The latter confirms our previous results from heterologous expression systems. Collectively, our results indicate that Zn^{2+} at low concentrations enhances LTP by modulating P2X receptors. Although it

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is not yet clear which purinergic receptor subtype(s) is responsible for these effects on LTP, the data presented here suggest that $P2X_4$ but not $P2X_7$ is involved.

Keywords

hippocampus; long-term potentiation; P2X; purinergic receptors; rat; zinc

Introduction

Long-term potentiation (LTP) in the CA1 region of the hippocampus is the most comprehensive model for studying activity-dependent synaptic modifications that underlie learning and memory (Malenka & Nicoll, 1999; Whitlock *et al.*, 2006). Hippocampal glutamatergic synapses play a critical role in LTP. Notably, the vesicles within these synapses co-store neurotransmitter with Zn^{2+} (Assaf & Chung, 1984; Howell *et al.*, 1984), a metal ion that inhibits both -aminobutyric acid (GABA) and *N*-methyl-ᴅ-aspartic acid (NMDA) receptor activity (Westbrook & Mayer, 1987), and potentiates -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor activity (Rassendren *et al.*, 1990).

Although the effect of Zn^{2+} on synaptic plasticity has been widely investigated within the mossy fiber-CA3 synapses of the hippocampus, it has not been possible to draw clear conclusions as to its role in LTP induction. Studies using dietary depletion of zinc (Lu *et al.*, 2000) and the Zn^{2+} chelators dithizone (Lu *et al.*, 2000) or Ca-EDTA (Li *et al.*, 2001) suggested that endogenous Zn^{2+} is required for the induction of LTP. However, another that analysed the effects of Ca-EDTA and the mouse *mocha* mutation (mutants lack vesicular Zn^{2+} within mossy fibers) on LTP suggested that Zn^{2+} is dispensible (Vogt *et al.*, 2000), and in another study exogenous application of 100–300 μ_M Zn^{2+} blocked LTP (Xie & Smart, 1994). The role of Zn^{2+} in the Schaffer collateral-CA1 synapses of the hippocampus is even less clear. Two reports (Xie & Smart, 1994; Izumi *et al.*, 2006) indicate that Zn^{2+} decreased NMDA receptor-mediated synaptic responses and reduced LTP, whereas others documented Zn^{2+}-induced increases in NMDA receptor responses but no corresponding effect on LTP (Kim *et al.*, 2002). Furthermore, a recent report showed that applying Zn^{2+} at low micromolar concentrations led to increased LTP induction (Takeda *et al.*, 2009). These conflicting results led us to hypothesize that within the CA1 region, Zn^{2+} modulates LTP by acting on receptors other than NMDA receptors.

P2X purinergic receptors represent another possible target for Zn^{2+} in the regulation of LTP. P2X receptors constitute a family of proteins that are homo- and heteromeric channels; each channel is composed of three subunits of the seven cloned subtypes and is activated by ATP and/or structurally related nucleotide analogs (North, 2002). P2X receptors are widely expressed in the hippocampus (Kanjhan *et al.*, 1999; Norenberg & Illes, 2000; Rubio & Soto, 2001) and contribute to ATP-mediated enhancement of LTP in the CA1 region (Wang *et al.*, 2004). Zn^{2+} applied at concentrations of 1–100 μ_M potentiates the ATP-evoked cationic currents generated by both $P2X_2$ and $P2X_4$ receptor subtypes when expressed in a heterologous system (Xiong *et al.*, 1999; Acuña-Castillo *et al.*, 2000; Lorca *et al.*, 2005; Huidobro-Toro *et al.*, 2008), but inhibits the ATP-evoked currents of the $P2X_7$ receptor expressed in the same context (Virginio *et al.*, 1997; Acuña-Castillo *et al.*, 2007).

Here we report that Zn^{2+} at low concentration enhances LTP, probably due to enhancement of P2X receptor function as two P2X receptor antagonists completely blocked this facilitatory effect. Higher Zn^{2+} concentrations reduced LTP, probably through inhibition of NMDA receptor activity. This dual role of Zn^{2+} may have important consequences for the

regulation of plasticity, in the contexts of both normal physiologic and disease-associated changes in conditions of the brain circuitry.

Materials and methods

Ethical guidelines

All animal care and procedures described below are in accordance with the Chilean Council for Science and Technology Research (CONICYT) guidelines, and were reviewed and approved by the University of Santiago de Chile Animal Care and Experimental Use Committee.

Hippocampal slice preparations

Male Sprague-Dawley rats 3–4 weeks of age were decapitated under halothane anesthesia (4% inhaled until stimulation of the limb withdrawal reflex failed to elicit a response). Hippocampi were then dissected and transverse slices (350 μm thick) were cut with a vibratome (Campden Instruments, London, UK), in ice-cold dissection buffer containing (in mm): 212.7 sucrose, 5 KCl, 1.25 NaH_2PO_4, 3 $MgSO_4$, 1 $CaCl_2$, 26 $NaHCO_3$ and 10 dextrose (pH 7.4, in 95% O_2/5% CO_2). Slices were transferred to a storage chamber and maintained at room temperature in artificial cerebrospinal fluid (ACSF) containing (in mm): 124 NaCl, 5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$ and 10 dextrose (pH 7.4, in 95% O_2/5% CO_2) for at least 1 h prior to recording.

Electrophysiology

In the recording chamber, slices were superfused with ACSF at a rate of 1–2 mL/min at 30 °C. Field excitatory postsynaptic potentials (fEPSPs) were evoked by square wave stimuli (0.2 ms) delivered with a concentric, bipolar stimulation electrode (200 μm diameter; FHC Inc., Bowdoinham, ME, USA) positioned in the Schaeffer collateral–commissural fibers, and recorded from the stratum radiatum in the CA1 region using a glass-pipette electrode (0.5–2 M) filled with ACSF. A stable baseline was established for 15 min, with test pulses applied every 15 s, adjusted to evoke 50% of the maximal response. While the same stimulus intensity was applied, LTP was induced with theta burst stimulation (TBS: 10 trains, each with 10 bursts at 5Hz, each burst consisting of four pulses at 100 Hz). In most experiments, the recordings were continued for 60 min after TBS. The evoked responses were filtered at 10 kHz and digitized at 5 kHz using Igor Pro (WaveMetrics Inc., Lake Oswego, OR, USA). Each drug studied was diluted in ACSF and was applied by superfusion for 20 min in total (10 min before and 10 min after TBS). In one set of experiments, d(−)-2-amino-5-phosphonopentanoic acid (AP5) was co-applied with Zn^{2+} prior to and following TBS; subsequently, AP5 and Zn^{2+} were washed out over the course of 55 min. The same slice was then superfused with 10 μM Zn^{2+} for 20 min. An additional TBS was applied in the presence of Zn^{2+}, and recordings were continued for a further 30 min. In the experiments using picrotoxin (PTX), a TBS was applied 20 min after PTX-superfused slices had produced stable basal responses; PTX was continuously applied throughout the course of the experiment. In these tests, the CA3 area of the hippocampus was dissected surgically under a microscope; the Schaffer collaterals were cut to avoid epileptiform activity induced by synaptic stimulation. In another set of experiments, NMDA receptor-mediated fEPSPs were isolated by blocking AMPA receptors with CNQX in Mg^{2+}-free ACSF, as previously described (Andreasen *et al.*, 1989). To differentiate between pre- and postsynaptic effects, we performed paired-pulse stimulation protocols: two pulses were given every 15 s, with an interstimulus interval of 20–2560 ms, and the time between pulses was doubled after each stimulation. This protocol, which assesses response facilitation by comparing the response to the second pulse with the response to the first pulse (paired pulse facilitation; PPF), was applied 20 min before and 30 min after TBS. PPF was also determined in the absence of

TBS (baseline responses) and in the absence and presence of Zn^{2+} (10 μM) or ATP (1 or 5 μM). The results are presented as the ratio between the fEPSP slopes of the second and first responses.

Data analysis

Data are presented as mean ± SEM and normalized relative to baseline (averaged fEPSP slope obtained during at least 15 min of baseline response) prior to drug application or TBS induction. LTP magnitude (percentage baseline) was measured as the average of the final 10 min of recording (50–60 min after TBS, unless otherwise noted). Student's *t*-test (for two groups) or ANOVA (for three or more groups) were used for statistical comparison of mean fEPSP slopes, and were carried out using GRAPH PAD PRISM software (San Diego, CA, USA); *P* < 0.05 was the cutoff for consideration as a significant result. Curve fitting and interpolation of the median effective concentration (EC_{50}) were also obtained using GRAPH PAD PRISM software. Amplitudes of responses during TBS were normalized to the maximal response of the initial burst and were fit with exponential functions of the form: $1 - Ae^{-B/}$, where *B* is the number of bursts in the train, is the number of bursts producing an *e*-fold decline in response amplitude, and $1 - A$ is the steady-state amplitude.

Drugs and chemicals

Zinc chloride ($ZnCl_2$), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), AP5, PTX, adenosine 5 -triphosphate (ATP), adenosine 5 -(3-thiotriphosphate) tetralithium (ATP S), 2-methylthio adenosine 5 -diphosphate (2-MeSADP), uridine-5 -triphosphate (UTP), adenosine, ivermectin (IVM), 2 ,3 -O-(2,4,6-trinitrophenyl) adenosine 5 -triphosphate (TNP-ATP), periodate-oxidized adenosine 5 -triphosphate (oATP), Brilliant Blue G (BBG), 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), 6-*N*,*N*-diethyl- - -dibromomethylene-D-adenosine-5 -triphosphate (ARL67156), as well as all salts used in ACSF and dissection solutions were purchased from Sigma (St. Louis, MO, USA). 4-(2-[7-amino-2-(2-furyl) [1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol (ZM241385) was purchased from Tocris (Ellisville, MO, USA). Stock solutions of PTX, DPCPX and ZM241385 were prepared in dimethyl sulfoxide (DMSO); the final concentration of DMSO in the perfusion solution was < 0.01%.

Results

The effect of Zn^{2+} on LTP is biphasic

Application of 10 μM Zn^{2+} enhanced the LTP evoked by TSB in comparison with that evoked under control conditions (*n* = 13–18, respectively, *P* = 0.0024, Fig. 1A and B, Table 1). Similar increases were observed when 5 and 50 μM Zn^{2+} were applied (*n* = 10, *P* = 0.02 and *n* = 7, *P* = 0.04, respectively, Fig. 1B, Table 1). Notably, application of 1 μM Zn^{2+} caused no significant change in LTP (*n* = 11, *P* = 0.48, Fig. 1B, Table 1), and application of Zn^{2+} at higher concentrations (100 and 300 μM) abolished LTP (*n* = 4, *P* = 0.01, and *n* = 6, *P* = 0.03, respectively, Fig. 1B, Table 1). Together, these results indicate that Zn^{2+} has a biphasic effect on LTP: one that is clearly facilitatory across the range 5–50 μM, and one that is inhibitory across the range 100–300 μM.

The facilitatory effect of Zn^{2+} is a consequence of postsynaptic events

The enhancement of LTP by Zn^{2+} was not attributable to changes in the recruitment of presynaptic fibers, as 10 μM Zn^{2+} did not modify the presynaptic fiber volley response (Fig. 1A). Moreover, this concentration of Zn^{2+} did not affect synaptic responses, either to basal stimulation (as shown by an unaltered baseline fEPSPs during Zn^{2+} application; Fig. 1A) or to TBS (Fig. 2A). In PPF experiments, the paired-pulse ratio during any interstimulus

interval remained unchanged by 10 μM Zn^{2+} facilitation of LTP (Fig. 2B); as in the case of the 20-ms interval, ratio values were not significantly modified (1.42 ± 0.04 before LTP vs. 1.48 ± 0.05 after Zn^{2+}-facilitated LTP, $n = 5$, $P = 0.21$, Fig. 2B). Additionally, in control, unstimulated samples (basal stimulation only) there were no statistical differences between paired-pulse ratios at 20-ms intervals, regardless of whether 10 μM Zn^{2+} was present (1.67 ± 0.16 vs. 1.62 ± 0.17, respectively, $n = 4$, $P = 0.44$, Fig. 2C). In further control experiments in which no Zn^{2+} was applied, no significant difference in the paired-pulse ratio during the 20-ms interval was observed, either before or after LTP (1.37 ± 0.07 vs. 1.33 ± 0.08, $n = 6$, $P = 0.35$, data not shown). These results strongly suggest that the mechanism underlying the observed facilitation of LTP by Zn^{2+} is postsynaptic in nature.

Zn^{2+}-dependent LTP facilitation does not depend on NMDA and $GABA_A$ receptors

To examine a possible role for the NMDA receptor in LTP facilitation by Zn^{2+}, we isolated the NMDA receptor-mediated component of the EPSP. Specifically, we blocked AMPA receptors with 1 μM CNQX and removed Mg^{2+} from the ACSF. Although basal NMDA-isolated fEPSPs were unaffected by application of 10 μM Zn^{2+} (a concentration that facilitates LTP), application of 100 μM Zn^{2+} noticeably inhibited these receptors ($n = 9$, $F_{2,24} = 39.02$, $P < 0.01$ compared with baseline, Fig. 3). In addition, we tested the effect of 10 μM Zn^{2+} on NMDA-isolated fEPSPs at several stimulation intensities, and discovered that Zn^{2+} did not change the input–output curve or the presynaptic volley–fEPSP correlation (Supporting Information Fig. S1). These observations suggest that the facilitation of LTP seen at low Zn^{2+} concentrations does not directly involve NMDA receptors. To assess whether NMDA receptors are necessary for the Zn^{2+}-induced facilitation of LTP, we applied the NMDA receptor-specific antagonist AP5. This drug reversibly blocked induction of LTP in the presence of 10 μM Zn^{2+} (from 196.3 ± 18% under control conditions to 84.2 ± 24.1% with 10 μM Zn^{2+} plus 50 μM AP5, $n = 6$, $F_{2,15} = 21.89$, $P < 0.01$; Fig. 4A). Following AP5 washout, however, LTP could again be generated and 10 μM Zn^{2+} clearly enhanced this effect (325.4 ± 48.9%, $n = 6$, $F_{2,15} = 21.89$, $P < 0.05$ compared with control LTP; Fig. 4A). Thus, the data derived from our experimental paradigm provide no evidence for a direct involvement of NMDA receptors in Zn^{2+}-induced LTP facilitation, even though NMDA receptors are necessary for LTP as such.

To assess whether GABAergic transmission influences the Zn^{2+}-induced increase in LTP, we superfused hippocampal slices with 10 μM PTX, a non-competitive $GABA_A$-receptor antagonist. This treatment increased basal responses by 32.9 ± 2.3% ($n = 5$, $P = 0.004$ compared with basal responses before PTX, Fig. 4B) but did not reduce the facilitatory effect of 10 μM Zn^{2+} on LTP (157.6 ± 3.4% with PTX vs. 207.3 ± 26.8% with PTX plus Zn^{2+}, $n = 5$, $P = 0.02$, Fig. 4B). Thus, it is unlikely that GABAergic interneurons play a relevant role in the facilitatory effect of Zn^{2+}.

The effect of exogenous ATP on LTP is biphasic

We next examined whether exogenous ATP or structurally related analogs could modify LTP induction. In slices superfused with 0.1 and 1 μM ATP, LTP was increased relative to that in controls ($n = 9$, $P = 0.047$ or $n = 15$, $P = 0.003$, respectively, Table 2, Fig. 5A and C). In slices superfused with 5 or 10 μM ATP, however, LTP was significantly reduced ($n = 12$, $P = 0.0086$ and $n = 11$, $P = 0.0015$, respectively; Table 2, Fig. 5B and C) as was the amplitude of the baseline response (64.6 ± 6.4% of inhibition, $n = 12$, $P < 0.0001$, and 72.5 ± 7.3% of inhibition, $n = 11$, $P < 0.0001$, respectively; Fig. 5B and C). The effect of ATP on baseline activity was concentration-dependent; neither 0.1 nor 1 μM ATP elicited a significant reduction in baseline activity (−3.4 ± 4.3% of inhibition, $n = 8$, $P = 0.4796$ and 12 ± 4.7% of inhibition, $n = 15$, $P = 0.0835$, respectively; Fig. 5A and C).

Investigation of the pre- or postsynaptic nature of the ATP effect on synaptic plasticity revealed that paired-pulse ratios obtained at all intervals after TBS in the context of 1 μM ATP were not significantly different from those observed before TBS. At a 20-ms interval, the ratio was 1.30 ± 0.06 before and 1.37 ± 0.1 after 1 μM ATP-facilitated LTP ($n = 12$, $P = 0.23$, data not shown). Furthermore, the paired-pulse ratio at a 20-ms interval obtained during baseline activity did not differ significantly from that obtained in the presence of 1 μM ATP (1.25 ± 0.04 vs. 1.30 ± 0.04, respectively, $n = 4$, $P = 0.1$, data not shown). However, 5 μM ATP induced a significant increase in the 20-ms interval paired-pulse ratio compared with baseline responses (1.39 ± 0.04 vs. 1.50 ± 0.03, respectively, $n = 5$, $P = 0.03$; Fig. 5D), but did not significantly change the 20-ms interval paired-pulse ratio before and after 5 μM ATP-induced inhibition of LTP (1.42 ± 0.11 vs. 1.44 ± 0.09, $n = 8$, $P = 0.34$, data not shown). Together, these results indicate that ATP-induced inhibition of basal activity might be due, at least in part, to a presynaptic component.

The facilitatory effect observed with 1 μM ATP was mimicked by application of the non-hydrolysable analog ATP S at the same concentration ($n = 5$, $P = 0.03$; Table 2, Supporting Information Fig. S2). Given that ATP S might undergo minute localized hydrolysis, which would lead to activation of adenosine receptors (Cunha *et al.*, 1998), we also tested the possibility that ATP might be hydrolysed by ecto-NTPDases, and found that the ATP-dependent facilitation of LTP was not affected by the application of 50 μM ARL67156, an NTPDase inhibitor ($n = 5$, $P = 0.13$; Fig. 5E and F); ARL67156 itself did not modify LTP induction ($n = 6$, $P = 0.2$; Table 2), suggesting that the facilitatory effect of exogenous ATP is due to ATP itself and not to any of its metabolites. In a further series of experiments, we assessed the latter possibility by applying structurally-related ATP agonists that selectively activate several different purinergic receptors: 2-MeSADP (0.1 μM, $n = 5$, $P = 0.28$; Table 2), an agonist of the $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors; UTP (1 μM, $n = 12$, $P = 0.30$; Table 2), an agonist of $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors; and adenosine (1 μM, $n = 12$, $P = 0.49$, Table 2). Notably, none of these agonists modified LTP. Furthermore, co-application of 1 μM ATP with both 50 nM DPCPX and 100 nM ZM241385 (selective antagonists of the A_1 and A_{2A} receptors, respectively) did not affect ATP-induced facilitation of LTP ($n = 7$, $P = 0.22$; Fig. 5E and F). Moreover, application of 50 nM DPCPX and 100 nM ZM241385 in the absence of exogenous ATP did not affect LTP (Table 2). Collectively, these results rule out the possibility that P2Y or adenosine receptors play roles in the facilitatory effect of ATP on LTP under the conditions used in our study. Interestingly, the P2X receptor antagonist TNP-ATP (North, 2002) fully abolished the ATP-induced facilitation of LTP ($n = 5$, $P = 0.04$; Fig. 5E and F), indicating that the facilitatory effect of ATP depends on the activation of P2X receptors. However, the P2 antagonist BBG, which is specific for the $P2X_7$ receptor subtype at 100 nM (Jiang *et al.*, 2000), failed to reduce ATP-dependent facilitation of LTP when applied ($n = 7$, $P = 0.31$; Fig. 5E and F), suggesting that $P2X_7$ receptors do not participate in LTP facilitation by ATP.

$P2X_4$ receptors play a role in LTP induction

Among the P2X receptor subtypes expressed in the hippocampus (Norenberg & Illes, 2000; Rubio & Soto, 2001), only the $P2X_4$ subtype has been directly reported to modulate LTP (Sim *et al.*, 2006). To evaluate the participation of $P2X_4$ receptors on LTP, we tested the effect of applying ivermectin (IVM), a $P2X_4$-specific positive allosteric modulator (Khakh *et al.*, 1999), to our assay system. As previously shown in mouse hippocampal slices (Sim *et al.*, 2006), we found that 3 μM IVM enhanced LTP induced in the CA1 region of the rat hippocampus ($n = 13$, $P = 0.04$; Table 2, Fig. 6). The facilitatory effect of IVM was fully prevented by the application of 10 μM TNP-ATP ($n = 4$, $P = 0.002$; Fig. 6). These data support the notion that $P2X_4$ receptors actively participate in LTP modulation, and highlight the role of endogenous extracellular ATP in synaptic plasticity.

The effect of ATP on LTP is modulated by Zn^{2+}

Based on the observation that Zn^{2+} is a positive modulator of the receptor function of $P2X_2$ and $P2X_4$ (Xiong *et al.*, 1999; Acuña-Castillo *et al.*, 2000), we evaluated whether superfusing hippocampal slices with Zn^{2+} plus ATP would further modulate LTP. We applied 5 μM Zn^{2+}, a concentration that enhanced LTP slightly (Table 1, Fig. 1B), with ATP at various concentrations. Indeed, the facilitation of LTP by 0.1 μM ATP plus 5 μM Zn^{2+} was greater than that observed in the presence of ATP alone (405.8 ± 70.5 vs. 255.4 ± 31.7%, *n* = 6 and 9, respectively, *P* = 0.03; Fig. 7A and C). Interestingly, increasing the ATP concentration to 1 μM or more resulted in a significant reduction of the ATP-induced facilitation of LTP in the presence of Zn^{2+} (236.1 ± 26.3 vs. 110.1 ± 19.5%, *n* = 12 and 10, respectively, *P* = 0.001; Fig. 7B and C). Plotting these results as an ATP concentration–response curve revealed a leftward shift in the curve, and an eight-fold reduction in the EC_{50}, from 3.18 ± 2.26 to 0.41 ± 0.45 μM (*n* = 6-12, *P* < 0.01; Fig. 7C), suggesting that Zn^{2+} increases the affinity of the P2X receptor for ATP, a notion consistent with data from *in vitro* assays (Acuña-Castillo *et al.*, 2000; Lorca *et al.*, 2005).

Role of the P2X receptors in Zn^{2+}-dependent facilitation of LTP

To assess whether nucleotide receptors play an active role in the Zn^{2+}-mediated facilitation of LTP, we applied TNP-ATP or oATP (Murgia *et al.*, 1993; North, 2002; Solini *et al.*, 2007), two antagonists of P2X receptors that are not subtype-selective. As shown in Fig. 8 and Table 2, neither 10 μM TNP-ATP nor 10 μM oATP significantly affected LTP induction (*n* = 4, *P* = 0.1, and *n* = 6, *P* = 0.3962, respectively). However, at 30 μM concentration each antagonist significantly inhibited LTP (*n* = 3, *P* = 0.02, and *n* = 9, *P* = 0.002, respectively, Table 2). Furthermore, when hippocampal slices were superfused with either 10 μM TNP-ATP or 10 μM oATP plus 10 μM Zn^{2+}, the Zn^{2+}-mediated facilitation of LTP was abolished (*n* = 6, *P* = 0.006 and *n* = 9, *P* = 0.002, respectively; Fig. 8). Therefore, a P2X purinergic receptor is probably involved in the facilitatory effect of Zn^{2+} on LTP. The joint application of 100 nM BBG and 10 μM Zn^{2+} did not reduce the facilitatory effect of the metal significantly (*n* = 4, *P* = 0.28; Fig. 8C and Supporting Information Fig. S3), indicating that the $P2X_7$ receptors do not play a major role in Zn^{2+}-dependent facilitation of LTP. To test whether Zn^{2+} modifies the activity of ecto-NTPDases, thus changing the levels of extracellular ATP rather than directly modulating P2X receptors, we superfused hippocampal slices with 10 μM Zn^{2+} in the presence of the NTPDase inhibitor 50 μM ARL67156. However, this NTPDase inhibitor did not affect the Zn^{2+}-dependent facilitation of LTP (*n* = 4, *P* = 0.32; Fig. 8C and Supporting Information Fig. S3). Together, these results support the idea that LTP facilitation by Zn^{2+} occurs through P2X receptors, probably of the $P2X_4$ subtype, and not by other purinergic mechanisms.

Discussion

Zn^{2+}-induced facilitation of LTP

The present study confirms that Zn^{2+} plays a role in LTP induction, and highlights a mechanism of action whereby this metal ion modulates P2X receptors in the Schaffer collateral–CA1 synapses of the rat hippocampus. Low micromolar concentrations of Zn^{2+}, applied before and during TBS, increased LTP, the largest increase being observed at 10 μM Zn^{2+}. This facilitation of LTP by Zn^{2+} does not have a presynaptic component, as the concentration of 10 μM did not modify the PPF curve during LTP or the TBS-evoked response amplitude.

Zn^{2+} modulates a wide array of receptors, including glutamate, GABA, glycine and purinergic receptors (Westbrook & Mayer, 1987; Bloomenthal *et al.*, 1994; Virginio *et al.*, 1997; Xiong *et al.*, 1999; Acuña-Castillo *et al.*, 2000; Mathie *et al.*, 2006; Huidobro-Toro *et*

al., 2008). Previously, we showed that micromolar concentrations of Zn^{2+} potentiate ATP-evoked currents produced by heterologously expressed $P2X_2$ and $P2X_4$ receptors (Acuña-Castillo *et al.*, 2000; Coddou *et al.*, 2002; Lorca *et al.*, 2005), but that they inhibit ATP-elicited currents produced by $P2X_7$ receptors (Acuña-Castillo *et al.*, 2007). In view of this evidence, and considering that puringeric receptors are widely distributed throughout the rat hippocampus (Ralevic & Burnstock, 1998; Kanjhan *et al.*, 1999; Norenberg & Illes, 2000; Rubio & Soto, 2001; Rodrigues *et al.*, 2005), we propose that P2X receptors are targets for the modulatory action of Zn^{2+} on LTP induction.

LTP facilitation by Zn $^{2+}$ requires activation of P2X receptors

Our demonstration here that the Zn^{2+}-dependent increase in LTP can be fully reversed by the P2X-receptor agonists TNP-ATP and oATP (Murgia *et al.*, 1993; North, 2002; Solini *et al.*, 2007) indicates that endogenous ATP, through P2X receptor activation, is involved in the Zn^{2+} facilitatory effect on LTP. Given that neither oATP nor TNP-ATP is selective for particular P2X receptor subtypes, our data do not pinpoint which P2X receptor subtypes, homomers or heteromers, are involved. Nevertheless, our finding that TNP-ATP blocks LTP facilitiation by the $P2X_4$ receptor-specific positive modulator IVM suggests that TNT-ATP may act by inhibiting at least the activity of $P2X_4$ receptors. In addition, as the Zn^{2+}-mediated LTP increase was not significantly modified by BBG, in spite of its application at a concentration that selectively inhibits the $P2X_7$ receptor (Jiang *et al.*, 2000), we can rule out a primary role for $P2X_7$ in this process.

Purinergic modulation of LTP

The endogenous activation of P2X receptors may be relevant to LTP modulation, consistent with the reduction in LTP by both 30 μ_M TNP-ATP or oATP and the increase by IVM. Thus, the endogenous activation of P2X receptors may be increased by the presence of Zn^{2+}, which enhances the ATP-evoked P2X currents several fold (Xiong *et al.*, 1999; Acuña-Castillo *et al.*, 2000; Lorca *et al.*, 2005). Furthermore, superfusion of hippocampal slices with exogenous ATP mimics the effects of activating nucleotide receptors that modulate LTP. The application of exogenous ATP revealed biphasic effects, suggesting that this nucleotide modulates LTP through more than one mechanism. These effects are postsynaptic, given that we did not observe differences in the paired-pulse ratio before or after LTP modulation in response to the application of 1 or 5 μ_M ATP. In addition, we consistently observed that 5 μ_M ATP reduced the magnitude of basal synaptic activity; the increase in the paired-pulse ratio experiments suggests that this effect might be explained, at least in part, by a presynaptic component. As extracellular ATP is rapidly metabolized to adenosine in the hippocampus, the newly formed adenosine may act through presynaptic A_1 receptors to reduce synaptic transmission, thereby decreasing neurotransmitter release in the CA1 area (Cunha *et al.*, 1998). Alternatively, ATP itself could act on P2 receptors to enhance the release of adenosine and facilitate LTP induction (Almeida *et al.*, 2003). However, in our experiments this latter possibility seems unlikely. First, the lack of an effect of exogenous adenosine or P2Y-selective agonists on LTP suggests that they have only a minor role or do not participate under the experimental conditions used here. Secondly, exogenous application of the nonhydrolysable analog ATP S, like that of ATP, facilitates LTP. Thirdly, the facilitatory effect of ATP on LTP did not change in the presence of the A_1 and A_{2A} receptor antagonists DPCPX and ZM241385, respectively.

The dual effect of ATP may account for the complex purinergic modulation of LTP and basal synaptic activity. In a recent review, Pankratov *et al.* (2009) proposed that both the facilitatory (Wang *et al.*, 2004; Sim *et al.*, 2006) and inhibitory (Pankratov *et al.*, 2002) roles of P2X receptors in LTP induction are consistent with the hypothesis of a bi-directional synaptic plasticity. Our results support this notion that P2X receptors may modulate synaptic

plasticity in one direction or another, as reflected by the biphasic effect of exogenous ATP application. We have considered that another potential target for ATP action might be glial cells, as P2X receptors are expressed in hippocampal astrocytes (Kukley *et al.*, 2001), and ATP can elicit currents in these cells through P2X receptor activation (Walz *et al.*, 1994) and also induce the release of glutamate from cultured astrocytes (Fellin *et al.*, 2006). However, a recent study reports that ATP does not evoke currents from astrocytes in acute hippocampal slices or freshly dissociated cell suspension (Jabs *et al.*, 2007). Thus, the contribution of P2X receptor activation in astrocytes during LTP induction is not yet clear and needs further elucidation.

The facilitatory effect of exogenous ATP on LTP is modulated by Zn^{2+}

Zn^{2+} reduces the EC_{50} derived from the ATP concentration–response curve on LTP. We propose that Zn^{2+} might exert this effect by increasing the affinity of certain P2X receptor subtypes for ATP. We previously reported that cultured cells expressing either $P2X_2$ or $P2X_4$ receptors show a similar modulation of the ATP concentration–response curve in the presence of Zn^{2+} (Acuña-Castillo *et al.*, 2000; Lorca *et al.*, 2005). The P2X receptor family contains seven subtypes ($P2X_{1-7}$), all of which are expressed in the hippocampus (Kanjhan *et al.*, 1999; Norenberg & Illes, 2000; Rodrigues *et al.*, 2005). Among these members, the $P2X_2$, $P2X_3$ and $P2X_4$ subtypes are putative targets for Zn^{2+} because the ATP-evoked cationic currents they evoke are facilitated by this metal (Wildman *et al.*, 1999; Huidobro-Toro *et al.*, 2008). However, it is unlikely that the facilitatory effect of Zn^{2+} on LTP induction is mediated by $P2X_3$ modulation, as the metal concentrations capable of facilitating ATP-evoked currents generated by this receptor are higher than those used in our study (Wildman *et al.*, 1999). Of the $P2X_2$ and $P2X_4$ receptor populations, the latter seems to be the better candidate for Zn^{2+} modulation, as in heterologous expression systems 10 μ_M Zn^{2+} has a 10-fold greater effect on ATP-evoked currents generated by $P2X_4$ than on those generated by the $P2X_2$ receptor (Huidobro-Toro *et al.*, 2008). Moreover, only the $P2X_4$ receptor has two Zn^{2+} binding sites – a high-affinity site that potentiates the ATP-induced current, and a low-affinity site that inhibits it (Coddou *et al.*, 2007). This may account for the biphasic effect observed in the modulation of LTP by Zn^{2+}. In addition, in experiments using IVM, a selective positive modulator of $P2X_4$ receptors (Khakh *et al.*, 1999), we observed a significant increase in LTP that was fully reversed by the application of TNP-ATP. These results, together with the findings of Sim *et al.* (2006) describing reduced LTP in mice lacking $P2X_4$ receptors, constitute strong evidence that $P2X_4$ receptors play a role in LTP facilitation, and in the mechanism of Zn^{2+}-mediated modulation of synaptic plasticity in the hippocampus. Furthermore, we hypothesize that a P2X-dependent increase of intracellular Ca^{2+} may be necessary to increase AMPA receptor insertion into postsynaptic membranes, as was demonstrated in other brain nuclei (Gordon *et al.*, 2005).

Other possible Zn^{2+} targets

Our data from hippocampal slices do not support the notion that the effects of Zn^{2+} on LTP involve the modulation of GABA receptors, a mechanism that has been proposed based on experiments carried out on cultured hippocampal neurons (Legendre & Westbrook, 1991). Specifically, neither the afferent volley nor synaptic transmission was modulated by the application of 10 μ_M Zn^{2+} and, more importantly, Zn^{2+}-induced LTP facilitation was not inhibited by the application of PTX. On the other hand, we cannot completely discard the possibility that Zn^{2+} inhibits the NMDA receptor at low concentrations, as such an effect could be masked by residual AMPA receptor activity. Nevertheless, such an inhibitory effect would not account for the facilitatory modulation of LTP observed in the presence of Zn^{2+}. Another reasonable target for Zn^{2+} modulation might be the AMPA/kainate receptors, which are potentiated by this metal at micromolar concentrations, but inhibited by millimolar concentrations (Rassendren *et al.*, 1990). Although we cannot exclude the

possibility that several neurotransmitter receptors may be integrated in Zn^{2+}-based modulation of LTP, our findings show a full reversion of Zn^{2+}-induced facilitation of LTP with P2X receptor antagonists, suggesting that these receptors are likely key targets for LTP facilitation. Patch-clamp recordings may provide further evidence of the specific participation of several postsynaptic neurotransmitter receptors, as well as a better understanding of the intracellular mechanisms involved in the Zn^{2+} and purinergic modulation of LTP.

The results from several studies, including those presented here, indicate that Zn^{2+} is an active modulator of synaptic strength and plasticity (Lu *et al.*, 2000; Li *et al.*, 2001; Izumi *et al.*, 2006; Lorca *et al.*, 2007; Morales *et al.*, 2008; Takeda *et al.*, 2008, 2009), yet the molecular mechanism(s) responsible for the Zn^{2+}-mediated modulation of LTP remain(s) unknown. Our findings on the role of Zn^{2+} as a facilitator of LTP induction in the CA1 area of the hippocampus confirm recent observations (Takeda *et al.*, 2009) and add mechanistic evidence for the role of this metal as a modulator of LTP. Our findings are not fully compatible with previously proposed mechanisms concerning the Zn^{2+}-dependent facilitatory effect (Takeda *et al.*, 2009). In particular, whereas Zn^{2+} has been proposed to act intracellularly by direct action on NMDA receptors, and thereby overcoming the negative modulator effect on NMDA receptors in the extracellular compartment, we hypothesize that Zn^{2+} facilitates LTP through the modulation of P2X receptors.

Conclusions

Based on the results presented here, we suggest that the Zn^{2+}-evoked enhancement of LTP is mainly mediated through the modulation of P2X receptors. At least two mechanisms seem to be involved in the biphasic effect of Zn^{2+}: (i) at low micromolar concentrations, Zn^{2+} facilitates $P2X_4$ receptor activity to increase LTP, possibly through binding to high-affinity sites in the extracellular domain of this receptor (Coddou *et al.*, 2007; Huidobro-Toro *et al.*, 2008); (ii) at higher micromolar concentrations, Zn^{2+} inhibits NMDA receptors (Westbrook & Mayer, 1987), resulting in decreased LTP – at these concentrations the facilitation of $P2X_4$ receptor activity by Zn^{2+} is significantly reduced, as reflected by the bell-shaped curve of the response to the metal of $P2X_4$ receptors expressed in heterologous systems (Coddou *et al.*, 2003, 2007). In conclusion, low concentrations of Zn^{2+} facilitate LTP induction in the CA1 region of the hippocampus at least partly by interacting with P2X receptors. This finding highlights the physiological role of Zn^{2+} as a synaptic plasticity modulator with certain implications for learning and memory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

2-MeSADP	2-methylthio adenosine 5 -diphosphate
ACSF	artificial cerebrospinal fluid

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AMPA	-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP5	D(−)-2-amino-5-phosphonopentanoic acid
ARL67156	6-*N,N*-diethyl-ᴮ- -dibromomethylene-D-adenosine-5 -triphosphate
ATP	adenosine 5 -triphosphate
ATP S	adenosine 5 -(3-thiotriphosphate)
BBG	Brilliant Blue G
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
DMSO	dimethyl sulfoxide
DPCPX	8-cyclopentyl-1,3-dipropylxanthine
EC$_{50}$	median effective concentration
fEPSP	field excitatory postsynaptic potential
GABA	-aminobutyric acid
IVM	ivermectin
LTP	long-term potentiation
NMDA	*N*-methyl-D-aspartic acid
oATP	periodate-oxidized adenosine 5 -triphosphate
PPF	paired-pulse facilitation
PTX	picrotoxin
TBS	theta-burst stimulation
TNP-ATP	2 ,3 -O-(2,4,6-trinitrophenyl) adenosine 5 -triphosphate
UTP	uridine-5 -triphosphate
ZM241385	4-(2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino]ethyl)phenol

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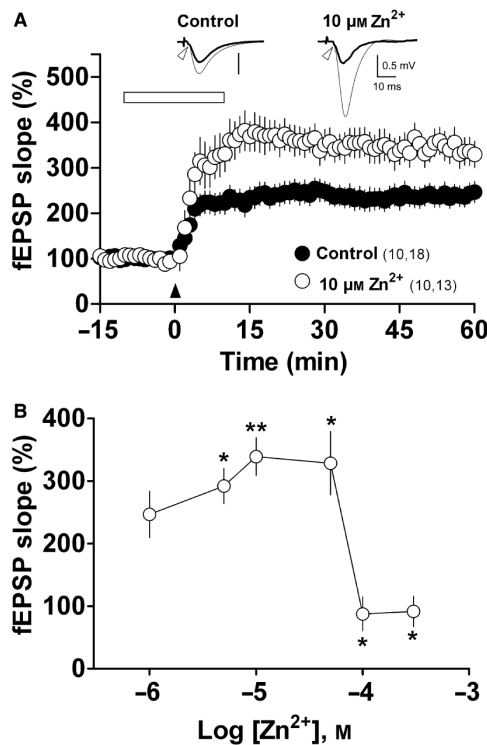
**Fig. 1.**
Zn^{2+} modulates LTP in a biphasic manner. (A) LTP induced in the absence (control) or presence of Zn^{2+}, with the metal applied to slices for 10 min before and 10 min after (open horizontal bar) the application of TBS (arrowhead). Symbols indicate the normalized fEPSP slope (means ± SEM). Numbers in parentheses: number of rats, number of slices. Inset – mean of five field responses obtained 12 min before (thick line) and 50 min after TBS (thin line), under both conditions. Open arrowheads indicate the presynaptic volley. (B) Zn^{2+} concentration–response curve. Each symbol represents the fEPSP slope obtained 50–60 min after TBS (means ± SEM). *$P < 0.05$ and **$P < 0.01$ compared with LTP in control slices for each condition.
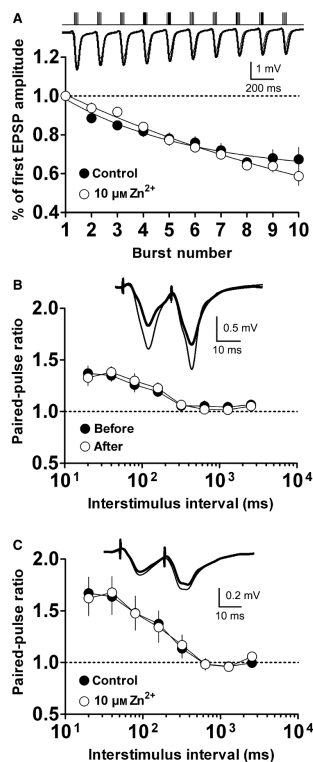
Fig. 2.
The facilitatory effect of Zn^{2+} on LTP is postsynaptic. (A) fEPSPs evoked during TBS application were normalized to the first fEPSP and are plotted as the normalized response. Open circles – 10 μM Zn^{2+} ($n = 6$), closed circles – control ($n = 6$); means ± SEM. Curves were fitted to a single exponential (see Materials and methods). Inset – representative recordings of TBS responses for control (thick line) and 10 μM Zn^{2+}- (thin line) treated slices; upper trace represents stimulus bursts. (B, C) Paired pulses separated by 20- to 2560-ms intervals recorded (B) before (closed circles, $n = 6$) or after Zn^{2+}-facilitated (open circles, $n = 6$) LTP induction; and (C) during baseline stimulation before (control, closed circles, $n = 4$) and during 10 μM Zn^{2+} application (open circles, $n = 4$). Symbols represent ratios of the fEPSP slope of the second vs. the first pulse ± SEM, for each interval. Insets – representative traces of three mean field responses at 20-ms intervals.
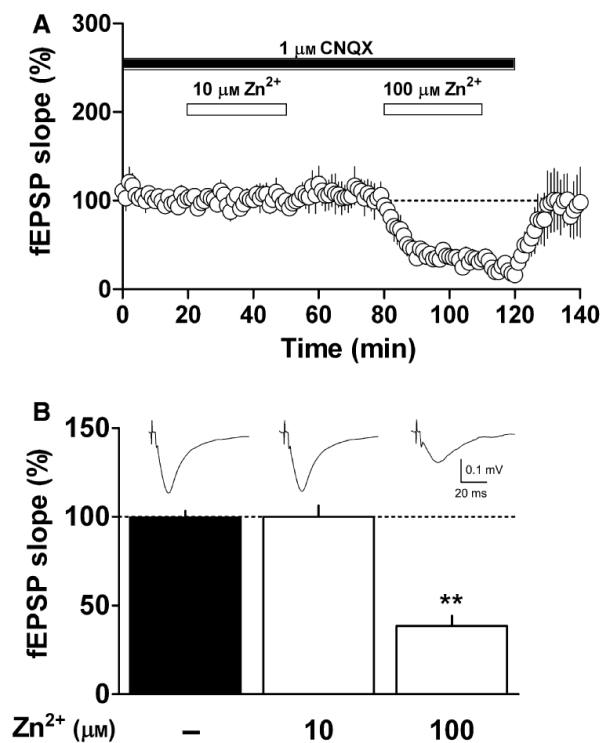
Fig. 3.
Effect of Zn^{2+} on isolated NMDA receptor-mediated responses. (A) Isolation of the NMDA receptor-mediated responses during superfusion of 1 μM CNQX in Mg^{2+}-free ACSF (closed bar). After NMDA responses had been monitored for 20 min, slices were superfused with 10 μM Zn^{2+} for 30 min (open bar). Following 30 min of metal washout, 100 μM Zn^{2+} was again applied for 30 min (open bar): mean fEPSP ± SEM. (B) Bar graph of the experimental data shown in A. Columns correspond to the average fEPSP slope (± SEM) calculated 10–20 min after CNQX application, 10–20 min after 10 μM Zn^{2+} application and 10–20 min after 100 μM Zn^{2+} application ($n = 9$, $**P < 0.01$ compared with fEPSP before Zn^{2+} superfusion). Inset – mean of five field responses obtained under each condition.
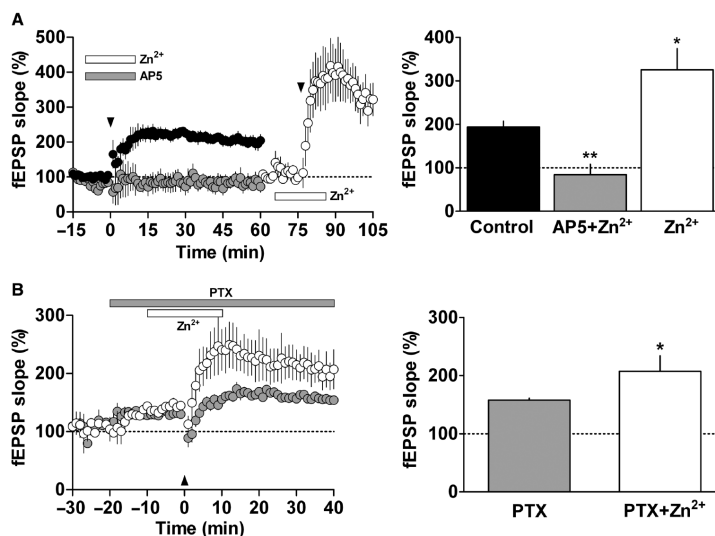
Fig. 4.
Influence of NMDA and $GABA_A$ receptors on the facilitatory effect of Zn^{2+} on LTP. (A) Left – LTP induced under control conditions (closed circles) or in the presence of 10 μ_M AP5 plus 10 μ_M Zn^{2+} (gray circles). AP5 and Zn^{2+} (gray and open horizontal bar, respectively) were applied by superfusion for 10 min before and 10 min after TBS (arrowhead). Sixty-five minutes after TBS slices were superfused with 10 μ_M Zn^{2+} for 20 min, a second TBS was applied (arrowhead): means ± SEM. Right – summary and statistical analysis of the experimental data shown in the left panel. Columns correspond to the normalized average fEPSP slope obtained 50–60 min after the first TBS (for control and AP5 + Zn^{2+}) or 20–30 min after the second TBS (for Zn^{2+} alone). Error bars, SEM; $n = 6$, *$P < 0.05$, **$P < 0.01$ compared with control. (B) Left – LTP induced in the presence of 10 μ_M PTX (gray circles) or 10 μ_M PTX plus 10 μ_M Zn^{2+} (open circles). Slices were superfused with PTX for 20 min before TBS was applied (arrowhead) and until the end of the experiment (gray horizontal bar). Zn^{2+} was applied 10 min before and 10 min after TBS (open horizontal bar). Right – summary and statistics of the experimental data shown in the left-hand panel. Columns correspond to normalized means of the fEPSP slope ± SEM of values 30–40 min after TBS ($n = 5$, *$P < 0.05$ compared with 10 μ_M PTX).
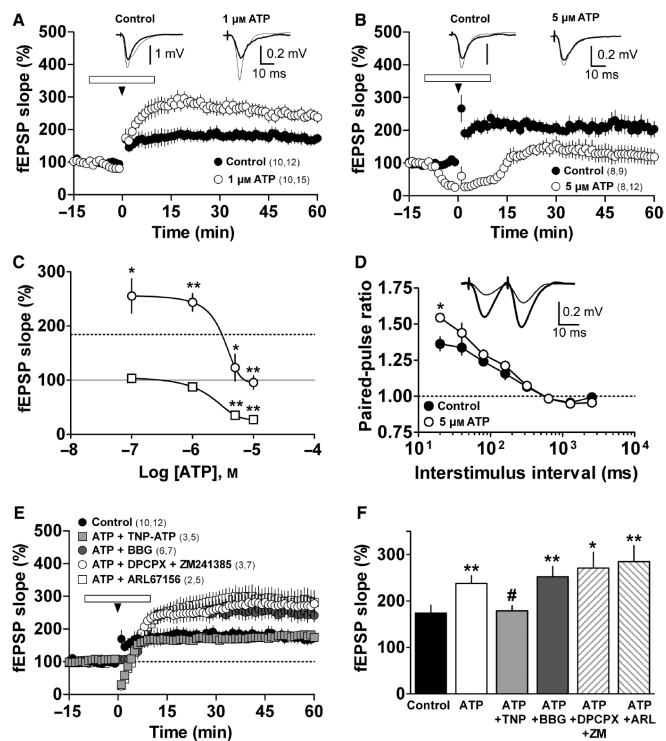
Fig. 5.

Biphasic effect of ATP on LTP induction. (A, B) LTP induced under control conditions (closed circles) and in the presence of 1 or 5 μ_M ATP (open circles), respectively. ATP was applied (open horizontal bar) for 10 min before and 10 min after TBS (arrowheads). Symbols indicate mean normalized fEPSP slope ± SEM. Numbers in parentheses: number of rats, number of slices. Insets – mean of five fEPSP responses obtained 12 min before (thick line) or 50 min after TBS (thin line, 1 or 5 μ_M ATP). (C) ATP concentration–response curves. Open circles represent the average fEPSP slope obtained 50–60 min after TBS (means ± SEM): *$P < 0.05$ and **$P < 0.01$ compared with control LTP for each experiment. Dotted line shows the average LTP value of all control experiments. Open squares represent the average fEPSP slope obtained 5–10 min after ATP superfusion and in the absence of TBS (means ± SEM): **$P < 0.01$ compared with fEPSP before superfusion of ATP (gray line). (D) Paired-pulse ratio interstimulus curve shows a significant difference in basal activity at 20-ms intervals in the presence of 5 μ_M ATP (open circles, $n = 5$, *$P < 0.05$), vs. no ATP superfusion (control, closed circles). Symbols represent ratios of the fEPSP slope of the second vs. the first pulse ± SEM for each interval. Inset – representative traces of three averaged field responses at 20-ms intervals recorded in the absence (thick line) or presence of 5 μ_M ATP (thin line). (E) LTP modulation in the presence of 1 μ_M ATP plus 10 μ_M TNP-ATP (light gray squares), 100 n$_M$ BBG (dark gray circles), 50 μ_M ARL67156 (open squares) or 50 n$_M$ DPCPX and 100 n$_M$ ZM241385 (open circles). (F) Summary and statistical results of the experimental data shown in E. Columns correspond to the average fEPSP slope obtained 50–60 min after TBS. Bars indicate SEM. *$P < 0.05$ and **$P < 0.01$ compared with control, #$P < 0.05$ compared with 1 μ_M ATP.
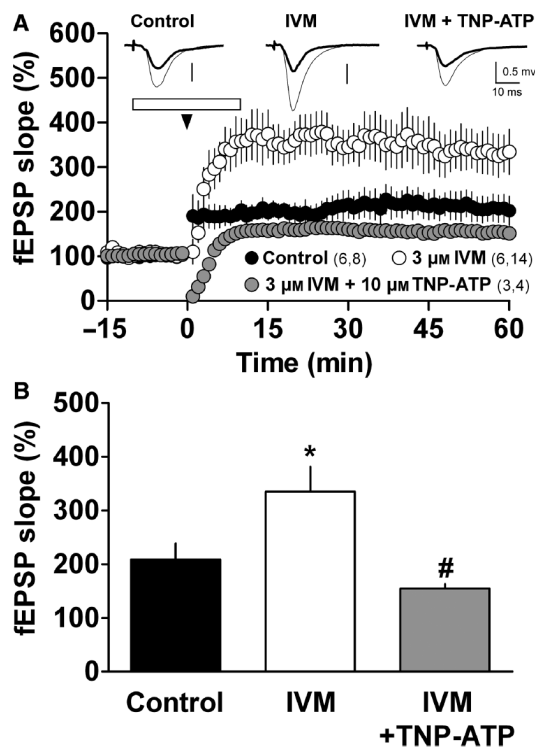
Fig. 6.
Ivermectin enhances LTP. (A) LTP induced under control conditions (closed circles), with superfusion of 3 μM IVM (open circles) or 3 μM IVM plus 10 μM TNP-ATP (gray circles) superfused 10 min before and 10 min after (open horizontal bar) TBS (arrowhead). Symbols indicate the normalized fEPSP slope (mean ± SEM). Numbers in parentheses: number of rats, number of slices. Inset – mean of five field responses obtained 12 min before (thick line) and 50 min after TBS (thin line), for each condition. (B) Summary of the experimental data shown in A. Columns correspond to the average fEPSP slope obtained 50–60 min after TBS. Bars indicate SEM. *$P< 0.05$ compared with control, #$P< 0.01$ compared with 3 μM IVM.
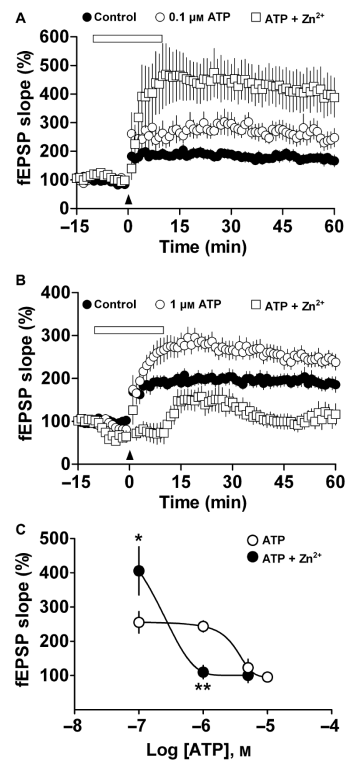
Fig. 7.
Zn^{2+} modifies ATP facilitation of LTP. (A,B) LTP modulation in the presence of 0.1 or 1 μ_M ATP (open circles) or ATP plus 5 μ_M Zn^{2+} (open squares). Slices were superfused with ATP or ATP plus Zn^{2+} (open horizontal bar) 10 min before and 10 min after TBS (arrowheads). (C) ATP concentration–response curves. Open circles represent the effect of ATP on LTP (as seen in Fig. 5C). Closed circles represent the effect of ATP plus 5 μ_M Zn^{2+} on LTP ($n =$ 6–12): $*P < 0.05$ and $**P < 0.01$ compared with ATP alone.
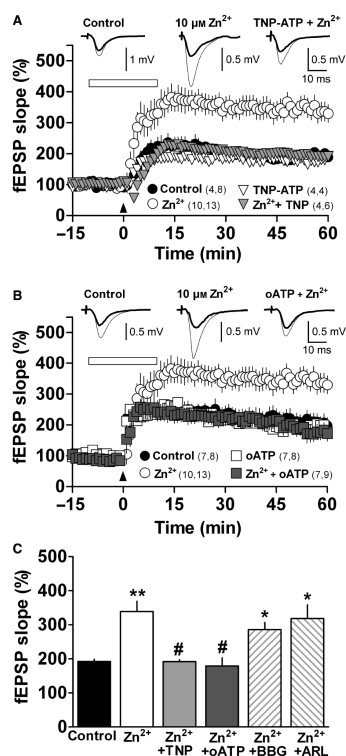
Fig. 8.
P2X antagonists abolish the facilitator effect of Zn^{2+}. (A) LTP induced under control conditions (closed circles), or in the presence of 10 μ_M Zn^{2+} (open circles), 10 μ_M TNP-ATP (open triangles) or 10 μ_M TNP-ATP plus 10 μ_M Zn^{2+} (gray triangles), with superfusion carried out for 10 min before and 10 min after (open horizontal bar) the TBS (arrowhead). (B) Control LTP (closed circles), LTP induced in the presence of 10 μ_M Zn^{2+} (open circles), 10 μ_M oATP (open squares) or 10 μ_M Zn^{2+} plus 10 μ_M oATP (gray squares), with superfusion carried out as described in A. Numbers in parentheses: number of rats, number of slices. Insets – mean of five field responses obtained 12 min before (thick line) and 50 min after TBS (thin line), under each condition. (C) Average fEPSP slope obtained 50–60 min after TBS for control slices ($n = 11$), and slices superfused with 10 μ_M Zn^{2+} ($n = 13$) and 10 μ_M Zn^{2+} plus either 10 μ_M TNP-ATP ($n = 6$), 10 μ_M oATP ($n = 9$), 100 nM BBG ($n = 5$) or 50 μ_M ARL67156 ($n = 4$). *$P < 0.05$ and **$P < 0.01$ compared with control, #$P < 0.01$ compared with 10 μ_M Zn^{2+}.

Table 1

Effects of several concentrations of Zn^{2+} on LTP

Zn^{2+} concentration (μM)	Percentage EPSP slope 50–60 min after TBS [mean ± SEM (n)]		
	Control	+Zn^{2+}	Significance[*]
1	220.1 ± 26.7 (8)	246.6 ± 37 (11)	NS
5	218.4 ± 24 (10)	292.1 ± 28.2 (10)	
10	226.7 ± 19.1 (18)	339.1 ± 30.2 (13)	
50	209.6 ± 14.2 (7)	328.5 ± 50.7 (7)	
100	227.3 ± 27.4 (4)	87.5 ± 27.1 (4)	
300	203.7 ± 27.2 (3)	91.5 ± 24.3 (6)	

[*] Key: and   , significant increase compared with control ($P < 0.05$ and $P < 0.01$, respectively);   , significant decrease compared with control ($P < 0.05$); NS, no significant difference compared with control.

Table 2

Effects of purinergic agonists, antagonists and a modulator on LTP

Concentration (µM)		Percentage EPSP slope 50–60 min after TBS [mean ± SEM (n)]		
		Control	+ Agonist/antagonist/ modulator	Significance[*]
Agonist				
ATP	0.1	182.4 ± 24.0 (9)	255.4 ± 31.7 (9)	
	1	173.9 ± 17.1 (12)	243.6 ± 16.4 (15)	
	5	209.1 ± 22.5 (9)	123.3 ± 24.7 (12)	
	10	180.8 ± 12.3 (9)	95.8 ± 12.3 (11)	
ATP S	1	190.6 ± 16.0 (3)	327.7 ± 47.5 (3)	
2-MeSADP	0.1	199.4 ± 33.7 (4)	162.4 ± 25.0 (5)	NS
UTP	1	170.5 ± 24.7 (6)	203.1 ± 23.8 (12)	NS
Adenosine	1	208.4 ± 14.3 (8)	213.7 ± 28.6 (12)	NS
Antagonist				
TNP-ATP	10	194.0 ± 6.1 (8)	178.0 ± 7.9 (4)	NS
	30	164.7 ± 14.2 (6)	119.6 ± 8.0 (3)	
oATP	10	208.2 ± 15.5 (10)	191.2 ± 19.8 (6)	NS
	30	200.2 ± 10.9 (7)	135.1 ± 13.8 (9)	
DPCPX+ZM241385	0.05 + 0.1	159.3 ± 18.4 (5)	157.7 ± 7.1 (4)	NS
ARL67156	50	190.5 ± 11.7 (6)	185.0 ± 27.3 (6)	NS
Modulator				
IVM	3	208.5 ± 30.3 (6)	335.1 ± 46.3 (13)	

[*] Key: and , significant increase compared with control ($P < 0.05$ and $P < 0.01$, respectively); and , significant decrease compared with control ($P < 0.05$ and $P < 0.01$, respectively); NS, no significant difference compared with control.