Coactivation of GABA$_A$ and GABA$_B$ Receptor Results in Neuroprotection During In Vitro Ischemia

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**Background and Purpose**—The possible neuroprotective effect of endogenous $\gamma$-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 $N_2$.

**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.$^3$–$^7$–$^11$ More recently, however, attention has been also focused on $\gamma$-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.$^{13}$–$^{19}$ 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.
Materials and Methods

Preparation and maintenance of rat corticostriatal slices have been previously described.3,24–26 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/ECC). Slices were kept in artificial cerebrospinal fluid, whose composition was as follows (in mmol/L): 126 NaCl, 2.5 KCl, 1.2 MgCl2, 1.2 Na2HPO4, 2.4 CaCl2, 11 glucose, and 25 NaHCO3. Artificial cerebrospinal fluid temperature was maintained at 34°C and was gassed with O2/CO2 (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% N2 and 5% CO2. 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 excitatory glutamatergic field potentials recorded 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/ECC). Slices were kept in artificial cerebrospinal fluid, whose composition was as follows (in mmol/L): 126 NaCl, 2.5 KCl, 1.2 MgCl2, 1.2 Na2HPO4, 2.4 CaCl2, 11 glucose, and 25 NaHCO3. Artificial cerebrospinal fluid temperature was maintained at 34°C and was gassed with O2/CO2 (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% N2 and 5% CO2. Ischemic and drug-containing solutions entered the recording chamber no later than 30 seconds after a 3-way tap was turned. 

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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 the peak negativity and the amplitude from the peak negativity 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.3 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 GABAα and GABAβ 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.22,23 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 (100 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 ± 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 5.0 ± 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% and 5±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_A and GABA_B receptors. To further investigate the receptor mechanisms of the neuroprotective action, we used 2 GABA agonists: muscimol (1 μmol/L), acting on the GABA_A receptor, and baclofen (1 μmol/L), acting on the GABA_B receptor (Figure 4). According to previous data, both of these agonists showed a neuroprotective effect only when coapplied (P<0.01 compared with GABA alone; n=4 for both).

Figure 4. Coactivation of GABA_A and GABA_B receptors is required to counteract the ischemia-induced effect. Neither muscimol, a GABA_A receptor agonist, nor baclofen, a GABA_B receptor agonist, was able to reverse the ischemia-induced loss of field potential. Conversely, coadministration of these agonists caused partial neuroprotection (**P<0.01 compared with control; n=4).

Our data support the view that the neuroprotective effects of tiagabine, vigabatrin, and GABA itself are mediated by the activation of both GABA_A and GABA_B 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_A agonist, and baclofen, a GABA_B 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_A autoreceptors. Thus, high levels of GABAmimetic drugs reduce the release of endogenous GABA. Alternatively, it can be speculated that excessive activation of GABA_A 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_A and GABA_B 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_A and GABA_B 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.
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