Is Pharmacological Neuroprotection Dependent on Reduced Glutamate Release?

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Background and Purpose—The aim of this study was to determine the possible role of the ionotropic glutamate receptor in the expression of irreversible electrophysiological changes induced by in vitro ischemia and to test whether the neuroprotective action of various neurotransmitter agonists and drugs of clinical interest is related to a presynaptic inhibitory action at glutamatergic synapses.

Methods—Intracellular and extracellular recordings have been performed in a rat corticostriatal slice preparation. Different pharmacological compounds have been tested on corticostriatal glutamatergic transmission in control conditions and in an in vitro model of ischemia (oxygen and glucose deprivation).

Results—In vitro ischemia lasting 10 minutes produced an irreversible loss of the field potential recorded from striatal slices after cortical stimulation. Preincubation of the slices with 3 μmol/L 6-cyano-7-nitroquinoxaline-2,3-dione (an α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid [AMPA] receptor antagonist) allowed a significant recovery of the field potential amplitude (P<0.05, n=6), whereas incubation with 30 μmol/L aminophosphonovaleric acid (an N-methyl-D-aspartate receptor antagonist) did not produce a significant recovery after 10 minutes of ischemia (P>0.05, n=7). Bath application of 3 mmol/L glutamate for 5 minutes produced a complete but reversible inhibition of the field potential amplitude. When a similar application was coupled with a brief period of ischemia (5 minutes), which produced, per se, only a transient inhibition of the field potential, it caused an irreversible loss of this parameter. We also tested the possible neuroprotective effect of neurotransmitter agonists reducing the release of glutamate from corticostriatal terminals. Agonists acting on purinergic (adenosine), muscarinic (oxotremorine), and metabotropic glutamate receptors (L-serine β-phosphate [L-SOP]) significantly (P<0.001, n=8 for each agonist) reduced glutamatergic synaptic potentials, with each showing different potencies. The EC50 was 26.4 μmol/L for adenosine, 0.08 μmol/L for oxotremorine, and 0.89 μmol/L for L-SOP. Concentrations of these agonists producing the maximal inhibition of the synaptic potential were tested on the ischemia-induced irreversible loss of field potential. Adenosine (P<0.05, n=9) and oxotremorine (P<0.05, n=8) showed significant neuroprotective action, whereas L-SOP was ineffective (P>0.05, n=10). Similarly, putative neuroprotective drugs significantly (P<0.001, n=10 for each drug) reduced the amplitude of corticostriatal potential, with different EC50 values (phenytoin, 33.5 μmol/L; gabapentin, 96.8 μmol/L; lamotrigine, 26.7 μmol/L; riluzole, 6 μmol/L; and sipatrigine, 2 μmol/L). Concentration of these drugs producing maximal inhibition of the amplitude of corticostriatal potentials showed a differential neuroprotective action on the ischemic electrical damage. Phenytoin (P<0.05, n=10), lamotrigine (P<0.05, n=10), riluzole (P<0.05, n=9), and sipatrigine (P<0.001, n=10) produced a significant neuroprotection, whereas gabapentin (P>0.05, n=11) was ineffective. The neuroprotective action of transmitter agonists and clinical drugs was not related to their ability in decreasing glutamate release, as detected by changes in the paired-pulse facilitation protocol.

Conclusions—Ionotropic glutamate receptors, and particularly AMPA-like receptors, play a role in the irreversible loss of field potential amplitude induced by ischemia in the striatum. Drugs acting by reducing glutamatergic corticostriatal transmission may show a neuroprotective effect. However, their efficacy does not seem to be directly related to their capability to decrease glutamate release from corticostriatal terminals. We suggest that additional modulatory actions on voltage-dependent conductances and on ischemia-induced ion distribution at the postsynaptic site may also exert a crucial role. (Stroke. 2000;31:766-773.)

Key Words: anticonvulsant ■ excitatory amino acids ■ ischemia ■ neuronal death

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It has been postulated that the abnormal release of excitatory amino acids plays a crucial role in triggering the cellular events leading to neuronal death after brain ischemia. The potential role of glutamate in the ischemia-induced acute electrical changes at the neuronal level has not been fully clarified. Corticostriatal afferents represent one of the major excitatory glutamatergic projections in the brain. Moreover, striatal neurons express high sensitivity to ischemic insults and energy deprivation.1-2 Prolonged in vitro ischemia causes irreversible electrophysiological changes in the striatum, reflecting the permanent disruption of ionic homeostasis.1-2

The role of ionotropic glutamate receptors in these changes has been challenged by electrophysiological studies.1 For this reason, we have analyzed the effect of either α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-like or N-methyl-D-aspartate (NMDA) glutamate receptor antagonists on the permanent loss of the field potential amplitude induced by in vitro (combined oxygen and glucose deprivation) ischemia in corticostriatal slices. Dealing with this issue, we have also tested whether application of exogenous glutamate is able to convert a transient depression of the field potential after brief ischemia into a permanent loss of this potential. Assuming that glutamate release during ischemia is a critical factor in the expression of the permanent electrical changes caused by this pathological event, we should expect that agents interfering with glutamate release might exert a neuroprotective effect in experimental ischemia.2 Lamotrigine represents a good example of this class of agents. This compound, which reduces the release of glutamate in various brain areas,3-5 also exerts a cerebroprotective effect after focal ischemia.6 Nevertheless, a large body of evidence demonstrates additional mechanisms underlying the action of lamotrigine7 and other neuroprotective compounds, such as phenytoin,8 gabapentin,9,10 riluzole,7,11 and sipatrigine, a new lamotrigine-like agent.12,13 All of these agents, in fact, have also been shown to modulate voltage-dependent sodium and calcium channels in the postsynaptic neuron. To correlate the neuroprotective efficacy of these pharmacological compounds with their presynaptic action at glutamatergic synapses, we performed electrophysiological experiments in corticostriatal slices. The possible pharmacological modulation of this pathological event in vitro might reflect the potential therapeutic efficacy of drugs of clinical interest in reducing neuronal damage after in vivo ischemia.14,15 Use of the in vitro preparation may allow us to quantify not only the neuroprotective efficacy of known doses of these drugs but also the presynaptic inhibitory effect of these concentrations of agents at corticostriatal glutamatergic synapses. In the present study, the pharmacological analysis of the neuroprotective effect of clinically related drugs was preceded by a detailed investigation of the possible neuroprotective effect of various neurotransmitter receptor agonists whose primary action is the reduction of corticostriatal glutamatergic transmission via a presynaptic mechanism.

Materials and Methods
Forty Wistar rats (150 to 250 g) were used. Animals were anesthetized with allotane and killed by severing the major blood vessels in the chest. The preparation and maintenance of slices have been described previously.1-2 Briefly, corticostriatal coronal slices (200 to 300 μm) were prepared from tissue blocks of the brain with the use of a vibratome. A single slice was transferred to a recording chamber and submerged in a continuously flowing Krebs solution (36°C, 2 to 3 ml/min) gassed with 95% O2/5% CO2. To study the effects of in vitro ischemia, slices were deprived of both glucose and oxygen. Glucose was totally removed from the perfusate, and the NaCl concentration was increased to balance the osmolarity. Oxygen deprivation was obtained by bubbling the perfusing solution in a gas mixture containing 95% N2/5% CO2. Ischemic solutions entered the recording chamber no later than 20 seconds after turning a 3-way tap. Complete replacement of the medium in the chamber took 90 seconds. The composition of the control solution was (in mmol/L) NaCl 126, KCl 1.2, NaH2PO4 1.2, CaCl2 1.2, CaCl2 2.4, glucose 11, and NaHCO3, 25.

For the extracellular experiments, the electrodes were filled with 2 mol/L NaCl (5 to 10 μl). The intracellular recording electrodes were filled with 2 mol/L KCl (30 to 60 μl) and placed in the striatum close (1 to 3 mm) to the cortical areas. An Axoclamp 2A amplifier (Foster City) was used for recordings. Traces were displayed on an oscilloscope and stored on a digital system. For synaptic stimulation, bipolar electrodes were used (0.03- to 0.01-millisecond duration, 1 to 5 V). These stimulating electrodes were located either in the cortical areas close to the recording electrode or in the white matter between the cortex and the striatum to activate corticostriatal fibers. 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 controls, with the latter representing the mean of responses recorded during a stable period (15 to 20 minutes) before the ischemic phase. Values given in the text and in the figures are mean±SD of changes in the respective cell populations. Student t test (for paired and unpaired observations) was used to compare the means. Drugs were applied by dissolving them to the desired final concentration in the saline and by switching the perfusion from control saline to drug-containing saline. Drugs used were as follows: adenosine (Sigma Chemical Co), aminophosphonovaleric acid (APV, Tocris), 6-cyano-7-nitroquinoline-2,3-dione (CNQX, Tocris), sipatrigine (BW619C89, kind gift from Dr M.J. Leach, University of Greenwich, London, UK), gabapentin (Parke-Davis), glutamate (Sigma), lamotrigine (Glaxo Wellcome), 1-serine-β-phosphate (L-SOP, Tocris), oxotremorine (RBI), phenytoin (Sigma), and riluzole (RBI). Adenosine, oxotremorine, L-SOP, gabapentin, and sipatrigine were solved in water; phenytoin, lamotrigine, and riluzole were solved in dimethyl sulfoxide. The maximal final concentration of the solvent was 1:300. This concentration of dimethyl sulfoxide did not produce, per se, detectable electrophysiological changes.

In some experiments in which the activity of the cytosolic enzyme LDH was determined during reperfusion in the medium to assess cellular necrosis, 0.2 mL of medium was collected at the indicated times, and the amount of LDH, expressed as percentage of total activity of the tissue, was calculated. The activity of LDH was determined spectrophotometrically from the change in absorbance at 340 nm, with 0.18 mmol/L NADH and 0.72 mmol/L pyruvate in 50 mmol/L phosphate buffer, pH 7.4 at 25°C, used as substrate. All the experiments were performed according to the Animal Research Guidelines of the European Communities Council Directive (86/609/EEC).

Results
Effect of Different Periods of Ischemia in Corticostriatal Slices
As shown in Figure 1A, a brief period (5 minutes) of in vitro ischemia produced a reversible inhibition of the field potential amplitude (n=10). Conversely, a longer period of ischemia (10 minutes) caused the irreversible disappearance of the corticostriatal field potential (n=12) (Figure 1B). Accord-
ingly, intracellular recordings have previously shown that striatal spiny neurons respond to in vitro ischemia with a reversible membrane depolarization when the period of energy deprivation lasts \( \leq 5 \) minutes. An ischemic period lasting \( \geq 10 \) minutes leads to a permanent loss of the resting membrane potential.1

Role of Ionotropic Glutamate Receptors in Irreversible Loss of Field Potential Induced by Ischemia

In the first part of the present study, we investigated the potential role of ionotropic glutamate receptors in the expression of the irreversible loss of the field potential amplitude after 10 minutes of ischemia. As shown in Figure 2A, preincubation of the slices with 3 \( \mu \)mol/L CNQX (an AMPA receptor antagonist) produced a significant depression of the field potential amplitude but allowed a significant recovery of this potential after ischemia \((P<0.05, n=6)\). Higher concentrations (5 and 10 \( \mu \)mol/L) of this antagonist did not produce further protection \((n=7\), data not shown). Conversely, incubation of the slices with 30 \( \mu \)mol/L APV (an NMDA receptor antagonist) produced minor changes of the field potential amplitude but induced only a mild recovery that was not statistically significant \((P>0.05, n=7)\) after 10 minutes of ischemia.

The potential role of glutamate receptors in the electrophysiological abnormalities induced by ischemia in the striatum was also investigated by using a different experimental approach. As shown in Figure 2B, bath application of 3 mmol/L glutamate (GLU, gray bar) induced a transient depression of the field potential amplitude. Similarly, a brief period of ischemia (5 minutes, black bar) also induced a transient depression of the potential. Note, however, that when the 2 treatments were combined, they induced a permanent loss of the field potential. The insert in panel B shows that in some experiments similar data were obtained before any pharmacological or experimental treatment of the slice to avoid the possible induction of sensitization.
In the second part of the present study, we tested the hypothesis that a drug-induced decrease of the release of glutamate from corticostriatal terminals is a major mechanism underlying the pharmacological neuroprotection that occurs after a period of in vitro ischemia lasting 10 minutes. To achieve this goal, we have analyzed the possible neuroprotective effects of various neurotransmitter agonists whose selective inhibitory actions at corticostriatal terminals have been previously demonstrated. 17–19 The results of the experiments clearly indicate that the activation of glutamate receptors plays a role in the irreversible disruption of an ionic gradient triggered by ischemia.

Effect of Neurotransmitter Receptor Agonists on Irreversible Loss of Field Potential Induced by Ischemia

In the second part of the present study, we tested the hypothesis that a drug-induced decrease of the release of glutamate from corticostriatal terminals is a major mechanism underlying the pharmacological neuroprotection that occurs after a period of in vitro ischemia lasting 10 minutes. To achieve this goal, we have analyzed the possible neuroprotective effects of various neurotransmitter agonists whose selective inhibitory actions at corticostriatal terminals have been previously demonstrated. 17–19

Figure 3A shows the dose-response curve and the EC₅₀ values obtained for adenosine, L-SOP, and oxotremorine (n=8 for each agonist). The pharmacological effects of all these drugs on the excitatory synaptic potential (EPSP) amplitude were fully reversed after 15 minutes of washout. Moreover, these inhibitory effects were coupled neither with changes of the resting membrane potential nor with alterations of the input resistance of the recorded neurons (data not shown). These data allowed us to chose for each drug a concentration producing the maximal inhibition of the synaptic potential to be used in the experiments dealing with ischemia. Drugs were applied 10 minutes before the onset of ischemia, and their application was also maintained during the period of ischemia. The drug-induced decrease of the field potential was stable after 5 minutes of application, and the amplitude of these depressions (expressed as percentage of the control values) was similar to the decrease of the intracellularly recorded EPSPs induced by each drug. Figure 3B shows that adenosine (100 μmol/L) produced a recovery of the field potential amplitude 30 minutes after the washout of the ischemic solution whose amplitude was significantly higher than that observed in the control medium (n=9). Oxotremorine (300 nmol/L), a muscarinic agonist acting mainly at M₂ receptors, 19 exerted a similar neuroprotective action (n=8). Conversely, L-SOP (30 μmol/L), an agonist acting at group III presynaptic metabotropic glutamate receptors, 18 did not show a significant neuroprotective action when measured 30 minutes after administration of the ischemic solution (n=10). The presynaptic effect of adenosine, oxotremorine, and L-SOP was further investigated by using a paired-pulse protocol. Paired-pulse modification of neurotransmission has been studied extensively and is attributed to a presynaptic change in release probability. 17,20,21 An increase in the ratio of the second pulse response to the first pulse response (EPSP₂/EPSP₁) indicates a decrease in the release probability. The decrease in transmitter release probability is consistent with the observations that manipulations depressing transmitter release usually increase the magnitude of this ratio. Therefore, we measured the magnitude of EPSP₂/EPSP₁ before and during the application of the tested compounds. Synaptic responses to a pair of stimuli were recorded with an interstimulus interval of 60 milliseconds. As shown in Figure 3C, adenosine (n=8), oxotremorine (n=9), and L-SOP (n=9) increased the magnitude of EPSP₂/EPSP₁ in the tested neurons, confirming for all of them a presynaptic site of action.
chose a concentration for each neuroprotective agent producing the maximal inhibition of the potential. This maximal concentration was tested on the ischemia-induced electrophysiological changes. As shown in Figure 4B, the most effective neuroprotective drug was sipatrigine (30 μmol/L, n=10). Pretreatment with this agent, in fact, allowed a recovery of the field potential amplitude to ~50% of the control value. A significant neuroprotection was also observed by pretreating the slices with 3 other putative neuroprotective drugs: lamotrigine (100 μmol/L, n=10), phenytoin (100 μmol/L, n=10), and riluzole (30 μmol/L, n=9). Conversely, gabapentin (300 μmol/L, n=11) did not significantly protect against the ischemic insult. The analysis of the paired-pulse facilitation revealed that the only drug that significantly increased the EPSP2/EPSP1 ratio measured under control conditions was lamotrigine (n=10), whereas phenytoin (n=11), gabapentin (n=10), riluzole (n=11), and sipatrigine (n=10) reduced the EPSP amplitude without affecting this electrophysiological parameter (Figure 4C). Neither lamotrigine nor the other studied compounds altered the resting membrane potential and the apparent input resistance of the intracellularly recorded cells (n=11 for each drug, data not shown).

**LDH Release During Ischemia**

To test an independent parameter to the slice “stress condition” in addition to electrophysiological variables, we measured LDH release in the perfusate after different periods of ischemia (5, 10, 20, and 30 minutes). Periods of ischemia lasting <30 minutes did not produce significant changes in LDH release compared with LDH release in control experiments (data not shown). A small but significant increase in the release of LDH from the slices was observed in the reperfusion phase after prolonged ischemic conditions (30 minutes). This release was time dependent, reaching a maximal value at 50 to 60 minutes of reperfusion.

**Discussion**

**Ionotropic Glutamate Receptors Play a Role in Expression of Irreversible Electrical Changes in the Striatum After Ischemia**

The striatum is the major afferent component of the basal ganglia and receives dense excitatory glutamatergic projections from the neocortex and thalamus. Glutamate receptor–mediated excitotoxicity has been proposed to cause striatal neuronal death after ischemia. It has been shown that glutamate receptor blockade reduces ischemic neuronal death within the striatum. A distinctive pathological feature of striatal ischemia is the specific neuronal vulnerability. In fact, ischemic insults cause a selective loss of striatal spiny neurons. This selective vulnerability has been related to the selective vulnerability to ischemic insults cause a selective loss of striatal spiny neurons. This selective vulnerability has been related to the selective vulnerability to ischemic insults cause a selective loss of striatal spiny neurons.

**Effect of Putative Neuroprotective Drugs on Irreversible Loss of Field Potential Induced by Ischemia**

In the third part of the present study, by using a similar electrophysiological approach, we analyzed the neuroprotective efficiency of various pharmacological agents expressed as the recovery of the field potential amplitude measured 30 minutes after the washout of the ischemic solution. As shown in Figure 4A, for each drug, we obtained a dose-response curve and an EC50 value showing the potency and the efficacy of the drug to inhibit the EPSP amplitude (n=10 for each drug). Also in this case, these experiments allowed us to...
suggest that activation of AMPA-like but not NMDA glutamate receptors plays a role in the expression of the long-term disruption of the ionic homeostasis in striatal neurons. The critical role of glutamate receptors in the generation of the irreversible loss of the corticostriatal field potential has also been approached by using a different experimental paradigm. Under control conditions, bath application of glutamate produced a transient depression of field potential amplitude. This depression was probably related to the massive depolarization induced by this transmitter in the postsynaptic neurons and to the activation of presynaptic autoreceptors located on corticostriatal terminals. We observed that brief periods of ischemia (5 minutes), which did not induce, per se, a long-term blockade of this potential, were able to cause permanent changes when administered in conjunction with bath application of glutamate. This finding strongly supports the idea that energy deprivation and activation of postsynaptic glutamate receptors may concur in the expression of the permanent alteration of striatal electrical activity. To correlate the permanent electrophysiological changes to the neuronal cell loss induced by ischemia, we have measured LDH release from the tissue. This parameter, in fact, is a biochemical marker of tissue necrosis. We found a significant increase of LDH release only in the reperfusion phase after 30 minutes of ischemia but not after shorter periods. This finding indicates that the irreversible loss of electrical signals is an early sign of neuronal damage, whereas changes in LDH release may be considered an indicator of neuronal injury after prolonged energy deprivation.

Neuroprotective Effect of Some Neurotransmitter Receptor Agonists Is Not Solely Related to Presynaptic Inhibition of Glutamate Release From Corticostriatal Terminals
In the present study, we have also analyzed the dose-response curves and the EC50 values of 3 neurotransmitter receptor agonists known to decrease the corticostriatal EPSP by presynaptic mechanisms: adenosine, L-SOP, and oxotremorine. According to their presynaptic mechanisms of action, all these agonists increased the paired-pulse facilitation (an index of a presynaptic inhibition). For each agonist, we tested a concentration producing the maximal inhibition of the EPSP on the ischemia-induced permanent suppression of the field potential. Surprisingly, we found that although adenosine was more effective than oxotremorine in reducing EPSP amplitude, it protected the field potential to the same extent as oxotremorine. Moreover, we also observed that L-SOP produced a significant depression of the synaptic potential but did not show a neuroprotective effect. These data seem to suggest that the neuroprotective action of neurotransmitter receptor agonists is not solely related to a presynaptic inhibition of glutamate release from corticostriatal terminals.

Does Modulation of Additional Postsynaptic Events Play a Role in Neuroprotective Effects of Various Drugs of Clinical Interest?
The finding that the neuroprotective effect of neurotransmitter receptor agonists does not correlate with the reduction of glutamate release is not in contrast with the hypothesis that the neuronal death caused by ischemia involves an excitotoxic mechanism. In fact, it is possible that although some of these agents do not directly affect the glutamate release within the striatum, they may modulate some events secondarily induced by glutamate receptor stimulation at the postsynaptic level, such as the activation of voltage-dependent inward conductances. It is also possible that some neuroprotective agents exert their pharmacological effect during ischemia by modulating a calcium-independent glutamate release. This pathological form of neurotransmitter release seems to be important during ischemia, and it may occur via different mechanisms. The pharmacological modulation of this calcium-independent glutamate release would not be detected by measuring the changes in paired-pulse facilitation, a calcium-dependent form of short-term synaptic plasticity.

To further explore the potential role of postsynaptic mechanisms of action in the pharmacological neuroprotection of the striatum after ischemia, we characterized the dose-response curve and the EC50 values of various putative neuroprotective drugs concerning their inhibitory action on the corticostriatal EPSP amplitude. The concentration of each agent producing the maximal inhibition of these potentials was tested on the permanent loss of field potential induced by ischemia. We observed that although all the putative neuroprotective drugs used in the present study depressed the EPSP amplitude, only lamotrigine increased the paired-pulse facilitation (suggesting a possible presynaptic action), whereas the neuroprotection exerted by phenytoin, sipatrigine, gabapentin, and riluzole was not coupled with significant changes of this parameter. Taken together, these data seem to suggest also that the modulation of postsynaptic membrane properties may play a crucial role in the mechanisms underlying neuroprotection in central neurons. Accordingly, inhibition of postsynaptic voltage-dependent calcium and/or sodium currents has been reported for phenytoin, lamotrigine, gabapentin, riluzole, and sipatrigine.

It is important to note that none of the neuroprotective agents used, at least not at the concentration used in the present study, affects resting membrane potential and apparent input resistance of the recorded spiny neurons. This observation clearly indicates that a possible postsynaptic site of action does not involve resting membrane conductances but rather implicates the modulation of currents operating at depolarized levels. Alternatively, it is also possible that these neuroprotective agents interact either with conductances that are specifically activated during ischemia, such as ATP-dependent potassium currents, or metabolic processes and conductances operated by intracellular calcium accumulation. We have recently shown that some neuroprotective agents used in the present study do not alter the amplitude of reversible membrane depolarizations recorded from striatal spiny neurons during brief (3- to 5-minute) periods of in vitro ischemia. The efficacy of these drugs in the present study might suggest that prolonged ischemia (10 minutes) triggers a more complex chain of events that can be modulated by these neuroprotective agents.

Limits of the Study and Future Perspectives
We are aware that the main limitation of the present study is that the mechanisms underlying the neuroprotection have
been investigated by using an in vitro model of ischemia. In fact, it is possible that the drugs that were used show a different pharmacological efficacy when used in vivo. However, the use of the in vitro model has allowed us to correlate the neuroprotective effect of known concentrations of drugs in a certain brain area with their efficacy in inhibiting glutamatergic transmission. This goal would never have been achieved by using an in vivo model of ischemia.

It is possible that concentrations of drugs higher than those tested in the present study could exert more pronounced neuroprotective effects in vitro. However, it should be noted that these higher concentrations might not have a clinical relevance, because they would be achieved in vivo only after the administration of doses of drugs exerting toxic effects.

It would be interestingly to measure, during in vivo experiments, glutamate concentrations with microdialysis during ischemia in control conditions and after treatment with the putative neuroprotective drugs. These experiments would allow researchers to correlate the decrease of glutamate release exerted by each pharmacological agent with its neuroprotective action. This interesting issue should be developed in future studies.

The pursuit of effective neuroprotective therapy has often been frustrating because the promise of efficacy in preclinical animal studies has not been realized in clinical trials. Nevertheless, it is worth noting that some of the drugs whose neuroprotective activity has been analyzed in the present study are currently under investigation in clinical trials.30

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One important criterion I keep in mind when reviewing papers for *Stroke* is whether the readers of the journal, both clinicians and experimentalists, are going to find the data relevant and useful to stroke research, in its broadest sense. In this respect, the difficulties in promoting glutamate receptor antagonists for clinical use for stroke have already resulted in serious doubts about whether glutamate receptor antagonists are important strategies for ischemic neuroprotection, as 2 decades of frustrations to materialize NMDA, AMPA, or other manipulation of the glutamatergic receptors/channels have passed. So, what can an in vitro, slice-based electrophysiological study still teach us in the art to keep us interested and even reenergized to continue to explore glutamatergic strategies in ischemic brain injury? In this report, the authors used a preparation that includes excitatory glutamatergic synapses to examine the effect of “ischemia” (glucose and oxygen deprivation) and an array of pharmacological agents on electrophysiological consequences (commonly believed to represent “en route” cell demise) of these neurons. An important component of the study is the use of pharmacological agents that have been in clinical development or are currently in clinical development for stroke. This is important, because it will allow, in due time, feedback from the clinical trials back to the “bench theory” for proof/disproof of the hypothesis. Several important points are made in the study by Calabresi et al, including the association of glutamate injury with ischemia; the role of the AMPA-like receptors in irreversible loss of cell potential during ischemia; and that neuroprotection (as assessed by preservation of electrophysiological parameters) may not correlate to glutamate release inhibition from presynaptic elements. Therefore, other features of drug interactions with the ionotopic glutamate channels need to be explored.

The article offers an interesting model system for pharmacological evaluation of compounds that needs to be further explored in conjunction with in vivo models. If the data generated in this in vitro system are confirmed in “main line” in vivo stroke models (eg, focal ischemia), including electrophysiological monitoring, coupled with direct monitoring of glutamate release and ultimately direct assessment of neuronal viability and neurobehavioral outcome, a powerful scheme to evaluate pharmacological agents could emerge. While the expertise of the authors does not span to the in vivo dimension, it is hoped that this in vitro “classical” pharmacological-electrophysiological study generates interest to extend the studies in view of seeking in vivo confirmation based on the in vitro results. Such database may be most valuable as it awaits results from clinical trials (negative or positive) which are the ultimate litmus test for the validity of the approach presented in the article by Calabresi et al. I hope that somewhere within the readers’ community of stroke this will happen.

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