Coactivation of GABA_A and GABA_B Receptor Results in Neuroprotection During In Vitro Ischemia

Cinzia Costa, MD; Giorgia Leone, MD; Emilia Saulle, MD; Francesco Pisani, MD; Giorgio Bernardi, MD; Paolo Calabresi, MD

Background and Purpose—The possible neuroprotective effect of endogenous γ-aminobutyric acid (GABA) on the irreversible electrophysiological changes induced by in vitro ischemia on striatal neurons was investigated. In particular, the aim of the study was the characterization of the neuroprotective action of 2 antiepileptic drugs increasing GABAergic transmission such as tiagabine, a GABA transporter inhibitor, and vigabatrin, an irreversible inhibitor of GABA transaminase.

Methods—Extracellular field potential recordings were obtained from rat corticostriatal slice preparations. In vitro ischemia was delivered by switching to an artificial cerebrospinal fluid solution in which glucose was omitted and oxygen was replaced with N2.

Results—An irreversible loss of the field potentials recorded from striatal neurons was observed after 10 minutes of ischemia in control solution. Conversely, tiagabine and vigabatrin partially prevented the ischemia-induced field potential loss. Surprisingly, both GABA_A and GABA_B receptor antagonists blocked these effects. Accordingly, neuroprotection could be obtained only when GABA_A and GABA_B receptor agonists were coapplied, but not when a single agonist was given in isolation.

Conclusions—Antiepileptic drugs targeting GABAergic transmission can exert neuroprotective effects against ischemia by increasing endogenous GABA levels and via the activation of both GABA_A and GABA_B receptors. (Stroke. 2004;35:596-600.)

Key Words: anticonvulsants • corpus striatum • electrophysiology • ischemia • receptors, GABA

Combined oxygen and glucose deprivation is a well-established in vitro model of ischemia for electrophysiological studies.1–3 The striatum and hippocampus are particularly vulnerable to ischemic insult, and neuronal damage is expressed as an alteration of both intrinsic membrane properties and synaptic transmission of the recorded cells.4–6

A large body of experimental work has been devoted to explore the neuroprotective efficacy of drugs blocking glutamate neurotransmission in animal models of cerebral ischemia.5,7–11 More recently, however, attention has been also focused on γ-aminobutyric acid (GABA) changes during ischemia and on possible neuroprotective effects of GABAergic drugs.12–15

Although a number of studies have suggested that increasing GABAergic synaptic transmission might display neuroprotective effects against brain ischemia,16–20 the exact mechanisms underlying these effects have yet to be elucidated.

Increasing GABA function might represent a beneficial therapeutic approach to acute ischemia for different reasons.13,19,20 First, endogenous GABA synthesis and release with consequent reduction in GABAergic transmission are decreased after an ischemic insult. Second, since glutamatergic and GABAergic transmissions work by each counterbalancing the function of the other, enhancing GABAergic activity should balance excessive glutamatergic excitation, which is the pivotal event leading to cell death.

The aim of the present study is to characterize the electrophysiological effects of 2 currently used GABAergic antiepileptic drugs, tiagabine and vigabatrin, and to examine the cellular sites at which they act by using recordings from rat corticostriatal slices. The mechanism of action of the 2 compounds is different. Vigabatrin is an irreversible inhibitor of GABA transaminase,21,22 whereas tiagabine blocks GABA reuptake into neurons and glia.23 Their possible differential abilities in protecting neurons against the permanent loss of electric activity caused by combined oxygen and glucose deprivation have been investigated. We have also analyzed the effects of other specific GABA_A and GABA_B agonists and antagonists.
Artificial cerebrospinal fluid temperature was maintained at 34 °C. D, The neuroprotective effect of tiagabine was antagonized by either bicuculline, a noncompetitive GABA A receptor antagonist, or CGP 46381, a GABA B receptor antagonist (*P<0.01 compared with tiagabine alone; n=4 for both).

Materials and Methods
Preparation and maintenance of rat corticostriatal slices have been previously described. Briefly, corticostriatal coronal slices were prepared from 2- to 3-month-old Wistar rats (thickness, 270 to 300 µm). All the experiments were conducted in conformity with the European Communities Council Directive of November 1986 (86/609/EEC). Slices were kept in artificial cerebrospinal fluid, whose composition was as follows (in mmol/L): 126 NaCl, 2.5 KCl, 1.2 MgCl 2, 1.2 NaH 2 PO 4, 2.4 CaCl 2, 11 glucose, and 25 NaHCO 3. Artificial cerebrospinal fluid temperature was maintained at 34°C and was gassed with O 2/CO 2 (95%/5%). In vitro ischemia was delivered by switching for 10 minutes to an artificial cerebrospinal fluid solution in which sucrose replaced glucose, gassed with 95% N 2 and 5% CO 2. Ischemic and drug-containing solutions entered the recording chamber no later than 30 seconds after a 3-way tap was turned.

Electrodes for extracellular recordings (15 to 20 MΩ) were filled with 2 mol/L NaCl. An Axoclamp 2B amplifier (Axon Instruments) was used for extracellular recordings. The field potential amplitude was defined as the average of the amplitude from the peak of the early positivity to peak late positivity. Quantitative data on modifications induced by ischemia are expressed as a percentage of the control values, the latter representing the mean of responses recorded during a stable period (15 to 20 minutes) before the ischemic phase. Tracings were displayed on a digital oscilloscope (Classic 6000, Gould) and digitally stored.

For data presented as mean±SEM, statistical analysis was performed with the use of Student’s t test. The significance level was established at P<0.05.

Drugs were applied by dissolving them to the desired final concentration in saline solution. Tiagabine was from Sanofi-Synthelabo; vigabatrin was from Camillo Corvi; GABA, muscimol, and CGP 46381 were from Tocris-Cookson; and bicuculline was from Sigma.

Results
Neuroprotective Effect of Tiagabine and Vigabatrin During In Vitro Ischemia
Excitatory glutamatergic field potentials were recorded from the striatum of corticostriatal rat slices. In electrophysiological experiments (n=97), the white matter between cortex and striatum was stimulated to obtain field potentials of 1.2±0.3 mV in amplitude and 2±0.5 ms in duration. Stimuli (0.03±0.01 ms and 1 to 5 V) were delivered at a frequency of 0.1 Hz to monitor the time course of field potential amplitude. After 10 to 15 minutes of stable baseline recording, ischemia was applied for 10 minutes. This treatment progressively reduced the amplitude of the field potential, which was completely suppressed in approximately 5 minutes. After the washout of the ischemic medium, the field potential did not recover (P<0.001; n=20) (Figure 1A).

The application of 30 µmol/L tiagabine for 10 minutes did not alter per se the amplitude of the field potential. However, in the presence of tiagabine, the field potential partially recovered 10 to 20 minutes after the washout of the ischemic solution (Figure 1A), reaching 41±5% of the preischemic value (P<0.01 compared with control; n=12). Similar results were obtained by the application of 10 µmol/L vigabatrin, which had a neuroprotective effect on field potential even stronger than tiagabine (Figure 2A). In fact, in the presence of vigabatrin, the field potential recovery after in vitro ischemia was approximately 53±6% of the preischemic value (P<0.01 compared with control; n=12).

The efficacy of tiagabine increased from 10 to 30 µmol/L (P<0.01, n=5; P<0.01, n=12, respectively), whereas at lower (3 µmol/L) or higher (100 and 300 µmol/L) doses it had no significant effect on postischemic field potential recovery (P>0.05; n=4 for all) (Figure 1C). Similarly, vigabatrin had a maximal neuroprotective effect at 10 µmol/L (P<0.01; n=12), while it decreased at 30 µmol/L (P<0.05, n=7). Doses of 3 or 100 µmol/L had no significant effects on postischemic field potential recovery (P>0.05; n=4 for both) (Figure 2C).

Coactivation of GABA A and GABA B Receptor Is Required for Neuroprotective Effects Against In Vitro Ischemia
Both tiagabine and vigabatrin are known to act on synaptic GABA levels with different mechanisms. Thus, to verify whether their neuroprotective effects were mediated by an
increased GABA_A and/or GABA_B receptor function, we applied these drugs in combination with either bicuculline (3 to 10 μmol/L), a GABA_A receptor antagonist, or CGP 46381 (100 μmol/L), a GABA_B receptor antagonist. Surprisingly, the neuroprotective effects of both tiagabine (30 μmol/L) (Figure 1D) and vigabatrin (10 μmol/L) (Figure 2D) were largely reduced by both of these GABA receptor antagonists. In particular, the tiagabine-mediated field potential recovery was reduced to 4±1.5% and 3.5±1.5% of preischemic values by bicuculline and CGP 46381, respectively (P<0.01 compared with tiagabine alone; n=4 for both), while the vigabatrin-mediated field potential recovery was reduced to 5±1.5% and 4.5±1.5% (P<0.01 compared with vigabatrin alone; n=4 for both).

Exogenous GABA and Direct GABAergic Agonist Mimic the Neuroprotective Effect of Tiagabine and Vigabatrin

We also measured the possible neuroprotective action caused by exogenous GABA. In the presence of GABA (100 μmol/L), the field potential partially recovered 10 to 20 minutes after the washout of the ischemic solution (Figure 3A), reaching 36±3% of the preischemic value (P<0.01 compared with control; n=5).

However, while GABA was protective at 100 μmol/L (P<0.01, n=5), lower (30 and 50 μmol/L) or higher (200 and 300 μmol/L) doses of this transmitter had no significant effect on postischemic field potential recovery (P>0.05; n=4 for both).
Interestingly, the neuroprotective effect of 100 μmol/L GABA (Figure 3C) was largely reduced by either bicuculline (3 to 10 μmol/L) or CGP (100 μmol/L). In particular, the GABA-mediated field potential recovery was reduced to 5.3±2.5% of preischemic values by bicuculline and CGP 46381, respectively (P<0.01 compared with GABA alone; n=4 for both).

Thus, our hypothesis was that the neuroprotective effect of tiagabine and vigabatrin is mediated by the activation of both GABA<sub>A</sub> and GABA<sub>B</sub> receptors. To further investigate the receptor mechanisms of the neuroprotective action, we used 2 GABA agonists: muscimol (1 μmol/L), acting on the GABA<sub>A</sub> receptor, and balofen (1 μmol/L), acting on the GABA<sub>B</sub> receptor (Figure 4). According to previous data, both of these agonists showed a neuroprotective effect only when coapplied (P<0.01 compared with control; n=4) (Figure 4), while no significant recovery was observed when a single agonist was applied (P>0.05 compared with control; n=4).

### Discussion

The data in the present study provide further support for the hypothesis that, by increasing GABAergic transmission, it is possible to counteract the neuronal death consequent to an ischemic insult. However, we also found that coactivation of both GABA<sub>A</sub> and GABA<sub>B</sub> receptors is required to achieve the GABA-mediated neuroprotection during energy deprivation.

Tiagabine and vigabatrin are 2 currently used antiepileptic drugs that are indicated against focal seizures with or without secondary generalization. Both drugs have been tested in experimental ischemia models. Vigabatrin showed protection of the hippocampal CA1 region and substantia nigra reticulata in a gerbil model of repetitive forebrain ischemia. Tiagabine significantly reduced the ischemic-induced elevation of glutamate levels in CA1 area during the posts ischemic period and protected the CA1 pyramidal cell layer in a gerbil model of transient ischemia. In these in vivo experiments, however, posts ischemic hypothermia occurred, and it may have played a role in the obtained results. Hypothermia is a well-known protective factor against cell damage. In our model, however, we found that both drugs were able to protect the field potential amplitude from the irreversible damage induced by ischemia independently from hypothermia. In our experimental condition the temperature of the slices was constantly maintained at 34°C. Preliminary in vitro experiments performed in our laboratory at 36°C showed that both drugs are neuroprotective even when given at “physiological temperature” (C. Costa, MD, et al, unpublished data, 2003).

In the presence of exogenous GABA, we obtained a neuroprotective effect that was similar to that obtained with tiagabine and vigabatrin. The effect of tiagabine, vigabatrin, and GABA was largely reduced in the presence of the GABAnergic antagonist such as bicuculline (a selective antagonist of GABA<sub>A</sub> receptor) or CGP 46381 (a selective antagonist of GABA<sub>B</sub> receptors).

GABA may protect neurons not only by directly inhibiting neurons but also by exerting an inhibitory influence on glutamate-mediated neuronal activity. In agreement with this hypothesis, it has been shown that the GABA<sub>A</sub> agonist muscimol inhibits N-methyl-D-aspartate–induced neurotoxicity in primary cell cultures. Conversely, less clear is the protective role induced by activation of GABA<sub>B</sub> receptors. Our data support the view that the neuroprotective effects of tiagabine, vigabatrin, and GABA itself are mediated by the activation of both GABA<sub>A</sub> and GABA<sub>B</sub> receptors. To further explore the potential role of presynaptic and/or postsynaptic mechanisms of action in the pharmacological neuroprotection of the striatum after ischemia, we used 2 GABA agonists: muscimol, a GABA<sub>A</sub> agonist, and balofen, a GABA<sub>B</sub> agonist. These agonists showed a neuroprotective effect only when coapplied.

Interestingly, we did not observe a linear relationship between the dose and the protective effect. In fact, for these drugs we obtained a bell-shaped dose-response curve. This finding may have different explanations. It is possible to speculate that high drug concentrations facilitate brain damage. It has been reported that endogenous GABA controls its own release via GABA<sub>B</sub> autoreceptors. Thus, high levels of GABA mimetic drugs reduce the release of endogenous GABA. Alternatively, it can be speculated that excessive activation of GABA<sub>A</sub> receptor may cause an overload of chloride ions into the neurons, leading to cell swelling.

The role of GABA in brain damage after energy deprivation has been investigated by using several experimental approaches. A number of studies demonstrated a neuroprotective role exerted by enhancing GABAergic transmission. Conversely, some in vitro studies found that GABA receptor agonists worsen the cerebral damage caused by energy deprivation.

Previous in vivo studies showed neuroprotective effects of GABA<sub>A</sub> and GABA<sub>B</sub> agonists given in isolation. Conversely, other studies either failed to show such neuroprotection or found relevant side effects associated with the administration of these agonists. Since our in vitro study clearly shows that coactivation of GABA<sub>A</sub> and GABA<sub>B</sub> receptors is required for GABA-mediated protection, future in vivo investigations should be performed to explore this issue further. It is possible that concomitant activation of both receptors would allow achievement of clinically relevant therapeutic effects even with low doses of agonists.
Acknowledgments

This study was supported by CNR and MIUR-Cofin grants to Drs Calabresi and Bernardi and by a Progetto Finalizzato Sanità grant to Dr Pisani. We wish to thank M. Tolu for technical assistance.

References

Coactivation of GABA<sub>A</sub> and GABA<sub>B</sub> Receptor Results in Neuroprotection During In Vitro Ischemia

Cinzia Costa, Giorgia Leone, Emilia Saulle, Francesco Pisani, Giorgio Bernardi and Paolo Calabresi

*Stroke*. 2004;35:596-600; originally published online January 15, 2004;
doi: 10.1161/01.STR.0000113691.32026.06

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/35/2/596

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/