A critical role of NO/cGMP/PKG dependent pathway in hippocampal post-ischemic LTP: Modulation by zonisamide

Cinzia Costa [a,b], Alessandro Tozzi [a,b], Sabrina Siliquini [a,b], Francesca Galletti [a,b], Gabriela Cardaioli [a,b], Michela Tantucci [a,b], Francesco Pisani [c], Paolo Calabresi [a,b,*]

[a] Clinica Neurologica, Università degli Studi di Perugia, Ospedale S.Maria della Misericordia, 06156 Perugia, Italy
[b] Fondazione Santa Lucia, Istituto di Ricovero e Cura a Carattere Scientifico, 00143 Rome, Italy
[c] Dipartimento di Neuroscienze, Università di Messina, Messina, Italy

ARTICLE INFO

ABSTRACT

Nitric oxide (NO) is an intercellular retrograde messenger involved in several physiological processes such as synaptic plasticity, hippocampal long-term potentiation (LTP), and learning and memory. Moreover NO signaling is implicated in the pathophysiology of brain ischemia. In this study, we have characterized the role of NO/cGMP signaling cascade in the induction and maintenance of post-ischemic LTP (iLTP) in rat brain slices. Moreover, we have investigated the possible inhibitory action of zonisamide (ZNS) on this pathological form of synaptic plasticity as well as the effects of this antiepileptic drug (AED) on physiological activity-dependent LTP. Finally, we have characterized the possible interaction between ZNS and the NO/cGMP/PKG-dependent pathway involved in iLTP.

Here, we provided the first evidence that an oxygen and glucose deprivation episode can induce, in CA1 hippocampal slices, iLTP by modulation of the NO/cGMP/PKG pathway. Additionally, we found that while ZNS application did not affect short-term synaptic plasticity and LTP induced by high-frequency stimulation, it significantly reduced iLTP. This reduction was mimicked by bath application of NO synthase inhibitors and a soluble guanyl cyclase inhibitor. The effect of ZNS was prevented by either the application of a NO donor or drugs increasing intracellular levels of cGMP and activating PKG. These findings are in line with the possible use of AEDs, such as ZNS, as a possible neuroprotective strategy in brain ischemia. Moreover, these findings strongly suggest that NO/cGMP/PKG intracellular cascade might represent a physiological target for neuroprotection in pathological forms of synaptic plasticity such as hippocampal iLTP.

© 2011 Elsevier Inc. All rights reserved.

Introduction

Nitric oxide (NO) is an intercellular retrograde messenger, originally described as an endothelial relaxation factor, which has been shown to be involved in several physiological processes such as hippocampal long-term potentiation (LTP), plasticity, learning, and memory (Arancio et al., 1996; Bohme et al., 1991). In particular, NO signaling is implicated in the pathophysiology of brain aging (Calabrese et al., 2000) and ischemia (Iadecola, 1997) and it is required for the generation of anoxia-induced LTP (Huang and Hsu, 1997). Synaptic and cellular events induced by brain ischemia share some pathophysiological mechanisms with those triggered by the abnormal neuronal discharge induced by epilepsy (Calabresi et al., 2003a,b). Therefore, drugs effective in minimizing seizure-induced brain damage might also be useful in reducing ischemic injury (Calabresi et al., 2003b).

Several antiepileptic drugs (AEDs) have been tested in animal models of focal or global ischemia and some of these compounds have also been tested in humans for possible neuroprotective effects through modulation of voltage-gated ion channels, enhancement of synaptic inhibition, and inhibition of synaptic excitation (Calabresi et al., 2003b; Costa et al., 2004, 2006). The new AED Zonisamide (ZNS) prevents hypoxic-ischemic damage in animal models (Hayakawa et al., 1994; Minato et al., 1997; Owen et al., 1997). Interestingly, its neuroprotective action is independent of its antiepileptic activity (Hayakawa et al., 1994).

Furthermore, the potential mechanisms of ZNS action comprise its inhibitory effect on the excessive NO production. In fact, experimental studies have shown that ZNS inhibits NO synthase in the hippocampus following NMDA administration suggesting a possible neuroprotective effect via a reduction of NO formation (Noda et al., 1999). Moreover, the neuroprotective effects of ZNS might be linked to its antioxidant and/or NO-modulating effects (Biton, 2007).

* Corresponding author at: Clinica Neurologica, Università di Perugia, Ospedale S. Maria della Misericordia, S.Andrea delle Fratte, 06156 Perugia, Italy. Fax: +39 0755784229.
E-mail address: calabre@unipg.it (P. Calabresi).
Available online on ScienceDirect (www.sciencedirect.com).

Thus, we have characterized the role of the NO/cGMP/PKG cascade in the induction and maintenance of post-ischemic LTP (iLTP). Moreover, we have also investigated the possible inhibitory action of ZNS on this pathological form of synaptic plasticity as well as the effects of this AED on physiological activity-dependent LTP. Finally, we have characterized the possible interaction between ZNS and the NO/cGMP/PKG dependent pathway involved in iLTP.

Materials and methods

Electrophysiology

All the experiments were conducted in conformity with the European Communities Council Directive of November 1986 (86/609/ECC). Hippocampal slices (thickness, 400 μm) were cut from 1 to 2-month-old male Wistar rats (n = 38) (Harlan, Italy) using a vibratome. Preparation and maintenance of hippocampal slices have been described previously (Costa et al., 2008).

A single slice was transferred to a recording chamber and submerged in a continuously flowing Krebs' solution (34 °C; 2.5–3 ml/min) bubbled with a 95% O_2–5% CO_2 gas mixture. The composition of the solution was as follows (in mol/L): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 10 glucose and 25 $NaHCO_3$.

An Axoclamp 2B amplifier (Molecular Devices, USA) was used for extracellular recordings (Costa et al., 2008). After acquiring a stable baseline for 20 min, LTP was induced by high-frequency stimulation (HFS) consisting of one train of one second at 100 Hz at baseline stimulation intensity. The initial slope of the response was used to assess changes in synaptic strength. In vitro ischemia was delivered by switching the standard Kreb's solution to an artificial cerebrospinal fluid solution in which sucrose replaced glucose, gassed with 95% N_2 and 5% CO_2 (oxygen and glucose deprivation, OGD). As previously reported, iLTP was induced in hippocampal slices by a brief OGD episode (2–2.5 min) (Crepel et al., 1993; Crepel and Ben-Ari, 1996). In these experiments, fEPSPs were evoked by the stimulation of Schaffer collaterals and recorded by an electrode filled with 2 mol/L NaCl (15–20 MΩ) inserted into the CA1 region in presence of an ACSF containing 10 μM bicuculline to block $GABA_A$ receptors, 0.3 mM Mg^{2+} to enhance NMDA receptor-mediated responses and 10 μM glycine to saturate the glycine allosteric site of the NMDA receptors (Crepel et al., 1993; Crepel and Ben-Ari, 1996).

Drugs were bath applied by switching the solution to one containing known concentrations of drugs. Quantitative data are expressed as a percentage of the fEPSP initial slope with respect to the relative control slope values, the latter representing the average response recorded during a stable period (15–20 min). All the drugs used in this study were applied 15–20 min before the onset of the bath application of ischemia solution.

Drugs

Zonisamide was from EISAI (U.K.); 7-NINA, L-NAME, SNAP, ODQ, 8Br-cGMP were from Tocris bioscience (U.K.); Zaprinast was from Sigma-Aldrich (Italy); Rp-8Br-cGMPS was from Merck Chemicals (Italy).

In the present study we have chosen a concentration of 1 μM ZNS because in a previous electrophysiological study on striatal slice preparations (Costa et al., 2010) we found that this dose reduced the effects induced by prolonged in vitro ischemia and rotenone without affecting the current-evoked firing discharge and the amplitude of excitatory postsynaptic potentials.

Statistical analysis

Off-line analysis was performed using Clampfit (Molecular Devices) and Microcal Origin software; Values given in the figures and in the text are mean ± S.E., n representing the number of the slices. Two-way ANOVA was used for statistical analysis. ANOVA for repeated measures was performed (group × time) for all the presented time-courses. The significance level was established at *p < 0.05, **p < 0.01, and ***p < 0.001.

Results

Zonisamide does not affect physiological synaptic transmission and plasticity in the CA1 hippocampal area

Extracellular field EPSPs (fEPSPs) were evoked by stimulating the Schaffer collateral fibers (10 μs duration; 30–50 V intensity) with a bipolar electrode placed in the stratum radiatum of the CA1 region and recorded in the same region with a glass microelectrode. The input–output (I–O) relationships, constructed by plotting the presynaptic fiber volley (FV) amplitude against the mean fEPSP slope, showed no significant difference between the I–O curve from slices incubated with 1 μM ZNS in respect to the one recorded in control condition (n = 12 for each group, p > 0.05; Fig. 1A, left).

To determine whether application of 1 μM ZNS affected the neurotransmitter release from presynaptic terminals at CA1 synapses, we applied a paired-pulse facilitation (PPF) protocol at various interpulse intervals (50–300 ms). These experiments showed no difference in PPF between the control condition and in the presence of ZNS (n = 10 for each group, p > 0.05; Fig. 1A, right). These findings suggest that the basal excitatory synaptic transmission and short-term plasticity were not modified in presence of 1 μM ZNS.

Having observed no changes in short-term forms of synaptic plasticity at the CA3–CA1 synapse, we turned our attention to a more sustained form of potentiation. Thus, we observed a robust LTP induced by high-frequency stimulation in control condition. Also in slices treated with 1 μM ZNS HFS-induced LTP was not altered (n = 14 for each experimental condition, p > 0.05; Fig. 1B).

Zonisamide blocks the induction but not the maintenance of iLTP

A brief episode (2–2.5 min) of OGD produces a transient depression of fEPSP. During reoxygenation, fEPSPs returned to control values within 7–8 min and were subsequently and persistently potentiated. In fact the slope of the fEPSP increased by 69.5 ± 7.7% at 60 min after OGD (n = 8, Fig. 1C). In presence of 1 μM ZNS, however, the amplitude of iLTP was significantly reduced (n = 16, p < 0.001; Fig. 1C). Conversely, when ZNS was applied after the induction of iLTP, it did not alter the amplitude of iLTP suggesting that this AED affects the induction but not the maintenance of this form of long-term synaptic plasticity (n = 16; Fig. 1D).

The NOS inhibitors, L-NAME and 7-NINA, block the induction but not the maintenance of iLTP

The NOS family consists of three isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) (Calabrese et al., 2007). In particular, nNOS, and eNOS have been suggested to play a role in LTP (Hopper and Garthwaite, 2006). Thus, we hypothesized that the blockade of the hippocampal NO production would interfere with the formation of iLTP.

In a first set of experiments we used L-NAME, an inhibitor of both nNOS and eNOS. Incubation of the slices (20 min) in the presence of 50 μM L-NAME prevented the formation of iLTP (n = 12, p < 0.001; Fig. 2A). Similar results were obtained by using 7-NINA, a more selective inhibitor of nNOS (Moore and Handy, 1997). In fact, the incubation of the slices in 30 μM 7-NINA blocked iLTP (n = 12, p < 0.001; Fig. 2A).

We also tested whether L-NAME and 7-NINA could interfere with the maintenance of iLTP. These drugs did not significantly alter the
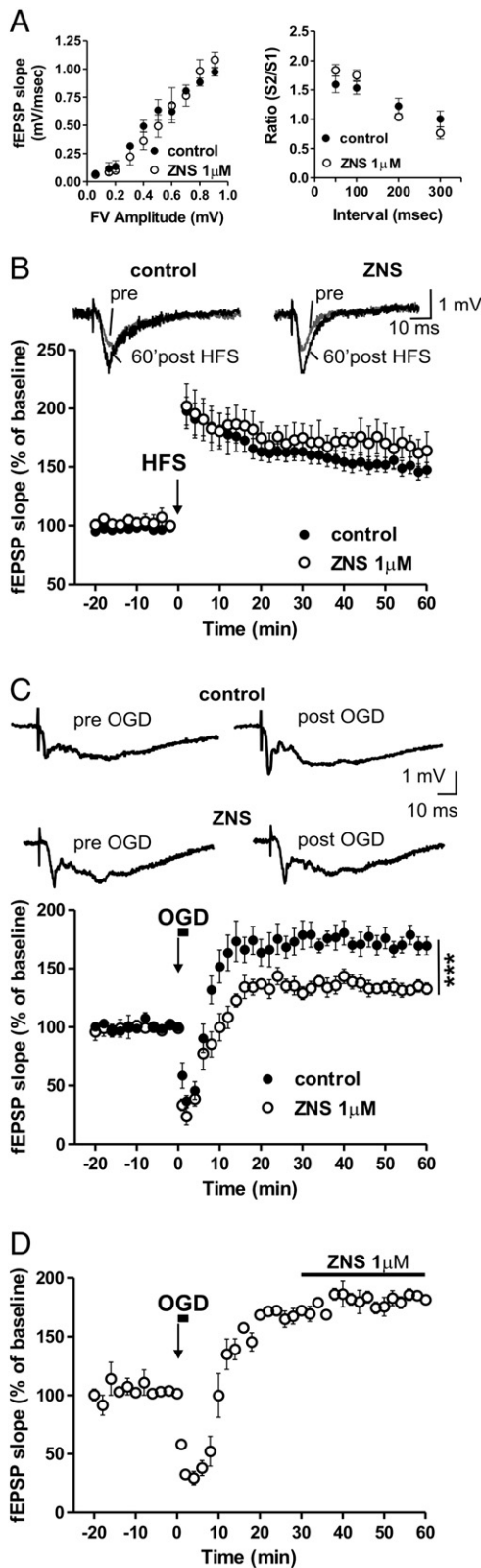
Fig. 1. Zonisamide reduces post-ischemic but not activity-dependent hippocampal LTP. (A) Left, input–output plot of field EPSPs (fEPSPs) recorded in the CA1 hippocampal area was not affected by 1 μM ZNS; Right, the paired-pulse ratio (fEPSP2/fEPSP1), measured at different inter-stimulus interval (50–300 ms), was not altered by ZNS. (B) ZNS did not reduce the physiological LTP induced by high frequency stimulation (HFS). Traces show examples of fEPSPs recorded before and 60 min after the delivery of HFS. (C) LTP induced by oxygen and glucose deprivation (OGD) was significantly reduced by ZNS (***p<0.001). (D) This AED, when applied after the induction of iLTP, did not affect its maintenance.
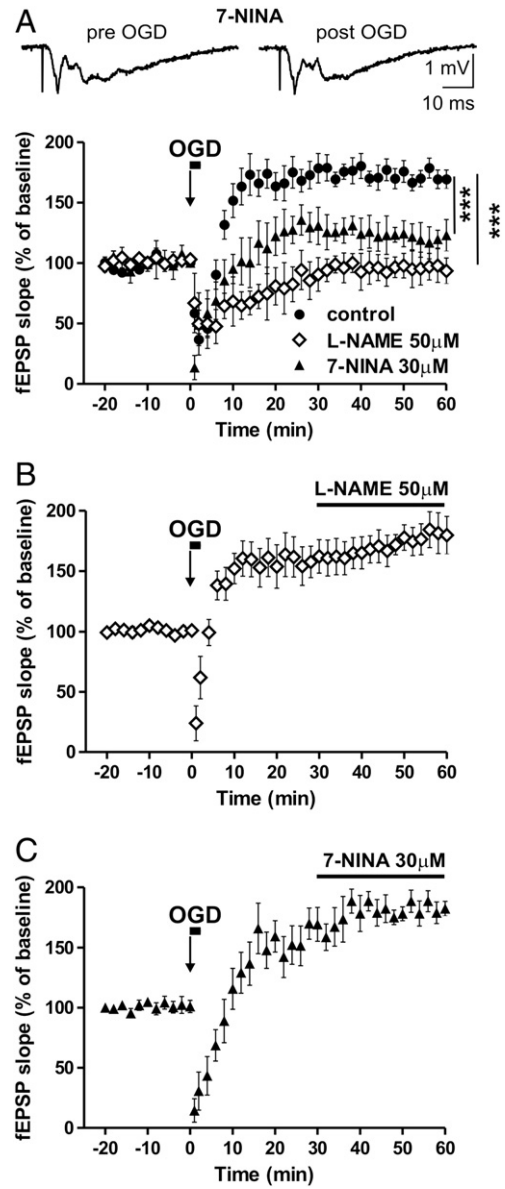

Fig. 2. The NOS inhibitors, L-NAME and 7-NINA, reduce iLTP. (A) The graph shows that both 50 μM L-NAME or 30 μM 7-NINA reduced the induction iLTP (***p<0.001). The upper traces show an example of fEPSP pre and post-OGD in the presence of 7-NINA. (B–C) The graphs show that neither L-NAME nor 7-NINA altered the maintenance of iLTP.

amplitude of iLTP after its induction suggesting that NO is not implicated in the maintenance of this form of synaptic plasticity (n = 12 for each group, p>0.05; Fig. 2BC).

SNAP, a NO donor, restores iLTP blocked by either zonisamide or NOS inhibitors

In order to further investigate the role of ZNS in the induction of the NO-dependent iLTP, we applied ZNS in association with the NO donor SNAP, a drug that did not alter per se the amplitude and the characteristics of iLTP (n = 12; Fig. 3A). Interestingly, while ZNS significantly reduced iLTP (Fig. 1C), the application of 100 μM SNAP plus 1 μM ZNS did not alter the iLTP. In fact, under this experimental condition the iLTP was similar to that observed in control condition (71 ± 17%, n = 12, p>0.05; Fig. 3A). The same result was observed when 100 μM SNAP was incubated with either 30 μM 7-NINA (73 ± 12%,
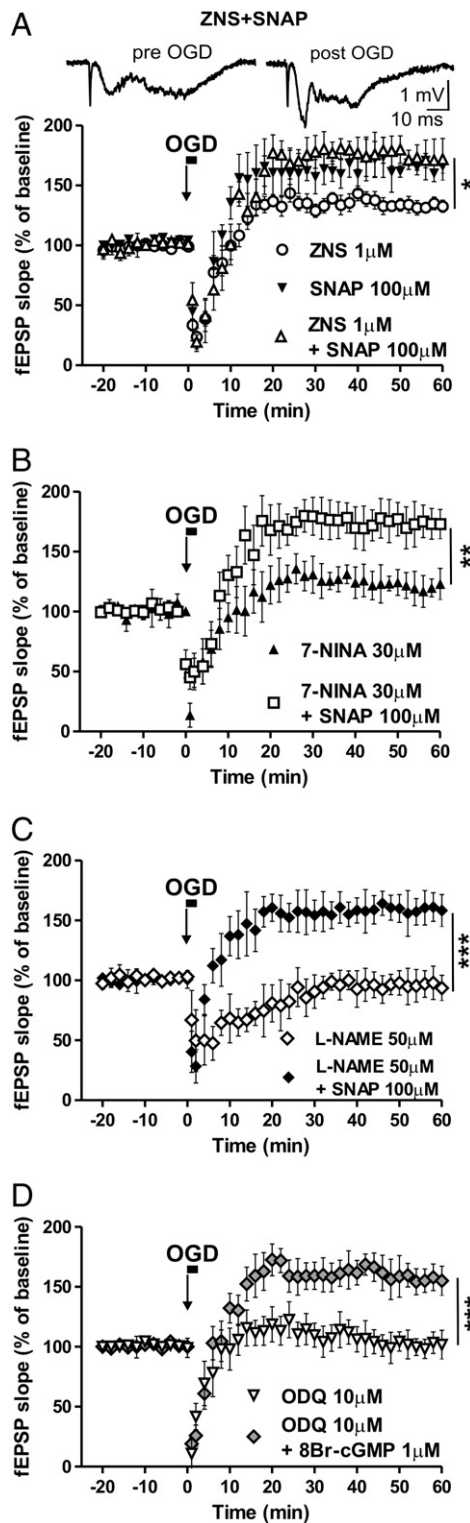
Fig. 3. SNAP, a NO donor, restores iLTP blocked by either zonisamide or NOS inhibitors while the guanylate cyclase inhibitor ODQ blocks it. (A) The graph shows that 100 μM SNAP in the presence of 1 μM ZNS applied before OGD restored iLTP (*p<0.05). The upper traces show an example of fEPSP pre and post-OGD in the presence of ZNS and SNAP. (B–C) This form of synaptic plasticity is also restored when 100 μM SNAP was incubated with either 30 μM 7-NINA (**p<0.01) or 50 μM L-NAME (***p<0.001). (D) The graph shows that 10 μM ODQ, an inhibitor of soluble guanylate cyclase, blocked iLTP; however, under this condition, iLTP was restored by 1 μM 8Br-cGMP, an activator of cGMP-dependent protein kinases.

n = 12, p<0.01; Fig. 3B) or 50 μM L-NAME (58.2 ± 13%, n = 12, p<0.001; Fig. 3C).

The block of iLTP by zonisamide or by a sGC inhibitor is restored by a cGMP analog

NO activates soluble guanylate cyclase (sGC), increasing the formation of cGMP (Boulton et al., 1995) suggesting that cGMP might be involved in the formation of hippocampal iLTP. To analyze this possibility, we inhibited the sGC with ODQ, a potent and selective inhibitor of this enzyme (Boulton et al., 1995), and we found that in presence of 10 μM ODQ, OGD was unable to induce iLTP (n = 12, p<0.001; Fig. 3D).

To further support the involvement of the NO/cGMP/PKG pathway in iLTP, we tested whether 8-Br-cGMP, an activator of cGMP-dependent protein kinases, was able to restore this form of synaptic plasticity even in the presence of either ODQ or ZNS. According to our hypothesis, 1 μM 8-Br-cGMP restored iLTP either in the presence of 10 μM ODQ (n = 12, p<0.001; Fig. 3D) or when co-applied with 1 μM ZNS (n = 12, p<0.001; Fig. 4A).

Zaprinast, a PDE inhibitor, reverses the zonisamide-induced blockade of iLTP

Because cGMP can regulate the activity of the cAMP degrading enzyme phosphodiesterase (PDE), we examined whether increases in cGMP levels can modulate iLTP, through effects on cAMP signaling. For this reason we used zaprinast, an inhibitor of cGMP-specific PDE, to elevate cGMP levels. We found that while 1 μM zaprinast did not affect per se iLTP, it was able to restore this pathological form of synaptic plasticity even in the presence of 1 μM ZNS (n = 16 for each group, p<0.001; Fig. 4B).

The PKG inhibitor Rp-8Br-cGMPS blocks iLTP

The final demonstration that iLTP requires the activation of a NO/cGMP/PKG dependent pathway, was achieved by utilizing the PKG-specific inhibitor, Rp-8Br-cGMPS. In the presence of 1 μM Rp-8Br-cGMPS iLTP was blocked (n = 8, p<0.001; Fig. 4C). Moreover, this inhibitor was also able to block the effects on iLTP induced by either 100 μM SNAP or 1 μM 8Br-cGMP (n = 8 for each experimental condition, p<0.001; Fig. 4C).

Discussion

The present study provides two major findings having both pathophysiological and clinical implications. Firstly, we have shown as a novel finding that the NO/cGMP/PKG pathway is involved in the induction of iLTP. As a second major result of this study, we suggest that a low concentration of the AED ZNS exerts a possible selective neuroprotective effect by reducing iLTP through its ability to modulate the NO/cGMP/PKG pathway.

NO/cGMP signaling cascade has been suggested to participate in LTP and a role for NO as a retrograde messenger has been postulated (O'Dell et al., 1991; Schuman and Madison, 1991). Furthermore, NO is involved in the pathophysiology of brain ischemia (Di Filippo et al., 2008; Dirnagl et al., 1999; Iadecola, 1997). Activation of NMDA receptors and the rise of intracellular Ca^{2+} are critical steps in the induction of this phenomenon both in the hippocampus and the striatum (Crepel et al., 2003; Di Filippo et al., 2008; Hsu and Huang, 1998). Accordingly, pharmacological antagonists, selectively targeting NR2B subunit containing NMDA receptors and uncompetitive NMDA receptor antagonists, such as memantine, block striatal i-LTP (Picconi et al., 2006; Tozzi et al., 2007). Moreover, the induction of anoxic LTP requires the activation of postsynaptic protein-kinase C (PKC) (Hsu and Huang, 1998).
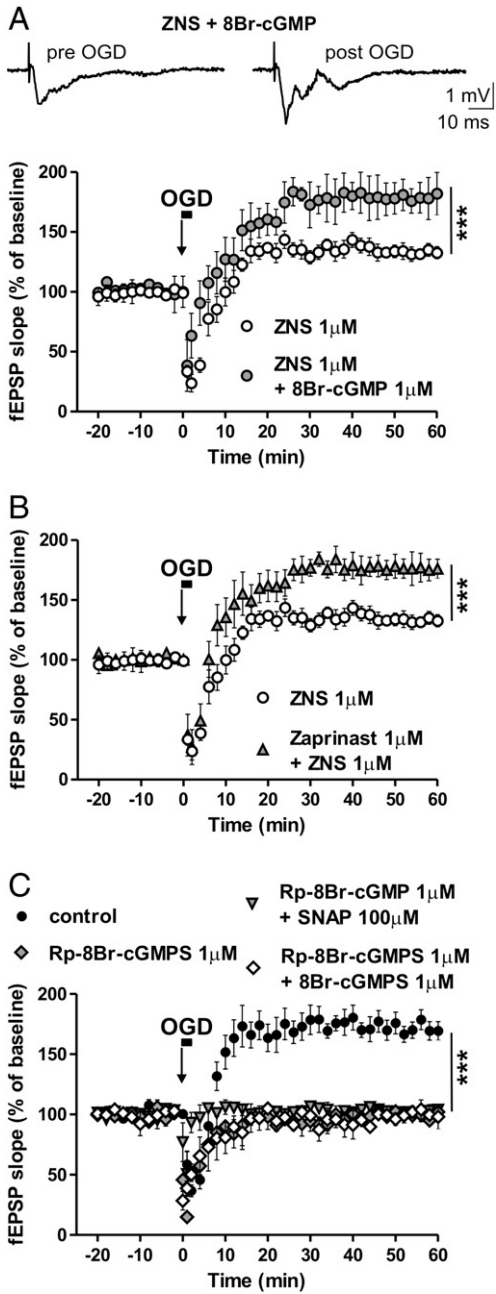
Fig. 4. cGMP and PKG play a critical role in the induction of iLTP. (A) The cGMP analog 8Br-cGMP was able to reverse the inhibitory effect of ZNS on iLTP. (B) Zaprinast (1 μM), an inhibitor of cGMP-specific phosphodiesterases, reversed the effect of ZNS on iLTP. (C). The PKG inhibitor Rp-8Br-cGMPS (1 μM) blocked iLTP and the effects of SNAP and 8Br-cGMP.

In the present study, we demonstrated that the endogenous NO plays a critical role in the induction of hippocampal iLTP. In fact, iLTP is blocked by either L-NAME, a non-selective NOS inhibitor, or 7-NINA, a relatively more selective inhibitor of nNOS. Furthermore, the finding that NOS inhibitors do not modify the iLTP once established indicates that NO production is necessary for the induction of this form of pathological synaptic plasticity, but not for its maintenance. Contradictory results supporting both toxic and protective effects of NOS inhibition have been published (Holscher, 1997). This dualism might be related to the different isoforms of NOS implicated in the pathophysiology of brain ischemia (Iadecola, 1997).

Interestingly, the extracellular levels of cGMP are increased in brain ischemia (Fedele and Raiteri, 1999) suggesting that in addition to NO, also the cGMP-dependent pathway operates in this condition.

Accordingly, in our study we found that the application of ODQ, a selective inhibitor of sGC (Garthwaite et al., 1995), abolished iLTP. Moreover, 8Br-cGMP, an analog of cGMP, was able to restore this pathological synaptic plasticity when applied in the presence of ODQ. In line with this finding, we observed that in the presence of Rp-8Br-cGMPS, an inhibitor of PKG, neither SNAP nor 8Br-cGMP could restore iLTP, further supporting the critical role of the NO/cGMP/PKG pathway.

Taken together these findings suggest a scenario in which iLTP, occurring at the CA3–CA1 synapse, is dependent on the activation of NOS in the postsynaptic neuron in agreement with previous studies on the involvement of NO in anoxia-induced LTP (Huang and Hsu, 1997). Nitric oxide acting as a retrograde messenger might diffuse to activate the cGMP-PKG pathway in the presynaptic neuronal terminals (Fig. 5) in line with previous studies reporting that anoxic hippocampal CA1 LTP involves both postsynaptic Ca^{2+} entry, via NMDA receptors and high voltage activated Ca^{2+} channels (HVA), and a presynaptic site of activity (Crepel et al., 2003; Hsu and Huang, 1997). NOS immunoreactivity has in fact been detected into the
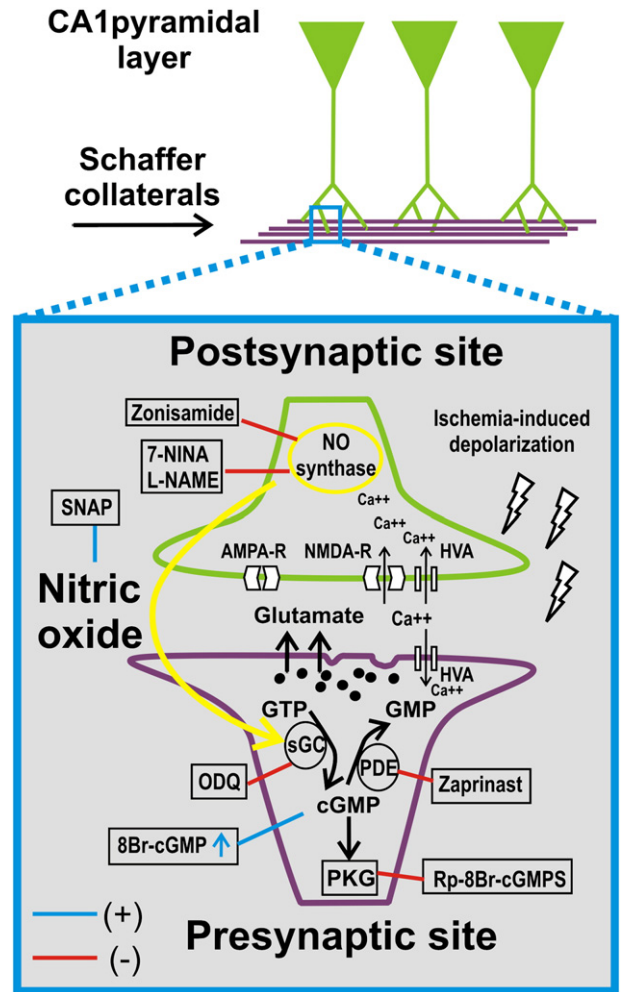

Fig. 5. Representation of the involvement of nitric oxide in the ischemic LTP in the CA1 hippocampal region. The scheme shows that an ischemia-dependent membrane depolarization triggers modifications in the pre-and post-synaptic neurons of the CA3–CA1 synapse. A calcium mediated activation of nitric oxide synthase (NOS) in postsynaptic sites of the hippocampal CA1 region determines nitric oxide (NO) production. The activation of cGMP/PKG intracellular pathway by the retrograde messenger NO in the presynaptic sites might modulate glutamate release and trigger ischemic LTP together with the modification occurring in the postsynaptic neuron. Drugs acting on NOS and on the cGMP/PKG intracellular pathway are shown in black boxes. Red line represents a blockade of enzymatic function; blue line represents the enhancement of the indicated molecule function.

pyramidal neurons of the CA1 hippocampal region (Wendland et al., 1994). Furthermore, immunogold electron microscopy revealed in the stratum radiatum of CA1 the presence of nNOS within the postsynaptic density of asymmetric axospinous synapses and the guanylate cyclase concentrated in the axon terminals thus providing a further neuroanatomical evidence in support of the hypothesis that NO can act as a synapse-specific retrograde messenger in CA1 (Burette et al., 2002). However, the release of NO from other hippocampal cell types, including CA1 interneurons and glial cells, can also not be excluded.

We also demonstrated that ZNS, a new antiepileptic drug, was able to exert a possible neuroprotective action by inhibiting iLTP. It is worth noting that this effect was achieved at a very low concentration (1 μM), possibly lower than that one required for its antiepileptic effect (Biton, 2007). In line with this hypothesis, we found that this low concentration of ZNS did not affect hippocampal basal excitatory synaptic transmission and PPF (present study) as well as the striatal synaptically-evoked and current-induced excitability (Costa et al., 2010). Moreover, in this study we have demonstrated that 1 μM ZNS is able to reduce the iLTP without affecting the physiological LTP, induced by the HFS protocol in hippocampal slices. Since HFS-induced CA1 hippocampal LTP has been considered a cellular substrate for learning and memory (Malenka and Nicoll, 1999), this latter finding might suggest that the ZNS-induced neuroprotection could be achieved in the absence of cognitive alterations. However, it is also possible that a single 100 Hz stimulation is not sufficient to produce NO-dependent LTP (Phillips et al., 2008).

We also found that ZNS reduced pathological iLTP, when administered before the ischemic insult while post-ischemic administration did not result in a significant modification of this form of plasticity. This result agrees with the observation that the pre-treatment is more effective than the administration after the ischemic insult (Hayakawa et al., 1994; Minato et al., 1997).

The mechanism of the neuroprotective action of ZNS is not completely understood yet. Some AEDs present neuroprotective effects modulating the excitatory synaptic transmission, the abnormal intrinsic excitability, and the inhibitory GABAergic transmission (Calabresi et al., 2003b; Costa et al., 2004, 2006). Conversely, ZNS seems to have a specific pharmacological profile. In fact, it reduces the production of free radicals in the hippocampus during kainate-induced seizures (Ueda et al., 2005) showing antioxidant properties (Biton, 2007).

A reduction of NO is one possible reason for the neuroprotective properties of ZNS since a low concentration of ZNS, in the presence of SNAP, a NO donors, is able to restore iLTP. The same results were obtained adding to ZNS either an analog of cGMP or an inhibitor of cGMP-degrading phosphodiesterase.

Conclusions

Our results suggest that ZNS is able to block pathological synaptic plasticity by modulation of the NO/cCMP/PKG pathway and they are in line with the observation that ZNS inhibits the initiation and propagation of hippocampal seizures by inhibiting NOS activity (Noda et al., 1999).

In conclusion we found that NO/cGMP/PKG pathway exerts a critical role in the induction the hippocampal iLTP and ZNS, at a non-anticonvulsant dose, reduces this pathological plasticity by modulating this biochemical pathway. Further in vivo studies are required to demonstrate that ZNS has a potential neuroprotective efficacy in the treatment of ischemic stroke and other neurological disorders (Rosler et al., 2010).

References

Arancio, O., Kiebler, M., Lee, C.J., Lev-Ram, V., Tsien, R.Y., Kandel, E.R., Hawkins, R.D., 1996. Nitric oxide acts directly in the presynaptic neuron to produce long-term potentiation in cultured hippocampal neurons. Cell 87, 1025–1035.
Biton, V., 2007. Clinical pharmacology and mechanism of action of zonisamide. Clin. Neuropharmacol. 30, 230–240.
Bohme, G.A., Bon, C., Stutzmann, J.M., Doble, A., Blanchard, J.C., 1991. Possible involvement of nitric oxide in long-term potentiation. Eur. J. Pharmacol. 199, 379–381.
Boulton, C.L., Southam, E., Garthwaite, J., 1995. Nitric oxide-dependent long-term potentiation is blocked by a specific inhibitor of soluble guanylyl cyclase. Neuroscience 69, 699–703.
Burette, A., Zabel, U., Weinberg, R.J., Schmidt, H.H.H.W., Valtschanoff, J.G., 2002. Synaptic localization of nitric oxide synthase and soluble guanylyl cyclase in the hippocampus. J. Neurosci. 22, 8961–8970.
Calabrese, V., Bates, T.E., Stella, A.M., 2000. NO synthase and NO-dependent signal pathways in brain aging and neurodegenerative disorders: the role of oxidant/antioxidant balance. Neurochem. Res. 25, 1315–1341.
Calabrese, V., Mancuso, C., Calvani, M., Rizzarelli, E., Butterfield, D.A., Stella, A.M., 2007. Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. Nat. Rev. Neurosci. 8, 766–775.
Calabresi, P., Centonze, D., Pisani, A., Cupini, L., Bernardi, G., 2003a. Synaptic plasticity in the ischaemic brain. Lancet Neurol. 2, 622–629.
Calabresi, P., Cupini, L.M., Centonze, D., Pisani, F., Bernardi, G., 2003b. Antiepileptic drugs as a possible neuroprotective strategy in brain ischemia. Ann. Neurol. 53, 693–702.
Costa, C., Leone, G., Saulle, E., Pisani, F., Bernardi, G., Calabresi, P., 2004. Coactivation of GABA(A) and GABA(B) receptor results in neuroprotection during in vitro ischemia. Stroke 35, 596–600.
Costa, C., Martella, G., Picconi, B., Prosperetti, C., Pisani, A., Di Filippo, M., Pisani, F., Bernardi, G., Calabresi, P., 2006. Multiple mechanisms underlying the neuroprotective effects of antiepileptic drugs against in vitro ischemia. Stroke 37, 1319–1326.
Costa, C., Belcastro, V., Tozzi, A., Di Filippo, M., Tantucci, M., Siliquini, S., Autuori, A., Picconi, B., Spillantini, M.G., Fedele, E., Pittaluga, A., Raiteri, M., Calabresi, P., 2008. Electrophysiology and pharmacology of striatal neuronal dysfunction induced by mitochondrial complex I inhibition. J. Neurosci. 28, 8040–8052.
Costa, C., Tozzi, A., Luchetti, E., Siliquini, S., Belcastro, V., Tantucci, M., Picconi, B., Ientile, R., Calabresi, P., Pisani, F., 2010. Electrophysiological actions of zonisamide on striatal neurons: selective neuroprotection against complex I mitochondrial dysfunction. Exp. Neurol. 221, 217–224.
Crepel, V., Ben-Ari, Y., 1996. Intracellular injection of a Ca²⁺ chelator prevents generation of anoxic LTP. J. Neurophysiol. 75, 770–779.
Crepel, V., Hammond, C., Chinestra, P., Diabira, D., Ben-Ari, Y., 1993. A selective LTP of NMDA receptor-mediated currents induced by anoxia in CA1 hippocampal neurons. J. Neurophysiol. 70, 2045–2055.
Crepel, V., Epsztein, J., Ben-Ari, Y., 2003. Ischemia induces short- and long-term remodeling of synaptic activity in the hippocampus. J. Cell. Mol. Med. 7, 401–407.
Di Filippo, M., Tozzi, A., Costa, C., Belcastro, V., Tantucci, M., Picconi, B., Calabresi, P., 2008. Plasticity and repair in the post-ischemic brain. Neuropharmacology 55, 353–362.
Dirnagl, U., Iadecola, C., Moskowitz, M.A., 1999. Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci. 22, 391–397.
Fedele, E., Raiteri, M., 1999. In vivo studies of the cerebral glutamate receptor/NO/cGMP pathway. Prog. Neurobiol. 58, 89–120.
Garthwaite, J., Southam, E., Boulton, C.L., Nielsen, E.B., Schmidt, K., Mayer, B., 1995. Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one. Mol. Pharmacol. 48, 184–188.
Hayakawa, T., Higuchi, Y., Nigami, H., Hattori, H., 1994. Zonisamide reduces hypoxic-ischemic brain damage in neonatal rats irrespective of its anticonvulsive effect. Eur. J. Pharmacol. 257, 131–136.
Holscher, C., 1997. Nitric oxide, the enigmatic neuronal messenger: its role in synaptic plasticity. Trends Neurosci. 20, 298–303.
Hopper, R.A., Garthwaite, J., 2006. Tonic and phasic nitric oxide signals in hippocampal long-term potentiation. J. Neurosci. 26, 11513–11521.
Hsu, K.S., Huang, C.C., 1997. Characterization of the anoxia-induced long-term synaptic potentiation in area CA1 of the rat hippocampus. British J. Pharmacol. 122, 671–681.
Hsu, K.S., Huang, C.C., 1998. Protein kinase C inhibitors block generation of anoxia-induced long-term potentiation. Neuroreport 9, 3525–3529.
Huang, C.C., Hsu, K.S., 1997. Nitric oxide signalling is required for the generation of anoxia-induced long-term potentiation in the hippocampus. Eur. J. Neurosci. 9, 2202–2206.
Iadecola, C., 1997. Bright and dark sides of nitric oxide in ischemic brain injury. Trends Neurosci. 20, 132–139.
Malenka, R.C., Nicoll, R.A., 1999. Long-term potentiation: a decade of progress? Science 285, 1870–1874.

Minato, H., Kikuta, C., Fujitani, B., Masuda, Y., 1997. Protective effect of zonisamide, an antiepileptic drug, against transient focal cerebral ischemia with middle cerebral artery occlusion-reperfusion in rats. Epilepsia 38, 975–980.

Moore, P.K., Handy, R.L., 1997. Selective inhibitors of neuronal nitric oxide synthase: is no NOS really good NOS for the nervous system? Trends Pharmacol. Sci. 18, 204–211.

Noda, Y., Mori, A., Packer, L., 1999. Zonisamide inhibits nitric oxide synthase activity induced by N-methyl-D-aspartate and buthionine sulfoximine in the rat hippocampus. Res. Commun. Mol. Pathol. Pharmacol. 105, 23–33.

O'Dell, T.J., Hawkins, R.D., Kandel, E.R., Arancio, O., 1991. Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. Proc. Natl. Acad. Sci. U. S. A. 88, 11285–11289.

Owen, A.J., Ijaz, S., Miyashita, H., Wishart, T., Howlett, W., Shuaib, A., 1997. Zonisamide as a neuroprotective agent in an adult gerbil model of global forebrain ischemia: a histological, in vivo microdialysis and behavioral study. Brain Res. 770, 115–122.

Phillips, K.G., Hardingham, N.R., Fox, K., 2008. Postsynaptic action potentials are required for nitric-oxide-dependent long-term potentiation in CA1 neurons of adult GluR1 knock-out and wild-type mice. J. Neurosci. 28, 14031–14041.

Picconi, B., Tortiglione, A., Barone, I., Centonze, D., Gardoni, F., Gubellini, P., Bonsi, P., Pisani, A., Bernardi, G., Di Luca, M., Calabresi, P., 2006. NR2B subunit exerts a critical role in postischemic synaptic plasticity. Stroke 37, 1895–1901.

Rosler, T.W., Arias-Carrion, O., Hoglinger, G.U., 2010. Zonisamide: aspects in neuroprotection. Exp. Neurol. 224, 336–339.

Schuman, E.M., Madison, D.V., 1991. A requirement for the intercellular messenger nitric oxide in long-term potentiation. Science 254, 1503–1506.

Tozzi, A., Costa, C., Di Filippo, M., Tantucci, M., Siliquini, S., Belcastro, V., Parnetti, L., Picconi, B., Calabresi, P., 2007. Memantine reduces neuronal dysfunctions triggered by in vitro ischemia and 3-nitropropionic acid. Exp. Neurol. 207, 218–226.

Ueda, Y., Doi, T., Tokumaru, J., Nakajima, A., Nagatomo, K., 2005. In vivo evaluation of the effect of zonisamide on the hippocampal redox state during kainic acid-induced seizure status in rats. Neurochem. Res. 30, 1117–1121.

Wendland, B., Schweizer, F.E., Ryan, T.A., Nakane, M., Murad, F., Sheller, R.H., Tsien, R.W., 1994. Existence of nitric oxide synthase in rat hippocampal pyramidal cells. Proc. Natl. Acad. Sci. U. S. A. 91, 2151–2155.