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# Electrophysiological actions of zonisamide on striatal neurons: Selective neuroprotection against complex I mitochondrial dysfunction

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ABSTRACT

Since the anti-epileptic drug Zonisamide (ZNS) seems to exert beneficial effects in Parkinson's (PD) disease, we have investigated the electrophysiological effects of ZNS in a rat corticostriatal slice preparation. ZNS affected neither the resting membrane potential nor the input resistance of the putative striatal spiny neurons. In contrast, this drug depressed in a dose-dependent manner the current-evoked repetitive firing discharge with a $EC_{50}$ value of 16.38 μM. ZNS also reduced the amplitude of glutamatergic excitatory postsynaptic potentials (EPSPs) with a $EC_{50}$ value of 32.5 μM.

Reduced activity of the mitochondrial respiratory chain, particularly complex I and II, is implicated in the pathophysiology of PD and Huntington's (HD) diseases, respectively. Thus, ZNS was also tested in two different *in vitro* neurotoxic models obtained by acutely exposing corticostriatal slices either to rotenone, a selective inhibitor of mitochondrial complex I, or to 3-nitropropionic acid (3-NP), an inhibitor of complex II. Additionally, we also investigated the effect of ZNS in an *in vitro* model of brain ischemia. Interestingly, low concentrations of ZNS (0.3, 1, 3 and 10 μM) significantly reduced the rotenone-induced toxicity protecting striatal slices from the irreversible loss of corticostriatal field potential (FP) amplitude *via* a GABA-mediated mechanism. Conversely, this drug showed no protection against 3-NP and ischemia-induced toxicity.

Our data indicate that relatively high doses of ZNS are required to decrease striatal neuronal excitability while low concentrations of this drug are sufficient to protect striatum against mitochondrial impairment suggesting its possible use in the therapy of basal ganglia neurodegenerative diseases.



## Introduction

Neuronal hyper-excitability, mitochondrial dysfunction, and oxidative stress have been implicated in the pathogenesis of different neurological disorders, including stroke, epilepsy, amyotrophic lateral sclerosis, Alzheimer's and Huntington's disease (HD) but the evidence is particularly strong in Parkinson's disease (PD) (Calabresi et al., 1996, 2007; Di Filippo et al., 2006; Mattson et al., 2008).

Glutamate-mediated excitotoxicity is believed to substantially contribute to neuronal death during the degenerative process (Greene and Greenamyre, 1996, Luetjens et al., 2000 and Calabresi et al., 2001). However, whether impaired activity of mitochondrial complex I and II leads to striatal neuron degeneration through similar or different mechanisms is unknown.

Advances in understanding the pathogenesis of the above-mentioned diseases allowed the development of new pharmacological approaches with different mechanisms of action. Antiepileptic drugs (AEDs) have been proposed as putative neuroprotective agents (Calabresi et al., 2003). The major common goal of the pharmacological treatment with AEDs is to counteract abnormal brain excitability by either decreasing the excitatory transmission or enhancing neuronal inhibition (Calabresi et al., 2000c; Rogawski and Loescher, 2004). Some AEDs have proven to modulate the excitatory corticostriatal transmission, the abnormal striatal excitability and/or the intrinsic striatal GABAergic transmission (Costa et al., 2004, 2006). Among the various AEDs, Carbamazepine (CBZ) exerts a potent neuroprotective action against rotenone-induced striatal neuronal dysfunction (Costa et al., 2008) and the use of various AEDs as a possible neuroprotective strategy against brain ischemia provided also encouraging experimental results (Calabresi et al., 2003; Costa et al., 2004, 2006).

Interestingly, in a recent double-blind controlled study the new AED Zonisamide (ZNS) has shown antiparkinsonian actions (Murata

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et al., 2007). An adjunctive treatment with low dose of ZNS to levodopa has induced improvement of all the cardinal symptoms of PD (Murata et al., 2007). Experimentally, a single ZNS dose increased intracellular and extracellular DOPA, dopamine (DA) and homovanillic acid (HVA) levels and decreased dihydroxyphenylacetic acid (DOPAC) levels in the rat striatum presumably through its moderate MAO-inhibiting effects (Okada et al., 1992, 1995). A ZNS-induced increase in dopamine synthesis has been also hypothesized (Murata et al., 2007).

Given these premises, the characterization of electrophysiological effects produced by ZNS on the striatal intrinsic and synaptic excitability might offer possible insights for the treatment of neurodegenerative disorders. In this study, therefore, we have analyzed the effects of ZNS in rat striatal slice preparations by means of intracellular and field potential recordings.

In addition, we have tested the neuroprotective effect of ZNS in two *in vitro* neurotoxic models obtained following the acute administration of selective inhibitors of complex I (rotenone) and II (3-nitropropionic acid, 3-NP) of the mitochondrial respiratory chain. In fact, a reduced activity of complex I has been found in PD patients (Schapira et al., 1998; Büeler, 2009), and chronic treatment with rotenone mimics PD in animal models (Ferrante et al., 1997; Betarbet et al., 2000; Höglinger et al., 2003; Sherer et al., 2007) while a defect of complex II has been found in HD (Browne et al., 1997; Beal, 1998; Di Filippo et al., 2006). We have also characterized the possible protective effects of ZNS against the permanent loss of electric activity caused by *in vitro* ischemia induced by combined oxygen and glucose deprivation.

## Materials and methods

### *Brain preparations and solutions*

All the experiments were conducted in conformity with the European Communities Council Directive of November 1986 (86/609/ECC) and in accordance with a protocol approved by the Animal Care and Use Committee at the University of Perugia. Preparation and maintenance of rat corticostriatal slices have been previously described (Calabresi et al., 1998; Picconi et al., 2003). Briefly, corticostriatal coronal slices (thickness, 270 μm) were cut from 1- to 2-month-old male Wistar rats (Harlan, Italy) using a vibratome. A single slice was then transferred to a recording chamber and submerged in a continuously flowing Kreb's solution (34 °C; 2.5–3 ml/min) bubbled with a 95% $O_2$ and 5% $CO_2$ gas mixture. The composition of the solution was (in mM) 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 $NaH_2PO_4$, 2.4 $CaCl_2$, 10 glucose, and 25 $NaHCO_3$. Drugs were bath-applied by switching the solution to one containing known concentrations of drugs. *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% $CO_2$. The ischemic solution was bath applied for 10 min during field potential recordings and for 2 min in a $Mg^{2+}$-free Kreb's solution during intracellular recordings. Rotenone for *in vitro* experiments was solved in DMSO. The final concentration was reached by dissolving it into the standard Kreb's solution 1 μM rotenone was applied on the slice by adding it to the standard medium for the duration of the experiment. DMSO alone, at least at the concentration used to solve rotenone, did not cause any detectable electrophysiological effect. Total replacement of the medium in the chamber occurred within 1 min.

### *Electrophysiology*

Intracellular recordings of striatal medium spiny neurons were obtained by using sharp microelectrodes pulled from borosilicate glass pipettes backfilled with 2 mol/l KCl (30–60 MΩ). An Axoclamp 2B amplifier (Molecular Devices, USA) was connected in parallel to an oscilloscope (Gould, USA) to monitor the signal in "bridge" mode and to a PC for acquisition of the traces using a pClamp9 software (Molecular Devices, USA). After the impalement of the neuron, a small amount of current (5–20 pA) might be injected via the recording electrode when necessary. Only neurons electrophysiologically identified as spiny neurons were considered for experiment (Calabresi et al., 1998).

Electrodes for extracellular recordings were filled with 2 mol/l NaCl (15–20 MΩ). An Axoclamp 2B amplifier (Molecular Devices, USA) was used for extracellular recordings. The field potential (FP) amplitude was defined as the average of the amplitude from the peak of the early positivity to the peak negativity and the amplitude from the peak negativity to peak late positivity. A glutamatergic corticostriatal synaptic excitatory postsynaptic potential (EPSP) or a FP was evoked every 10 s by means of a bipolar electrode connected to a stimulator unit (Grass Telefactor, USA). The stimulating electrode was located in the white matter between the cortex and the striatum to activate corticostriatal fibers. The recording electrodes were invariably placed within the dorso-lateral striatum. Quantitative data are expressed as a percentage of the field potential or of the EPSP amplitudes in respect to the relative control amplitude values, the latter representing the mean of responses recorded during a stable period (15 to 20 min). Off-line analysis was performed using Clampfit (Molecular Devices, USA) and GraphPad Prism 3.0 (GraphPad software) software.

### *Drugs*

Powders were dissolved in water or DMSO and then stored at −20 °C in aliquots. Each aliquot was only used the day of experiment and then discarded. Drugs were applied by dissolving them to the desired final concentration in the external Kreb's solution. Zonisamide was from EISAI (London, UK); Rotenone, (−)-Bicuculline methiotide and 3-Nitropropionic acid were from Sigma-Aldrich (Milan, Italy).

### *Statistical analysis*

Analysis of variance (ANOVA), followed by Bonferroni's *post hoc* test, and Student's *t*-test were used for statistical analysis. Values given in the figures and text are mean ± standard error of the mean (SEM). The significance level was established at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

## Results

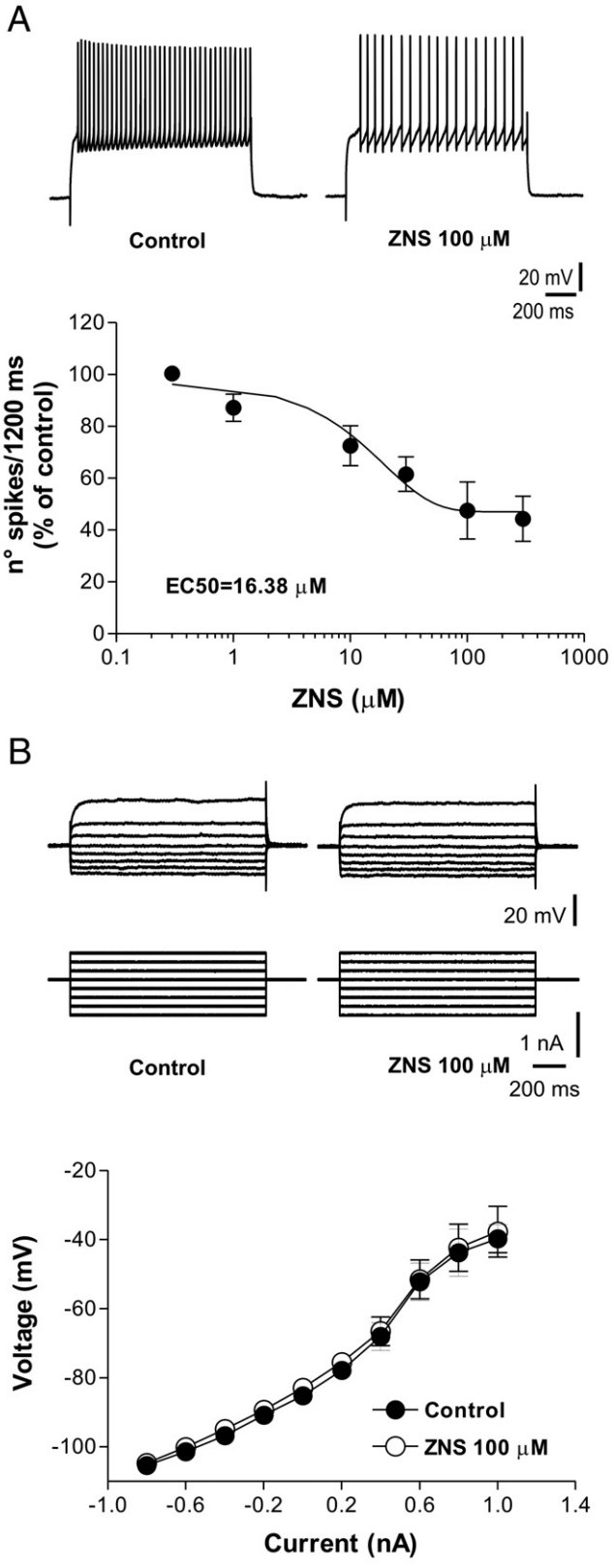
### *Effects of ZNS on intrinsic membrane properties of striatal spiny neurons*

In this study we have analyzed the electrophysiological properties of ZNS on principal spiny neurons recorded in rat corticostriatal slice preparations.

Conventional sharp microelectrode intracellular recordings were obtained from 75 electrophysiologically identified striatal spiny neurons. These cells had high resting membrane potential (−84 ± 5 mV), relatively low apparent input resistance (38 ± 8 MΩ) when measured at the resting potentials from the amplitude of small (<10 mV) hyperpolarizing electrotonic potentials, action potentials of short duration (1.1 ± 0.3 ms) and high amplitude (102 ± 4 mV).

Striatal spiny neurons respond to a depolarizing pulse of current with a sustained repetitive firing of action potentials (Calabresi et al., 1998). ZNS reduced, in a dose-dependent manner (1–300 μM), the repetitive firing discharge frequency produced by depolarizing steps of current ($n=7$ for each concentration). The action potential frequency discharge was, in fact, reduced by 47.53 ± 10.97 % in the presence of 100 μM ZNS, with respect to control conditions ($n=7$, $p<0.001$; Fig. 1A). Lower doses failed to significantly reduce firing

discharge. The potency of ZNS on the inhibition of firing discharge, expressed as the extrapolated $EC_{50}$ value, was 16.38 μM. ZNS, similarly to other AEDs (phenytoin, lamotrigine and gabapentin) (Calabresi et al., 1999), affected neither the membrane potential nor the input resistance and the current–voltage relationship of the recorded cells ($n=7$ for each concentration, $p=0.98$; Fig. 1B).




*Effects of ZNS on glutamatergic excitatory synaptic transmission*

Excitatory glutamatergic FPs were recorded from the striatum of corticostriatal rat slices as previously described (Costa et al., 2004). In electrophysiological experiments ($n=55$), the white matter between cortex and striatum was stimulated to obtain FP of $1.2\pm0.3$ mV in amplitude and $2\pm0.5$ ms in duration. Stimuli ($0.03\pm0.01$ ms and 1 to 5 V) were delivered at a frequency of 0.1 Hz to monitor the time course of FP amplitude. The application of 0.3 μM, 1 μM, 3 μM, 10 μM and 30 μM ZNS for 10 min did not alter the amplitude of the FP ($n=7$ for each concentration $p>0.05$). Conversely, 100 μM and 300 μM of ZNS were able to decrease the FPs by about $11\pm1.3\%$ and $23\pm6.9\%$, respectively ($n=12$ for each concentration $p<0.05$; Fig. 2A).

Striatal spiny neurons respond to a single cortical stimulation by producing an excitatory postsynaptic potential (EPSP). In this study, we have also analyzed the effects of ZNS on EPSP amplitude and we have found that 10–300 μM of ZNS, bath-applied for 10–20 min, reduced the corticostriatal EPSP in a dose-dependent manner ($n=7$ for each concentration, $p<0.05$; Fig. 2B). Also, this effect was concentration-dependent and the potency of ZNS, expressed as the extrapolated $EC_{50}$ value, was 32.5 μM.

In order to investigate whether the depression of EPSPs by ZNS was dependent on pre- and/or postsynaptic sites of action, we measured synaptic responses to a pair of stimuli before and during the applications of two different concentrations of ZNS (Fig. 2C). In these experiments, the inter-stimulus interval was 60 ms. Paired-pulse modification of neurotransmission has been studied extensively and is attributed to a presynaptic change in release probability (Manabe et al., 1993; Schulz et al., 1994; Calabresi et al., 1999). The analysis of the paired-pulse ratio (EPSP2/EPSP1) revealed that the depression of the EPSP amplitude, induced by either 30 or 100 μM ZNS, was not associated with a significant increase of the EPSP ratio indicating that the effect of ZNS does not involve only a presynaptic site of action ($n=8$; $p=0.98$; Fig. 2C).

*Effects of ZNS on the electrophysiological action induced by acute intoxication with rotenone*

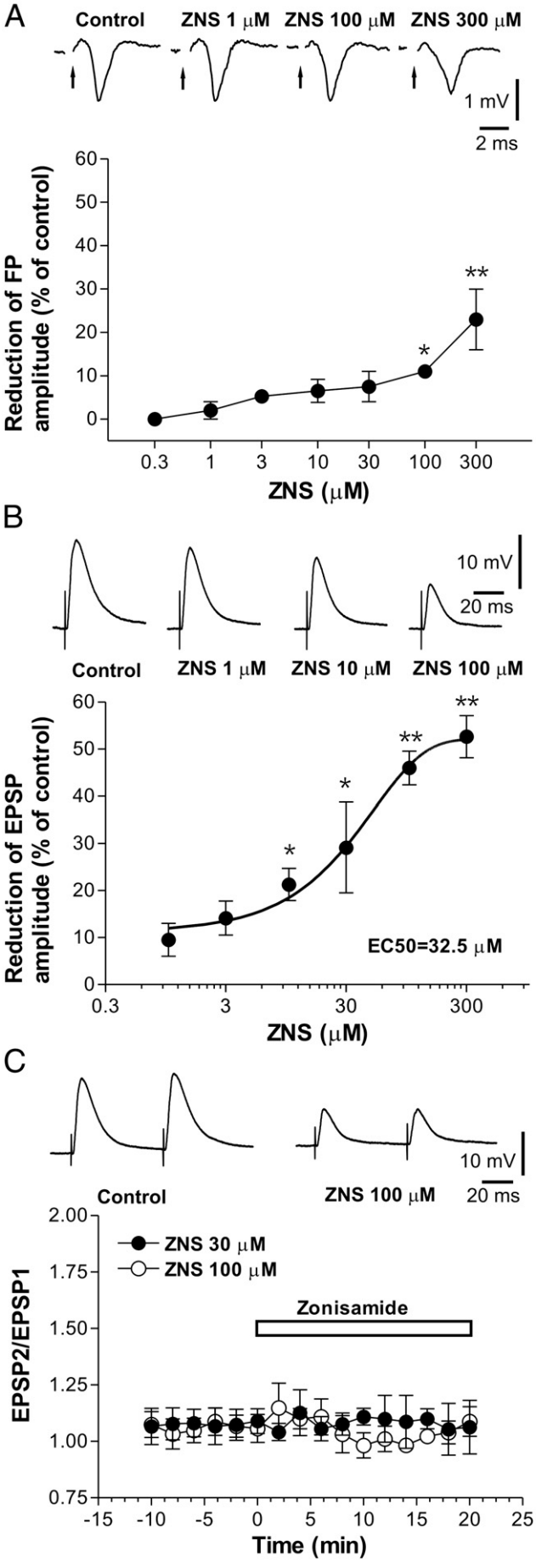
To test whether inhibition of mitochondrial complex I by rotenone was able to alter the electrical neuronal activity in a corticostriatal brain slice preparation, FPs were recorded from the striatum after the activation of glutamatergic inputs.

As shown in Fig. 3A, rotenone induced a progressive reduction of the FP amplitude and after 25–30 min of 1 μM rotenone application, the loss of the FP amplitude was almost complete.

The possible neuroprotective effect of ZNS on the FP amplitude in the presence of acute intoxication with rotenone was investigated by treating slices with different concentrations of ZNS (Fig. 3A). The application of 1 μM ZNS did not alter the amplitude of the FP ($n=22$). However, this concentration of ZNS, applied before and during the administration of 1 μM rotenone, was neuroprotective ($n=7$, $p<0.001$; Figs. 3A, B).

The neuroprotective action of ZNS was evident at concentrations of 0.3, 1, 3, and 10 μM ($n=7$, $p<0.05$; $n=7$, $p<0.01$; $n=7$, $p<0.05$; $n=5$, $p<0.05$, respectively) and undetectable at 0.1 and 30 μM ($n=7$,

**Fig. 1.** ZNS inhibits in a dose-dependent manner the current-evoked firing discharge of striatal spiny neurons while does not alter current-voltage relationship. (A) The graph shows the dose–response curve obtained at various concentrations of ZNS on the firing discharge. Each data point was obtained from 7 single experiments. The upper part of the figure shows single voltage responses to a depolarizing pulse (1.0 nA) recorded from a striatal spiny neuron under control condition and during the application of 100 μM ZNS. (B) The graph and electrophysiological traces from a medium spiny neuron showing current–voltage relationship in control condition and in the presence of 100 μM ZNS. This antiepileptic drug induced no significant changes of resting membrane potential and did not modify current drops caused by voltage steps in both depolarizing and hyperpolarizing directions.




$p>0.05$; $n=7$, $p>0.05$, respectively). High doses of ZNS showed a detrimental effect. In fact, in the presence of 100 μM ZNS we observed, after 15 min of the application 1 μM rotenone, a reduction of the FP amplitude larger than the effect obtained in the presence of rotenone alone ($n=7$, $p<0.001$; Figs. 3A, B).

To expand our understanding on the mechanisms underlying the neuroprotective action of ZNS, we analyzed the role of endogenous GABA. A $GABA_A$-mediated mechanism was, in fact, involved in the protection against rotenone by the AED carbamazepine (Costa et al., 2008). In particular, we tested whether a low concentration of bicuculline was able to block the neuroprotective action of ZNS. This $GABA_A$ receptor antagonist, at a concentration of 3 μM, did not change either the FP amplitude or the rotenone-induced electrophysiological changes while it fully blocked the neuroprotective effects of ZNS on the FP amplitude ($n=6$, $p<0.001$; Fig. 3C).

### *Lack of effect of ZNS against toxicity induced by 3-NP*

3-NP, an irreversible inhibitor of succinate dehydrogenase (SD, complex II) has been used as an experimental model of HD (Beal et al., 1993; Brouillet and Hantraye, 1995; Brouillet et al., 1999). As we have previously described (Centonze et al., 2006; Tozzi et al., 2007), bath application of the slices with 3-NP (10 mM) irreversibly reduced the FP amplitude in the absence of external $Mg^{2+}$, but not in the presence of physiological (1.2 mM) concentration of this ion. Incubation of the slices with a different concentrations of ZNS (1 μM, 30 μM and 100 μM) before (10 min) and during the application of 3-NP did not prevent the irreversible electrophysiological changes induced by this mitochondrial toxin ($n=5$ for each concentration, $p>0.05$; Fig. 4A).

### *Lack of effect of ZNS against the toxicity induced by oxygen and glucose deprivation*

As previously reported (Costa et al., 2004), a period of *in vitro* ischemia (oxygen and glucose deprivation) produced an irreversible loss of corticostriatal FPs. After 10–15 min of a stable baseline recording, *in vitro* ischemia was applied for 10 min. This treatment progressively reduced the amplitude of the FP, which was completely suppressed in about 5 min. After the washout of the ischemic solution, the FP did not recover and remained undetectable until the end of the recording, suggesting an irreversible loss of physiological excitatory synaptic transmission and a permanent ionic disruption in striatal neurons. Application of ZNS (0.3 to 100 μM) for 10 min before and during the ischemic period did not show any neuroprotective action on the FP reduction ($n=15$, $p>0.05$; Fig. 4B).

## Discussion

In the present study we have investigated the effects of ZNS on the electrical activity of striatal spiny neurons. Our findings can be summarized in three major points.

First, we have shown that the intrinsic resting properties (membrane potential and input resistance) of striatal spiny neurons

**Fig. 2.** ZNS inhibits excitatory glutamatergic field potentials (FPs) and excitatory postsynaptic potentials (EPSPs) evoked by cortical stimulation in striatal spiny neurons without affecting paired-pulse facilitation. (A) The graph shows the dose–response curve obtained at various concentrations of ZNS on the amplitude of coticostriatal FPs. The upper traces shows averages (6 single sweeps) of FPs recorded from a striatal slices under control condition and during the application of 1 μM, 100 μM and 300 μM ZNS. (B) The graph shows the dose–response curve obtained at various concentrations of ZNS on the amplitude of EPSPs. The upper traces show averages (6 single sweeps) of EPSPs recorded from a striatal spiny neuron under control condition and during the application of 1 μM, 10 μM and 100 μM ZNS. (C) The traces show synaptic responses to a pair of stimuli recorded with interstimulus interval of 60 ms under control condition and in the presence of 100 μM ZNS. The graph shows the ratio of the second pulse response to the first pulse response (EPSP2/EPSP1) before and during the application of two different concentrations of ZNS.

are not significantly modified by ZNS even at high concentrations. The second observation was that relatively high concentrations (30–300 μM) of ZNS are required to significantly decrease both the current-evoked repetitive firing discharge and the amplitude of striatal glutamatergic transmission. The third finding was that ZNS, at low concentrations (0.3–10 μM), exerts a selective neuroprotective effect on the irreversible loss of the FP amplitude induced by the complex I mitochondrial toxin rotenone. No effects were observed against the neurotoxicity induced either by the complex II mitochondrial toxin 3-NP or by *in vitro* ischemia.

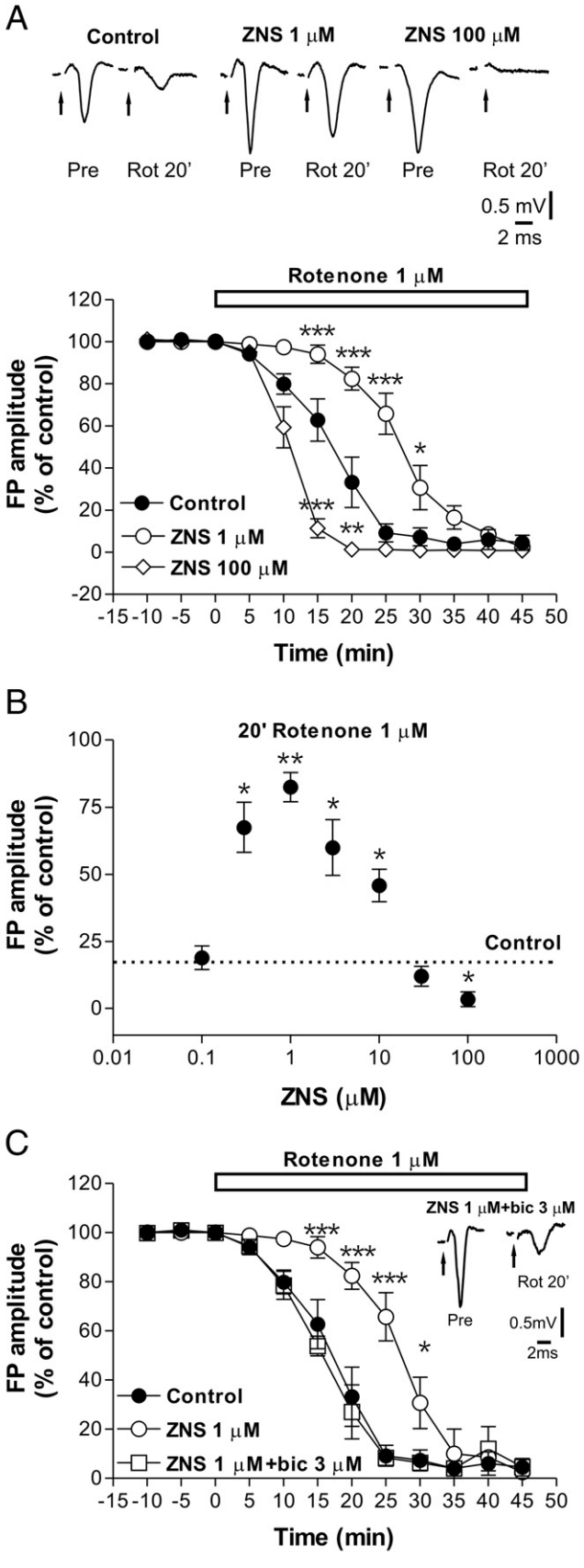



**Fig. 3.** ZNS exerts differential effects on rotenone-induced toxicity depending on the utilized dose. (A) Example traces of corticostriatal field potentials recorded in control condition and at two different concentration of ZNS. The rotenone-mediated reduction of the field potential amplitude was significantly reduced by ZNS 1 μM while 100 μM ZNS exerts a detrimental effect. The graph shows the time course of the mean field potential amplitude recorded in control condition and in the presence of 1 and 100 μM ZNS in corticostriatal slices. Incubation of the slices in 1 μM ZNS increased the neurotoxic effect induced by rotenone. (B) The dose–response curve shows that neuroprotective effect of ZNS was dose related. (C) The graph shows that the neuroprotective effect of 1 μM ZNS was abolished by the application of 3 μM bicuculline. The insert shows the single electrophysiological traces.

ZNS is a multi-target antiepileptic drug, exhibiting a broad combination of complementary mechanisms of actions which may offer a clinical advantage in the treatment of both partial and generalized seizures. ZNS stabilizes neuronal membranes, and disrupts synchronized firing through combined effects on ion channels, thereby limiting the spread of seizures (Rock et al., 1989; Kito et al., 1996). By altering the fast inactivation threshold of voltage-dependent sodium channels, ZNS reduces the sustained high-frequency repetitive firing of action potentials. Suppression of voltage-dependent sodium currents, detected as an inhibition of the firing activity, is a common action shared by many neuroprotective compounds and anticonvulsant drugs, including phenytoin (Yaari et al., 1986), carbamazepine (McLean and Macdonald, 1986a), oxcarbazepine (Calabresi et al., 1995b), gabapentin (Calabresi et al., 1999), and sodium valproate (McLean and Macdonald, 1986b).

ZNS may also alter the process of synaptic transmission through its effect of attenuation of neuronal excitability. Glutamate is a primary mediator of neuronal excitatory pathways of the brain, which have been implicated in neurodegenerative diseases (Beal, 1992a; Browne et al., 1997; Chase and Oh, 2000). Excitotoxicity seems to play a role in a variety of neuropathological conditions and neurodegenerative diseases with distinct genetic etiologies may share excitotoxicity as a common pathogenic pathway (Beal, 1992b; Calabresi et al., 2000a,b; Dong et al., 2009). Excessive activation of glutamate receptors by excitatory amino acids leads to a number of deleterious consequences, including generation of free radicals, activation of the mitochondrial permeability transition, and secondary excitotoxicity (Beal, 1998; Albers and Beal, 2002).

We found that ZNS exerts a concentration-related reduction of glutamatergic corticostriatal excitatory transmission without affecting the paired-pulse facilitation. Since this parameter is considered a good index of a presynaptic mechanism of action (Manabe et al., 1993; Schulz et al., 1994; Calabresi et al., 1997) it can be argued that the inhibitory effect of ZNS on glutamatergic transmission does not exclusively involve a presynaptic site of action but it could also affect a postsynaptic mechanism.

Interestingly, low doses of ZNS are sufficient to exert a neuroprotective effect against rotenone-induced electrophysiological alterations.

It is known that neurodegenerative disorders affecting the basal ganglia, such as PD and progressive supranuclear palsy (PSP), have been associated with defects in mitochondrial complex I function (Swerdlow et al., 2000; Albers and Beal, 2002; Albers et al., 2002; Chirichigno et al., 2002). Moreover, experimental studies have reported that generalized complex I inhibition by rotenone damages both nigral and striatal neurons, in a pattern reminiscent of PSP pathology (Ferrante et al., 1997; Höglinger et al., 2003; Champy et al., 2004; Zhu et al., 2004). Rotenone might lead to striatal damage

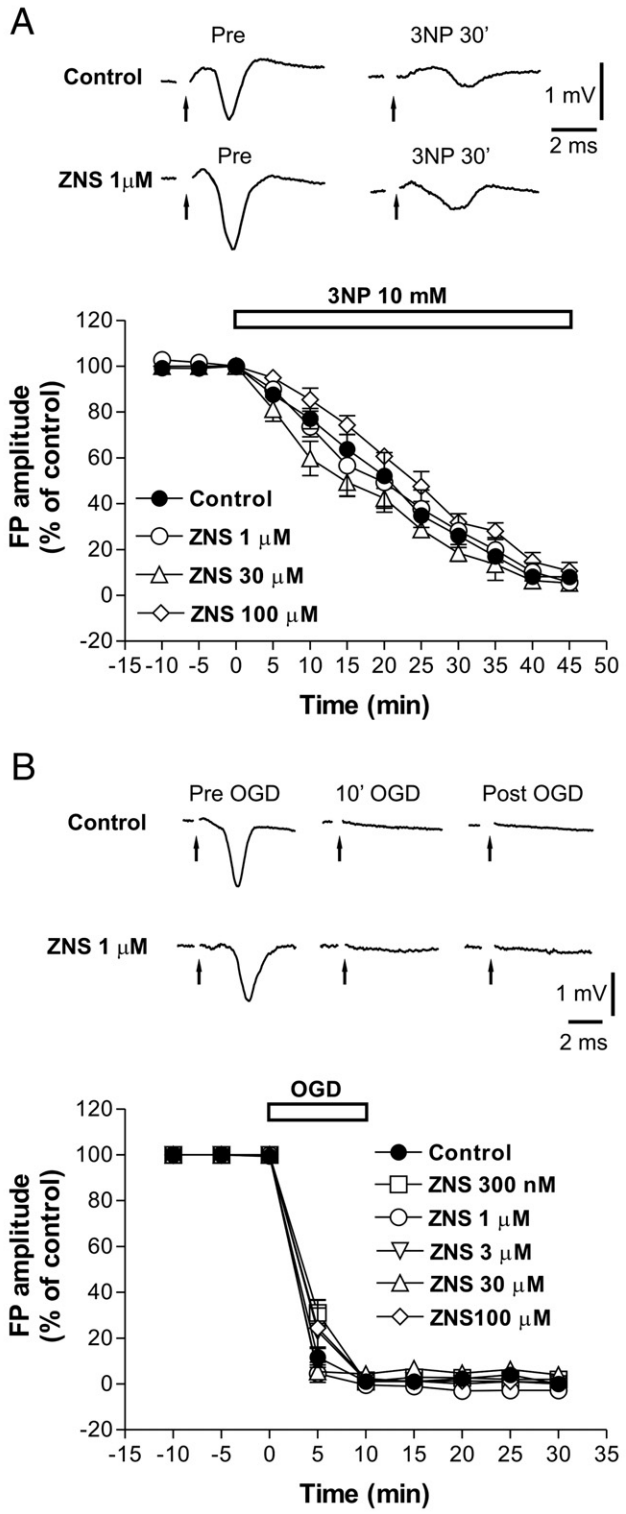



**Fig. 4.** ZNS does not exert neuroprotective action on neurotoxicity induced either by mitochondrial complex II inhibition or by oxygen and glucose deprivation (OGD). (A) The traces represent a field potential recorded before and after 30 min of application 10 mM 3NP in control condition (upper traces) and in the presence of 1 μM ZNS (lower traces). The graph shows the time course of the mean field potential amplitude recorded in the presence of 1, 30, and 100 μM ZNS in corticostriatal slices. (B) Single experiments showing the changes of field potential amplitude following OGD in control medium (upper traces) and in the presence of 1 μM ZNS (lower traces). The graph shows the time-course of the OGD-induced changes of field potential amplitude in control condition and in the presence of 300 nM, 1 μM, 3 μM, 30 μM and 100 μM ZNS.

through various NMDA-independent mechanisms, including the inhibition of cellular ATP production, intraneuronal sodium and calcium accumulation, and free radical generation (Höglinger et al., 2003; He et al., 2003; Bonsi et al., 2004; Bao et al., 2005).

Different mechanisms have been proposed to explain the beneficial effects of AEDs, in particular of ZNS on PD, including an inhibitory effect on voltage-dependent $Na^+$ channel (Schauf, 1987) and depolarization-induced glutamate release (Okada et al., 1998). ZNS also enhances GABAergic and monoaminergic transmission (Kawata et al., 1999; Okada et al., 2002; Yoshida et al., 2005). Additionally, ZNS is a specific T-type $Ca^{2+}$ channel blocker (Suzuki et al., 1992; Kito et al., 1996) that increases the burst firing of dopaminergic neurons in the substantia nigra. Recently, Yano and collaborators have demonstrated, in mice after MPTP treatment, that one of the neuroprotective actions of ZNS may involve elevation of tyrosine hydroxylase on the dopaminergic system (Yano et al., 2009).

We have recently reported that CBZ is able to exert a neuroprotective effect against rotenone, by enhancing the GABA-mediated inhibition *via* the activation of $GABA_A$ receptors (Costa et al., 2008). The present experiments are in line with this result and show that the neuroprotective action of ZNS is blocked by bicuculline. This finding provides further support to the hypothesis that neuroprotective mechanisms against rotenone involve an enhancement of GABA-mediated inhibition via activation of $GABA_A$ receptors. In line with this hypothesis, ZNS interacts with the $GABA_A$ receptor complex (Mimaki et al., 1990) and a similar mechanism has also been proposed for phenytoin (Granger et al., 1995). Interestingly, we did not observe a linear relationship between the dose and the protective effect. In fact, for this drug we obtained a bell-shaped dose–response curve that was also obtained for the neuroprotective action of tiagabine and vigabatrin (Costa et al., 2004). Since these two AEDs exert their neuroprotection by enhancing endogenous GABA it could be speculated that excessive activation of $GABA_A$ receptor may cause an overload of chloride ions into the neurons, leading to cell swelling.

The inhibition of the sodium currents represents a well-recognized mechanism for the therapeutic action of several AEDs as well as a possible target for neuroprotection (Rogawski and Loescher, 2004). Although we have demonstrated that the concentration of ZNS exerting the neuroprotective action is much lower than that one required to block sodium-dependent action potential discharge, it is still possible that such low doses of this drug modulate sodium channels expressed by GABAergic interneurons but not those on spiny neurons. Future studies to test the effects of rotenone and ZNS on GABAergic striatal interneurons are required.

Interestingly, there is a relationship between serum ZNS concentrations and clinical effect in animal models (Masuda et al., 1979). In fact, ZNS was effective at serum concentrations above 10 mg/l (47 μmol/l) and toxicity was likely to occur at concentrations above 70 mg/l (Patsalos et al., 2008; Striano et al., 2008).

ZNS did not exert neuroprotection effects against 3-NP-induced electrical alterations. Different lines of evidence support the view that glutamate NMDA receptors play a primary role in toxic effects of mitochondrial complex II inhibition by 3-NP (Greene and Greenamyre, 1996; Kim et al., 2000; Lee et al., 2002; Kanki et al., 2004). An antagonist of NR2B-containing NMDA receptors, like ifenprodil, was able to protect striatal neurons from 3-NP-induced toxicity but not against the effects of rotenone (Centonze et al., 2006). Similarly, memantine, a low-affinity uncompetitive NMDA receptor antagonist, displays some protection against irreversible FP loss induced by 3-NP without influencing toxicity induced by rotenone (Tozzi et al., 2007). On the basis of these data, it can be hypothesized that ZNS is protective mainly in experimental models of neurotoxicity not involving NMDA receptors.

The lack of neuroprotective effects of ZNS against *in vitro* ischemia models is rather surprising. ZNS, in fact, has been shown to prevent

ischemic damage in adult rodents (Minato et al., 1997; Owen et al., 1997). Additionally, ZNS attenuated neonatal hypoxic–ischemic damage in animals by a mechanism independent of its anticonvulsant properties (Hayakawa et al., 1994). A possible explanation for the lack of effect of ZNS in our *in vitro* model of ischemia in the striatum is the peculiar selectivity of ZNS on the various subtypes of voltage gated calcium channels. In fact, ZNS blocks the T-type voltage-gated calcium channel (Matar et al., 2009) that are not prominently expressed in striatal spiny neurons (Foehring et al., 2000). Moreover, ZNS does not seem to affect the high-threshold L-type $Ca^{2+}$channel (Suzuki et al., 1992), one of the major targets of putative neuroprotective therapies (Pisani et al., 1998, 2004). Finally, the concomitant inhibition of fast $Na^{+}$ and high voltage-activated $Ca^{2+}$conductances could be critically important for the neuroprotection induced by both the old and new AEDs against the acute effects of ischemia (Costa et al., 2006).

The neuroprotective effects of ZNS may be linked to its antioxidant properties (Sobieszek et al., 2003; Biton, 2007). In fact, ZNS is known to scavenge hydroxyl radicals and nitric oxide radicals in a cell-free system (Mori et al., 1998), and inhibits lipid peroxidation and oxidative DNA damage in the rat brain (Komatsu et al., 1995, 2000). Moreover, recent findings indicate that pretreatment with ZNS reduces the production of free radicals in the hippocampus during kainate-induced seizures (Ueda et al., 2005). In line with these data, we have also found evidence of the neuroprotective effects of ZNS on differentiated neuroblastoma cell cultures exposed to rotenone toxin. Our preliminary data indicate that ZNS pre-incubated 30 min before to rotenone treatment significantly reduced, in these cells, ROS production in comparison to control condition (in preparation). However, further studies should be carried out to characterize the mechanisms leading to protein oxidation induced by free radicals during rotenone application and the possible neuroprotective effects of antioxidants.

In addition, ZNS, as well as topiramate, is an inhibitor of carbonic anhydrase and it may thus influence neuronal activity via changes of pH (Biton, 2007). However, this mechanism is not believed to contribute to the antiepileptic activity of this drug (Thöne et al., 2008) and the possible involvement of this mechanism in the neuroprotective action of ZNS remains to be established.

In conclusion, our experiments on the electrophysiological effects of ZNS on striatal neurons suggest that low concentrations of ZNS are sufficient to significantly reduce membrane alterations induced by mitochondrial inhibition *via* $GABA_A$ receptors. Based on these experimental results, it might be interesting to verify whether ZNS exerts neuroprotective actions using *in vivo* models of basal ganglia neurodegenerative diseases.

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## References

Albers, D.S., Beal, M.F., 2002. Mitochondrial dysfunction in progressive supranuclear palsy. Neurochem. Int. 40, 559–564.
Albers, D.S., Swerdlow, R.H., Manfredi, G., Gajewski, C., Yang, L., Parker, W.D., Beal, M.F., 2002. Further evidence for mitochondrial dysfunction in progressive supranuclear palsy. Exp. Neurol. 168, 196–198.
Bao, L., Avshalumov, M.V., Rice, M.E., 2005. Partial mitochondrial inhibition causes striatal dopamine release suppression and medium spiny neuron depolarization via $H_2O_2$ elevation, not ATP depletion. J. Neurosci. 25, 10029–10040.
Beal, M.F., 1992a. Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? Ann. Neurol. 31, 119–130.
Beal, M.F., 1992b. Role of excitotoxicity in human neurological disease. Curr. Opin. Neurobiol. 2, 657–662.
Beal, M.F., 1998. Mitochondrial dysfunction in neurodegenerative diseases. Biochim. Biophys. Acta 10, 211–223.
Beal, M.F., Brouillet, E., Jenkins, B.G., Ferrante, R.J., Kowall, N.W., Miller, J.M., Storey, E., Srivastava, R., Rosen, B.R., Hyman, B.T., 1993. Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. J. Neurosci. 13, 4181–4192.
Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., Greenamyre, J.T., 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat. Neurosci. 3, 1301–1306.
Biton, V., 2007. Clinical pharmacology and mechanism of action of zonisamide. Clin. Neuropharmacol. 30, 230–240.
Bonsi, P., Calabresi, P., De Persis, C., Papa, M., Centonze, D., Bernardi, G., Pisani, A., 2004. Early ionic and membrane potential changes caused by the pesticide rotenone in striatal cholinergic interneurons. Exp. Neurol. 185, 169–181.
Brouillet, E., Conde, F., Beal, M.F., Hantraye, P., 1999. Replicating Huntington's disease phenotype in experimental animals. Prog. Neurobiol. 59, 427–468.
Brouillet, E., Hantraye, P., 1995. Effects of chronic MPTP and 3-nitropropionic acid in nonhuman primates. Curr. Opin. Neurol. 8, 469–473.
Browne, S.E., Bowling, A.C., MacGarvey, U., Baik, M.J., Berger, S.C., Muqit, M.M.K., Bird, E.D., Beal, M.F., 1997. Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann. Neurol. 41, 646–653.
Büeler, H., 2009. Impaired mitochondrial dynamics and function in the pathogenesis of Parkinson's disease. Exp. Neurol. 218, 235–246.
Calabresi, P., De Murtas, M., Stefani, A., Pisani, A., Sancesario, G., Mercuri, N.B., Bernardi, G., 1995b. Action of GP47779, the active metabolite of oxcarbazepine, on corticostriatal system. I. Modulation of corticostriatal synaptic transmission. Epilepsia 36, 990–996.
Calabresi, P., Pisani, A., Mercuri, N.B., Bernardi, G., 1996. The corticostriatal projection: from synaptic plasticity to dysfunctions of the basal ganglia. Trends Neurosci. 19, 19–24.
Calabresi, P., Centonze, D., Pisani, A., Bernardi, G., 1997. Endogenous adenosine mediates the presynaptic inhibition induced by aglycemia at corticostriatal synapses. J. Neurosci. 17, 4509–4516.
Calabresi, P., Centonze, D., Pisani, A., Sancesario, G., Gubellini, P., Marfia, G.A., Bernardi, G., 1998. Striatal spiny neurons and cholinergic interneurons express differential ionotropic glutamatergic responses and vulnerability: implications for ischemia and Huntington's disease. Ann. Neurol. 43, 586–597.
Calabresi, P., Centonze, D., Marfia, G.A., Pisani, A., Bernardi, G., 1999. An *in vitro* electrophysiological study on the effects of phenytoin, lamotrigine and gabapentin on striatal neurons. Br. J. Pharmacol. 126, 689–696.
Calabresi, P., Centonze, D., Bernardi, G., 2000a. Cellular factors controlling neuronal vulnerability in the brain: a lesson from the striatum. Neurology 14, 1249–1255.
Calabresi, P., Centonze, D., Gubellini, P., Marfia, G.A., Pisani, A., Sancesario, G., Bernardi, G., 2000b. Synaptic transmission in the striatum: from plasticity to neurodegeneration. Prog. Neurobiol. 61, 231–265.
Calabresi, P., Picconi, B., Saulle, E, Centonze, D., Hainsworth, A.H., Bernardi, G., 2000c. Is pharmacological neuroprotection dependent on reduced glutamate release. Stroke 31, 766–772.
Calabresi, P., Gubellini, P., Picconi, B., Centonze, D., Pisani, A., Bonsi, P., Greengard, P., Hipskind, R.A., Borrelli, E., Bernardi, G., 2001. Inhibition of mitochondrial complex II induces a long-term potentiation of NMDA-mediated synaptic excitation in the striatum requiring endogenous dopamine. J. Neurosci. 21, 5110–5120.
Calabresi, P., Cupini, L.M., Centonze, D., Pisani, F., Bernardi, G., 2003. Antiepileptic drugs as a possible neuroprotective strategy in brain ischemia. Ann. Neurol. 53, 693–702.
Calabresi, P., Picconi, B., Tozzi, A., Di Filippo, M., 2007. Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci. 30, 211–219.
Centonze, D., Prosperetti, C., Barone, I., Rossi, S., Picconi, B., Tscherter, A., De Chiara, V., Bernardi, G., Calabresi, P., 2006. NR2B-containing NMDA receptors promote the neurotoxic effects of 3-nitroproprionic acid but not of rotenone in the striatum. Exp. Neurology 202, 470–479.
Champy, P., Höglinger, G.U., Feger, J., Gleye, C., Hocquemiller, R., Laurens, A., Guerineau, V., Laprevote, O., Medja, F., Lombes, A., Michel, P.P., Lannuzel, A., Hirsch, E.C., Ruberg, M., 2004. Annonacin, a lipophilic inhibitor of mitochondrial complex I, induces nigral and striatal neurodegeneration in rats: possible relevance for atypical parkinsonism in Guadeloupe. J. Neurochem. 88, 63–69.
Chase, T.N., Oh, J.D., 2000. Striatal dopamine-and glutamate-mediated dysregulation in experimental parkinsonism. Trends Neurosci. 23, S86–91.
Chirichigno, J.W., Manfredi, G., Beal, M.F., Albers, D.S., 2002. Stress-induced mitochondrial depolarization and oxidative damage in PSP cybrids. Brain Res. 951, 31–35.
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.
Di Filippo, M., Picconi, B., Costa, C., Bagetta, V., Tantucci, M., Parnetti, L., Calabresi, P., 2006. Pathways of neurodegeneration and experimental models of basal ganglia disorders: downstream effects of mitochondrial inhibition. Eur. J. Pharmacol. 545, 65–72.

Dong, X.X., Wang, Y., Qin, Z.H., 2009. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. Acta Pharmacol. Sin. 30, 379–387.
Ferrante, R.J., Schulz, J.B., Kowall, N.W., Beal, F.M., 1997. Systemic administration of rotenone produces selective damage in the striatum and globus pallidus, but not in the substantia nigra. Brain Res. 753, 157–162.
Foehring, R.C., Mermelstein, P.G., Song, W.J., Ulrich, S., Surmeier, D.J., 2000. Unique properties of R-type calcium currents in neocortical and neostriatal neurons. J. Neurophysiol. 84, 2225–2236.
Granger, P., Biton, B., Faure, C., Vige, X., Depoortere, H., Graham, D., Langer, S.Z., Scatton, B., Avenet, P., 1995. Modulation of the gamma-aminobutyric acid type A receptor by the antiepileptic drugs carbamazepine and phenytoin. Mol. Pharmacol. 47, 1189–1196.
Greene, J.G., Greenamyre, J.T., 1996. Bioenergetics and glutamate excitotoxicity. Prog. Neurobiol. 48, 613–634.
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.
He, Y., Imam, S.Z., Dong, Z., Jankovic, J., Ali, S.F., Appel, S.H., Le, W., 2003. Role of nitric oxide in rotenone-induced nigro-striatal injury. J. Neurochem. 86, 1338–1345.
Höglinger, G.U., Féger, J., Prigent, A., Michel, P.P., Parain, K., Champy, P., Ruberg, M., Oerte, W.H., Hirsch, E.C., 2003. Chronic systemic complex I inhibition induces a hypokinetic multisystem degeneration in rats. J. Neurochem. 84, 491–502.
Kanki, R., Nakamizo, T., Yamashita, H., Kihara, T., Sawada, H., Uemura, K., Kawamata, J., Shibasaki, H., Akaike, A., Shimohama, S., 2004. Effects of mitochondrial dysfunction on glutamate receptor-mediated neurotoxicity in cultured rat spinal motor neurons. Brain Res. 1015, 73–81.
Kawata, Y., Okada, M., Mizuno, T., Wada, K., Murakami, K., Kondo, T., Kaneko, S., 1999. Effects of zonisamide on K+ and Ca2+evoked release of monoamine as well as $K^+$evoked intracellular $Ca^{2+}$mobilization in rat hippocampus. Epilepsy Res. 35, 173–182.
Kim, G.W., Copin, J.C., Kawase, M., Chen, S.F., Sato, S., Gobbel, G.T., Chan, P.H., 2000. Excitotoxicity is required for induction of oxidative stress and apoptosis in mouse striatum by the mitochondrial toxin, 3-nitropropionic acid. J. Cereb. Blood Flow Metab. 20, 119–129.
Kito, M., Maehara, M., Watanabe, K., 1996. Mechanisms of T-type calcium channel blockade by zonisamide. Seizure 5, 115–119.
Komatsu, M., Okamura, Y., Hiramatsu, M., 1995. Free radical scavenging activity of zonisamide and its inhibitory effect on lipid peroxide formation in iron-induced epileptogenic foci of rats. Neurosciences 21, 23–29.
Komatsu, M., Hiramatsu, M., Willmore, L.J., 2000. Zonisamide reduces the increase in 8-hydroxy-2 deoxyguanosine levels formed during iron-induced epileptogenesis in the brains of rats. Epilepsia 41, 1091–1094.
Lee, W.T., Itoh, T., Pleasure, D., 2002. Acute and chronic alterations in calcium homeostasis in 3-nitropropionic acid-treated human NT2-N neurons. Neuroscience 113, 699–708.
Luetjens, C.M., Bui, N.T., Sengpiel, B., Münstermann, G., Poppe, M., Krohn, A.J., Bauerbach, E., Krieglstein, J., Prehn, J.H., 2000. Delayed mitochondrial dysfunction in excitotoxic neuron death: cytochrome *c* release and a secondary increase in superoxide production. J. Neurosci. 20, 5715–5723.
Manabe, T., Wyllie, D.J.I., Nicoll, R.A., 1993. Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. J. Neurophysiol. 70, 1451–1459.
Matar, N., Jin, W., Wrubel, H., Hescheler, J., Schneider, T., Weiergräber, M., 2009. Zonisamide block of cloned human T-type voltage-gated calcium channels. Epilepsy Res. 83, 224–234.
Masuda, Y., Utsui, Y., Shiraishi, Y., Karasawa, T., Yoshida, K., Shimuzu, M., 1979. Relationship between plasma concentrations of diphenylhydantoin, phenobarbital, carbamazepine and 3-sulfamoylmethyl-1,2-benzisoxazole (AD-810), a new anti-convulsant agent, and their anticonvulsant or neurotoxic effects in experimental animals. Epilepsia 20, 623–633.
Mattson, M.P., Gleichmann, M., Cheng, A., 2008. Mitochondria in neuroplasticity and neurological disorders. Neuron 60, 748–766.
McLean, M.J., Macdonald, R.L., 1986a. Carbamazepine and 10,11-epoxycarbamazepine produce use-and voltage-dependent limitation of rapidly firing action potentials of mouse central neurons in cell culture. J. Pharmacol. Exp. Ther. 238, 727–737.
McLean, M.J., Macdonald, R.L., 1986b. Sodium valproate, but not ethosuximide, produces use- and voltage-dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. J. Pharmacol. Exp. Ther. 237, 1001–1011.
Mimaki, T., Suzuki, Y., Tagawa, T., Yabuuchi, H., 1990. Interaction of zonisamide with benzodiazepine and GABA receptors in rat brain. Med. J. Osaka Univ. 39, 13–17.
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.
Mori, A., Noda, Y., Packer, L., 1998. The anticonvulsivant zonisamide scavenger free radicals. Epilepsy Res. 30, 153–158.
Murata, M., Hasegawa, K., Kanazawa, I., 2007. Zonisamide improves motor function in Parkinson disease: a randomized, double-blind study. Neurology 68, 45–50.
Okada, M., Kaneko, S., Hirano, T., Ishida, M., Kondo, T., Otani, K., Fukushima, Y., 1992. Effects of zonisamide on extracellular levels of monoamine and its metabolite, and on $Ca^{2+}$dependent dopamine release. Epilepsy Res. 13, 113–119.
Okada, M., Kaneko, S., Hirano, T., Mizuno, K., Kondo, T., Otani, K., Fukushima, Y., 1995. Effects of zonisamide on dopaminergic system. Epilepsy Res. 22, 193–205.
Okada, M., Kawata, Y., Mizuno, K., Wada, K., Kondo, T., Kaneko, S., 1998. Interaction between Ca2+, K+, carbamazepine and zonisamide on hippocampal extracellular glutamate monitored with amicrodialysis electrode. Br. J. Pharmacol. 124, 1277–1285.
Okada, M., Zhu, G., Yoshida, S., Kanai, K., Hirose, S., Kaneko, S., 2002. Exocytosis mechanism as a new targeting site for mechanisms of action of antiepileptic drugs. Life Sci. 72, 465–473.
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.
Patsalos, P.N., Berry, D.J., Bourgeois, B.F., Cloyd, J.C., Glauser, T.A., Johannessen, S.I., Leppik, I.E., Tomson, T., Perucca, E., 2008. Antiepileptic drugs-best practice guidelines for therapeutic drug monitoring: a position paper by the subcommission on therapeutic drug monitoring, ILAE Commission on Therapeutic Strategies. Epilepsia 49, 1239–1276.
Picconi, B., Centonze, D., Håkansson, K., Bernardi, G., Greengard, P., Fisone, G., Cenci, M.A., Calabresi, P., 2003. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat. Neurosci. 6, 501–506.
Pisani, A., Bonsi, P., Calabresi, P., 2004. Calcium signaling and neuronal vulnerability to ischemia in the striatum. Cell. Calcium 36, 277–284.
Pisani, A., Calabresi, P., Tozzi, A., D'Angelo, V., Bernardi, G., 1998. L-type Ca2+ channel blockers attenuate electrical changes and $Ca^{2+}$rise induced by oxygen/glucose deprivation in cortical neurons. Stroke 29, 196–201.
Rock, D.M., Macdonald, R.L., Taylor, C.P., 1989. Blockade of sustained repetitive action potentials in cultured spinal cord neurons by zonisamide (AD 810, CI 912), a novel anticonvulsant. Epilepsy Res. 3, 138–143.
Rogawski, M.A., Loescher, W., 2004. The neurobiology of antiepileptic drugs. Nat. Rev. Neurosci. 5, 553–564.
Schapira, A.H., Gu, M., Taanman, J.W., Tabrizi, S.J., Seaton, T., Cleeter, M., Cooper, J.M., 1998. Mitochondria in the etiology and pathogenesis of Parkinson's disease. Ann. Neurol. 44, S89–98.
Schauf, C.L., 1987. Zonisamide enhances slow sodium inactivation in Myxicola. Brain Res. 413, 185–188.
Sherer, T.B., Richardson, J.R., Testa, C.M., Seo, B.B., Panov, A.V., Yagi, T., Matsuno-Yagi, A., Miller, G.W., Greenamyre, J.T., 2007. Mechanism of toxicity of pesticides acting at complex I: relevance to environmental etiologies of Parkinson's disease. J. Neurochem. 100, 1469–1479.
Schulz, P.E., Cook, E.P., Johnston, D., 1994. Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation. J. Neurosci. 14, 5325–5337.
Sobieszek, G., Borowicz, K.K., Kimber-Trojnar, Z., Małek, R., Piskorska, B., Czuczwar, S.J., 2003. Zonisamide: a new antiepileptic drug. Pol. J. Pharmacol. 55, 683–689.
Striano, S., Striano, P., Capone, D., Pisani, F., 2008. Limited place for plasma monitoring of new antiepileptic drugs in clinical practice. Med. Sci. Monit. 14, 73–78.
Suzuki, S., Kawakami, K., Nishimura, S., Watanabe, Y., Yagi, K., Seino, M., Miyamoto, K., 1992. Zonisamide blocks T-type calcium channel in cultured neurons of rat cerebral cortex. Epilepsy Res. 12, 21–27.
Swerdlow, R.H., Golbe, L.I., Parks, J.K., Cassarino, D.S., Binder, D.R., Grawey, A.E., Litvan, I., Bennett, J.P., Wooten, G.F., Parker, W.D., 2000. Mitochondrial dysfunction in cybrid lines expressing mitochondrial genes from patients with progressive supranuclear palsy. J. Neurochem. 75, 1681–1684.
Thöne, J., Leniger, T., Splettstösser, F., Wiemann, M., 2008. Antiepileptic activity of zonisamide on hippocampal CA3 neurons does not depend on carbonic anhydrase inhibition. Epilepsy Res. 79, 105–111.
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.
Yano, R., Yokoyama, H., Kuroiwa, H., Kato, H., Araki, T., 2009. A novel anti-Parkinsonian agent, zonisamide, attenuates MPTP-induced neurotoxicity in mice. J. Mol. Neurosci. 39, 211–219.
Yaari, Y., Seltzer, M., Pincus, J., 1986. Phenytoin: mechanism of its anticonvulsivant action. Ann. Neurol. 20, 171–184.
Yoshida, S., Okada, M., Zhu, G., Kaneko, S., 2005. Effects of zonisamide on neurotransmitter exocytosis associated with ryanodine receptors. Epilepsy Res. 67, 153–162.
Zhu, C., Vourc'h, P., Fernagut, P.O., Fleming, S.M., Lacan, S., Dicarlo, C.D., Seaman, R.L., Chesselet, M.F., 2004. Variable effects of chronic subcutaneous administration of rotenone on striatal histology. J. Comp. Neurol. 478, 418–426.