The Rise and Fall of NMDA Antagonists for Ischemic Stroke

L. Hoyte, P.A. Barber, A.M. Buchan and M.D. Hill*

From the Calgary Stroke Program, Department of Clinical Neurosciences, University of Calgary, Calgary, Ab, Canada

Abstract: It has long been accepted that high concentrations of glutamate can destroy neurons, and this is the basis of the theory of excitotoxicity during brain injury such as stroke. Glutamate N-methyl-D-aspartate (NMDA) receptor antagonists such as Selfotel, Aptiganel, Gavestinel and others failed to show neuroprotective efficacy in human clinical trials or produced intolerable central nervous system adverse effects. The failure of these agents has been attributed to poor studies in animal models and to poorly designed clinical trials. We also speculate that NMDA receptor anatagonism may have hindered endogenous mechanisms for neuronal survival and neuroregeneration. It remains to be proven in human stroke whether NMDA receptor antagonism can be neuroprotective.

INTRODUCTION

Glutamate receptors in the central nervous system can be divided into ionotropic and metabotropic types. Among the ionotropic glutamate receptors, the N-methyl-D-aspartate (NMDA) receptor is a ligand-gated calcium (Ca^{2+}) channel. It is a pentameric or tetrameric heteromer of NR1 and NR2 subunits [1]. Mutliple alternately spliced variants of a single gene make up the NR1 subunits and four genes yield the NR2 transcripts. The receptor has co-agonist sites for glycine, which are usually occupied at physiological glycine concentrations meaning that the receptor is usually "glycine-primed" [2]. Affinity for glycine varies by NR2 subunit [3]. Mg^{2+} blocks the channel in a voltage dependent manner at physiological concentrations, but blockade is lost with depolarization [4].

The NMDA receptor is a pivotal ion channel in the process of excitotoxicity. Excitotoxicity has been implicated as a mechanism for neuronal damage in the central nervous system (CNS) in multiple disease states with ischemia being the paradigm [5]. Following transient complete cerebral ischemia, there is an increase in the levels of the excitatory amino acid glutamate [6], which has been shown to activate the NMDA receptor to neurotoxic levels during energy deprived situations [7]. This excitotoxic neurotransmission is due to the inward flux of Ca^{2+} through the NMDA channels following cerebral ischemia [8]. It was proposed by Simon *et al.* [9] in 1984 that blockade of the NMDA receptor may protect neurons in the brain from ischemic damage.

Blockade of the NMDA channel by competitive antagonism requires high concentrations of the antagonist because of the high affinity of the NMDA receptor for glutamate. This is relevant because adverse events due to competitive NMDA receptor antagonists such as hallucinations, delirium, psychosis etc. are dose-dependent. Non-competitive antagonism is the method of action of the street drug phencyclidine (PCP) and the anesthetic agent ketamine. Both are effective at reducing the Ca^{2+} inward current and have been examined in both focal and global models of ischemia.

During global ischemia there are intracellular and extracellular Ca^{2+} concentration changes in the hippocampus [8,10, 11]. These Ca^{2+} concentration changes have the largest effect on the CA-1 neurons of the hippocampus [8, 9]. Injection of NMDA receptor antagonists into the hippocampus greatly reduced Ca^{2+} influx during ischemia [8]. Systemic administration of NMDA antagonists such as ketamine or MK-801, also slowed Ca2+ influx during ischemia [10]; however a secondary rise in intracellular Ca^{2+} concentrations two hours post-ischemia was not attenuated by MK-801 [11] suggesting that alternate secondary mechanisms may also require blockade to prevent cytotoxic intracellular rises in Ca^{2+} concentrations.

It appears that it is the large calcium influx that is the immediate precursor to neuronal cell death [12, 13]. This may be due to the role of calcium as a

*Address correspondence to this author at the Calgary Stroke Program, Department of Clinical Neurosciences, University of Calgary, Foothills Hospital, Rm 1242A, 1403 29th Street NW, Calgary, Alberta, T2N 2T9, Canada; Fax: 403 283 2270; E-Mail: michael.hill@calgaryhealthregion.ca

Current affiliations
Lisa Hoyte, BSc., Calgary Stroke Program, Department of Clinical Neurosciences, University of Calgary, Calgary, Alberta, CANADA
Philip A. Barber, MB MRCP, Calgary Stroke Program, Department of Clinical Neurosciences, University of Calgary, Calgary, Alberta, CANADA
Alastair M. Buchan, MB FRCPC, Calgary Stroke Program, Department of Clinical Neurosciences, University of Calgary, Foothills Hospital, Calgary, Alberta, CANADA
Michael D. Hill, MD MSc FRCPC – Departments of Clinical Neurosciences, Medicine and Community Health Sciences, Faculty of Medicine, University of Calgary, Calgary, AB CANADA

Conflicts of interest:
None to declare
Statement of Authorship
LH and PAB researched and drafted the text on experimental models of stroke. MDH researched and drafted the text on clinical aspects of neuroprotective trials. AMB edited the final manuscript and contributed constructive comments. MDH edited the overall manuscript.

1566-5240/04 $45.00+.00

second messenger and its importance in inducing early immediate genes in apoptotic mechanisms of cell death [14]. Genetic determinants to the excitotoxic death phenomenon have been identified [15]. Different mouse strains have exhibited varying levels of glutamate excitotoxicity, an indication that there may be genes that regulate excitotoxic cell death [15]. Calcium is also toxic to the mitochondrial inner membrane and may ~~further~~ impair energy metabolism.

Current treatment of ischemic stroke involves the use of tissue-plasminogen activator (tPA), which may enhance the activity of NMDA receptors and increase excitotoxic lesions in rats [16]. This same toxicity has not been observed with desmoteplase [17] and it has been suggested that the toxicity of tPA is not due to the recombinant protein but instead due to nitric oxide produced from the L-arginine carrier present in the preparation, particularly in permanent focal ischemia (Buchan AM 2003 personal communication).

Most of the clinical trials have tested the hypothesis that NMDA antagonism, either non-competitively or by blocking the glycine or polyamine sites, would reduce clinical injury and result in better neurological outcomes at three months. All of these trials have failed [18]. It has been proposed by Choi *et al.* [19] that treatment following thrombolysis be broadened beyond targeting the excitotoxic mechanism for a more effective therapy. The corollary is that agonizing inhibitory receptors such as serotonin and the GABA-A receptors will abrogate the levels of calcium seen in the intracellular compartment, but these trials have also faltered.

Traditionally evidence for neuroprotection is sought first in *in vitro* models of neuronal cell culture exposed to either anoxia/aglycemia or glutamate insults. Promising candidates are then developed *in vivo* in rodent and murine models of both global and focal ischemia. Currently the evidence that neuroprotection works, lending support for clinical trials, is based on the *in vivo* models of focal stroke. In view of the failure of neuroprotective strategies in clinincal trials we propose an alternative process of assessing these agents in the preclinical phase. Putative neuroprotective agents should show efficacy in models of both “cytoprotection” (global ischemia) by reducing CA1 injury, and infarct volume (focal ischemia) prior to phase II and III clinical trials.

ANIMAL MODELS OF STROKE

Animal models of cerebral ischemia have been well developed in the rat and mouse. Rabbit, dog and primate models exist but are less commonly used. Transient forebrain ischemia in the rat involves occlusion of the vertebral arteries and both common carotid arteries [20, 21] and results in a selective neuronal death. This selective neuronal death is influenced by ischemia duration [22]. A shorter duration of ischemia results in a slower progression of CA-1 damage in the hippocampus [20]. These models create a transient oligaemia (no blood flow) to the hippocampus, cortex and striatum during ischemia followed, after 5, 10, 15 or even 30 minutes, by a prompt recovery of both blood flow and energy levels (ATP) [23]. The forebrain ischemic insult although brief is very severe. Importantly, drugs given following reperfusion cannot influence the degree of blood flow reduction during the ischemic insult. In this model, hypothermia even 6 to 24 hours after reperfusion allows for long-term recovery of cells [24]. There is a prolonged interval of depressed energy in the hippocampal neurons prior to cell death [25]. Given the extraordinary sensitivity of CA_1 cells compared with CA_3 cells, any agent that reverses this selective vulnerability could be defined as a neuroprotectant. Drugs that prevent CA_1 cell death are likely to attenuate cortical and striatal injury resulting in reduced infarction following either permanent or transient focal ischemia.

Focal neocortical ischemia can be induced in the rat with a micro-aneurysm clip on the distal middle cerebral artery in conjunction with tandem common carotid artery occlusion [26]. Mechanical models of focal ischemia use either an intraluminal suture, which induces severe ischemia in the striatum and relatively milder ischemia in the cortex [27], or a more distal extravascular clip, which may spare the striatum but results in more severe cortical ischemia [21]. The infarction in the cortex is associated with a breakdown in the blood brain barrier and edema [28]. Components of excitotoxicity, apoptosis and a subsequent neuro-inflammatory reaction all work in parallel, resulting in necrosis and the full evolution or maturation of the infarct.

In lissencephalic species (rodents) there is very little white matter, the striatum is hard to protect, and while 50% infarct volume reductions are often seen in cortex, this reduction in injury may not be static. In the same way that brief ischemia results in less infarction, if a longer interval is allowed to elapse this small infarct will evolve, recruiting the ischemic penumbra . The ischemic penumbra is defined as *fundamentally reversible* and is perhaps pragmatically characterised by a response to pharmacological agents [29]. When infarct volume reductions are seen at 24 or 48 hours, it remains unclear if a drug induced protection can be maintained out to 7–28 days or even to three months, although it has been demonstrated for post-ischemic hypothermic neuroprotection [30].

Buchan and Pulsinelli used the NMDA antagonist MK-801 to demonstrate that the neuroprotective effect of NMDA antagonism may be due to hypothermia and not the agent itself [22]. Temperature unregulated gerbils treated with MK-801 showed a lower grade of damage than untreated. These animals also showed a prolonged hypothermia. Similar protection was observed when untreated animals were maintained at 34.5˚C, but both saline and MK-801 treated gerbils showed

similar damage when rectal temperatures were maintained at 38.5˚C. These effects seem to be due to the hypothermia induced by MK-801 and not the agent itself. The effect of hypothermia on calcium uptake has raised questions regarding the treatment of excitotoxic cell death [31].

Further work by Buchan *et al.* [32], failed to demonstrate MK-801 induced neuroprotection of the CA-1 neurons following global ischemia in rats when temperature is controlled. Rats were temperature controlled to a minimum of 37.5˚C during ischemia and recovery. No neuroprotective effect of NMDA antagonism by MK-801 was observed following transient forebrain ischemia.

NMDA antagonists have been assessed experimentally in focal ischemia. The focal infarct reductions are in part related to changes in vasoactivity (increased rCBF) and increases in tachycardia and blood pressure (also resulting in a relative change of cerebral blood flow) [33]. Furthermore, much of the earlier work did not use temperature regulation, and very short periods of survival were used to assess either histological injury or behavioural outcome (<3 days). Valtysson *et al.* addressed this issue and found that a single dose of MK-801 simply postponed the stroke injury [34]. Indeed it appears not much has changed in recent studies with 66% using survival periods of less than 48 hours [35].

NMDA RECEPTOR AND INJURY RECOVERY

Glutamate mediated synaptic transmission is essential for neuronal survival. Choi *et al.* ~~intriguenely~~ discovered that NMDA antagonists can cause proteosome inhibition resulting in selective apoptosis in striatal and cortical neurons associated with intracellular calcium starvation [36]. When NMDA receptors are blocked in the fetal or neonatal brain apoptosis is triggered [37]. Blockade of NMDA receptors also increases neuronal death in the adult brain following injury [38]. The importance of the NMDA receptors to neuronal survival indicates that blockade of these receptors may hypothetically result in decreased neuronal survival long term.

The NMDA receptor has been implicated in regulating neurogenesis [39]. NMDA receptor antagonism has been shown to block neurogenesis in the sub-ventricular zone of rats following global ischemia [40]. This NMDA mediated neurogenesis may operate through a common pathway to adrenal steroids. Blocking of NMDA receptor activation results in a consequent decrease in corticosteroid effects on cell proliferation. NMDA receptor may be downstream to the corticosteroid in this pathway [41]. Arvidsson *et al.* [42] found that administration of MK-801 to rats following transient focal ischemia resulted in a decreased neurogenesis in the dentate gyrus. In fact, the number of Bromo-deoxy-uridine (BrdU) labeled cells on the ipsilateral side in MK-801 treated animals was similar to the contralateral side and to sham operated animals. Although reduced cortical neuronal injury was seen with MK-801, degree of injury did not correlate with the amount of neurogenesis, perhaps suggesting that the reduced neurogenesis in the MK-801 was independent of the size of stroke injury.

In conclusion, there is evidence to suggest that that synaptic activity mediated by NMDA receptors promotes survival of neurons. Blockade of NMDA-mediated synaptic transmission must be detrimental in situations when support by endogenous measures is required, as occurs after stroke. Further evidence is required to determine the significance of dentate gyrus neurogenesis in clinical stroke, and the importance of NMDA receptor regulation of neurogenesis.

PROBLEMS WITH DRUG TESTING FOR NEUROPROTECTION

While several good animal model of ischemia exist, rat models of both focal and global ischemia have been the most used and it is instructive to re-emphasize that rats are a lissencephalic species with proportionally less white matter than humans. Drug testing in animal models has suffered from a lack of physiological control resulting in spurious outcomes [22]. In the ensuing clinical trials there have also been a series of unexpected physiological perturbations, which have resulted in deleterious side effects either leading to excessive toxicity or causing futility in terms of improving outcome.

A second major problem has been the difficulty of achieving drug levels in humans comparable to those that achieved neuroprotection in animal models. Particularly, using competitive NMDA receptor antagonists, drug development was abandoned early in human trials because of dose-limiting toxicity [43].

A third difficulty is that in the animal models, there is a slow maturation of injury during the first few days. As a result, observed “neuroprotection” in the first 48 hours to 7 days is in reality is only a mirage because injury development has merely been postponed [23]. In humans, where three month clinical outcomes are the gold standard, early neuroprotection will likely have evaporated in tandem with the slow maturation of injury.

Fourth, the timing of intervention has been relaxed for human studies, primarily to allow for adequate recruitment of patients. In most animal models, benefit has been shown only in the first 1-3 hours after ischemia. Most human studies have used a 6-hour window or longer. NMDA receptor agonism by glutamate occurs very early after ischemia onset such that pharmacological intervention in human stroke might only be potentially effective within the first hour of onset or less [44].

Finally, little attention has been paid to the interspecies differences between rats and humans. Preclinical neuroprotective studies have

concentrated almost exclusively on the protection of cerebral grey matter from ischemic injury. The effects of neuroprotective therapy, such as NMDA antagonists, are largely unknown [45, 46]. The human brain contains a greater proportion of white matter compared with the rat brain, and the failure of some neuroprotective trials may be due to an inability of certain agents to protect against axonal injury. NMDA antagonsists have never been shown to protect white matter and they do not protect the striatum experimentally. Approximately a third of human stroke is lacunar, yet there are no animal models of this lacunar stroke. With relatively much more white matter, little has been done to assess this important limitation of these drugs in humans.

MAJOR CLINICAL TRIALS OF NMDA RECEPTOR ANTAGONISTS

Dizocilpine (MK801), the first drug thought to show consistent neuroprotective effects in pre-clinical studies, never reached the human clinical trial development stage because of safety concerns over histopathological changes in rats [47]. Several NMDA-R antagonists have reached evaluation in human Phase III clinical trials and full discussion of each drug is beyond the scope of this review. The reader is referred to a review by Saver *et al.* [48]. Two examples of prominent direct NMDA antagonist drugs studied in Phase IIB or Phase III trials were selfotel (CGS 19755) and aptiganel hydrochloride (CNS 1102). Gavestinel (GV150526) and $MgSO_4$, both of which non-competitively inhibit the NMDA receptor by binding at non-glutamate receptor sites, have or are being investigated in phase III clinical trials.

Selfotel is a lipophillic competitive NMDA-R antagonist. Tolerable side effects, such as mild confusion, dysarthria, hallucinations were observed in initial trials. Two trials with combined enrolment of 567 patients were suspended by the Data Safety and Monitoring Board at 31% target enrolment because of increased mortality in the selfotel treatment group. While 90-day mortality was not different between groups, early mortality at 30d was increased in the selfotel-treated group [RR 1.41 95%CI 0.96-2.1]. This difference was larger for patients with more severe stroke. No morbidity benefit was seen among surviving patients. Survival analysis suggested a very early trend, beginning within 24 hours, to increased mortality in the selfotel-treated patients [49, 50].

A majority of patients were treated late; only 13% were treated within 3 hours of symptom onset. Further, reperfusion strategies were not yet approved and hence not used. Many reasons are postulated for the failure to reproduce pre-clinical results in the human setting. However, the essential conclusion is that intravenous selfotel given within 6 hours of stroke onset is probably neurotoxic in the setting of acute ischemia in humans.

Aptiganel hydrochloride similarly showed promise in pre-clinical studies and entered a large Phase IIB study [51]. After 628 patients were enrolled, the Data Safety and Monitoring Board combined with the sponsor stopped the trial. The trial assessed two dose tiers of aptiganel compared to control. A trend to higher mortality in the treatment arms was seen across the three groups ($p=0.03$). There was no evidence of benefit among surviving patients.

As in the selfotel trials, more than 90% of patients were enrolled in the 3-6h time window. Heterogeneity of stroke type was assured by the unwise decision to allow enrolment prior to CT scan so that 9% of the cohort had an intracerebral hemorrhage. Again, the major conclusion was that aptiganel hydrochloride is probably harmful for human acute stroke patients. These two negative trials may be linked in part to interference by NMDA receptor antagonists with mechanisms of regeneration and repair.

Gavestinel antagonizes the glycine site on the NMDA receptor. It was shown to be safe in dose escalation studies in humans [52], and drug dosing was similar to that which showed neuroprotection in the rat model. Two large phase III trials were planned and completed and both were neutral. Both trials were designed to include a heterogenous group of stroke patients including intracerebral hemorrhage since imaging was not mandated prior to enrolment.

The GAIN International study randomized 1804 patients to treatment or placebo within 6 hours of stroke onset with absolutely neutral results on the primary outcome defined by the Barthel Index score at 90 days. Subset analysis failed to identify any treatment effect by group. The authors noted that the stricter criteria for the identification of drugs that would be neuroprotective in humans were developed [53] during the phase III trial and that gavestinel would not have m*et al.*l of the proposed criteria.

The GAIN Americas study met a similar fate among 1646 randomized patients [54]. One subgroup of patients younger than age 75 with milder strokes did marginally better with treatment. Although the two GAIN studies were applauded for innovative design features, the combined conclusion is that gavestinel is not neuroprotective in humans when given approximately 5 hours after stroke onset.

The final compound which is undergoing ongoing studies is magnesium sulfate. Magnesium is a non-competitive inhibitor of the NMDA receptor, blocking the ion channel. Several large studies have examined its efficacy in reducing mortality in the setting acute myocardial infarction. It has shown to be effective for managing neurological complications of eclampsia and is a well-known drug with an excellent safety profile. It shows moderate neuroprotective properties after focal ischemia in rats [55].

The IMAGES study ($MgSO_4$ in acute stroke study) is ongoing and has a MRI sub-study component

[56]. A new study examining the utility of giving $MgSO_4$ in the ambulance prior to neurological assessment and prior to CT scanning is just about to get underway in the city of Los Angeles. The entire city has been mobilized to be involved in this trial. Further data should be available soon on this simple, potentially widely applicable and possibly effective therapy.

CURRENT PATH OF RESEARCH

Much of the current research has moved away from directly blocking the NMDA receptor and instead focuses on interactions between the receptor and intracellular signaling mechanisms. The NMDA receptor subunits have been shown to interact with the postsynaptic density protein 95 (PSD-95) [57]. In fact, transient global ischemia induces a change in the normal interaction of the PSD-95 receptor with the NMDA receptor subunits [58]. Tymianski *et al.* [59] showed that disrupting the PSD-95 protein blocked nitric oxide production induced by Ca2+ without affecting normal NMDA receptor function, suggesting that the PSD-95-NMDA receptor interaction is specific to excitotoxic signaling. Following transient focal ischemia, perturbation of the NMDA receptor-PSD-95 interaction seems to decrease infarct size, improve neurological score and attenuate the fraction of dead cells [60].

Current animal studies use homogenous strains of animals with full physiological controls. These studies are also finished at early time points and do not accurately depict the long term outcome of clinical trials and the heterogeneity of the human population. Future animal experiments require long-term survival and a more detailed evaluation of functional recovery, including behavioural testing over time. Any new agent must be proven to be cytoprotective with the global model of cerebral ischemia, and also organoprotective using the focal model. Organoprotection must be defined by behavioural outcomes similar to those used in humans. Further assessment of the role of these agents on white matter is required, something that can be achieved by using multiple animal models beyond the rat. Finally, with the recent observation stem cells exist and cell regeneration is part of brain recovery from any insult, any new agent must be shown not to interfere with mechanisms of repair and regeneration.

Standards have been published discussing the necessary steps for pre-clinical drug assessment prior to moving ahead with large human trials [53]. These include many of the suggestions above. A recent review suggests that many investigators have not heeded these suggestions. Simple control of physiological parameters such as temperature and post-ischemia temperature have been inadequate. Failure to use both male and female animals, the failure to examine behavioural outcomes, and the failure to examine outcomes beyond the first few days after stroke are key inadequacies of recent studies [61]. Finally, because thrombolysis has become a standard of care for stroke therapy, pre-clinical studies must now look at the combination of thrombolysis and neuroprotection.

Clinical trials should evaluate the agent in approximately the same conditions that it was proven effective in the animal model. For example, agents proven effective in MCA occlusion in animals need to be tested in patients suffering from a similar ischemia. The heterogeneity of clinical stroke in humans may be a strong factor in the multiple trial failures of the recent past. Timing must be considered. Relaxation of time windows to improve recruitment or influence the future potential market are powerful inducements but have lead to failure.

In summary, it remains quite possible that NMDA receptor antagonism is neuroprotective in humans early in the ischemic process. Quite clearly, at later time windows, it is toxic leading to worse outcomes. Future development must heed the lessons of past failures. In the clinical world, it takes at least one hour for a patient to arrive at the Emergency room after stroke onset. Many patients arrive late, many hours after stroke onset. The application of such NMDA receptor antagonism as a neuroprotective treatment for stroke will only work in pre-clinical administration by paramedics in the field. Such an agent must be incredibly safe because it will be given to stroke mimics, patients with intracerebral hemorrhage as well as to patients with ischemic stroke. Magnesium is currently the only agent that looks promising in this regard and the large trial being launched in Los Angeles County, California should help us answer this question definitively.

ACKNOWLEDGEMENTS

MDH was funded by the CIHR and the Heart & Stroke Foundation of Alberta/NWT/Nunavut.

AMB was funded by the Heart & Stroke Foundation of Alberta/NWT/Nunavut.

REFERENCES

[1] Behe, P., Stern, P., Wyllie, D.J.A., Nassar, M., Schoepfer, R. and Colquhoun, D. (1995). *Proc. Roy. Soc. Lond. Ser. B,* **262**, 205-213.

[2] Small, D.L., Morley, P., and Buchan, A.M. (1999). In: Miller LP (ed). Stroke Therapy: Basic, Preclinical and Clinical Directions. Wiley-Liss USA 1999.

[3] Stern, P., Behe, P., Schoepfer, R., and Colquhoun, D. (1992) *Proc. Roy. Soc. (Lond).,* **250**, 271-277.

[4] Johnson, J.W. and Ascher, P. (1990) *Biophys.,* **57**, 1085-1090.

[5] Chen, Z.L., Indyk, J.A., Bugge, T.H., Kombrinck, K.W., Degen, J.L., and Strickland, S. (1999) *J. Neurosci.,* **19**, 9813-9820.

[6] Benveniste, H., Drejer, J., Schousboe, A., and Diemer, N.H. (1984) *J. Neurochem.*, **43**, 1369-1374.

[7] Novelli, A., Reilly, J.A., Lysko, P.G., and Henneberry, R.C. (1988) *Brain Res.,* **451**, 205-212.

[8] Benveniste, H., Jorgensen, M.B., Diemer, N.H. and Hansen, A.J. (1988) *Acta Neurol. Scand.*, **78**, 529-536.

[9] Simon, R.P., Swan, J.H., Griffiths, T., and Meldrum, B.S. (1984) *Science.,* **226**, 850-852.

[10] Silver, I.A. and Erecinska, M. (1990) *J. Gen. Physiol.,* **95**, 837-866.
[11] Silver, I.A. and Erecinska, M. (1992) *J. Cereb. Blood Flow. Metab.*, **12**, 759-772.
[12] Tymianski, M. and Tator, C.H. (1996) *Neurosurgery,* **38**, 1176-1195.
[13] Bennett, M.R. and Huxlin, K.R. (1996) *Gen. Pharmacol.*, **27**, 407-419.
[14] Morley, P., Hogan, M.J., and Hakim, A.M. (1994) *Brain Pathol.*, **4**, 37-47.
[15] Schauwecker, P.E. and Steward, O. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 4103-4108.
[16] Nicole, O., Docagne, F., Ali, C., Margaill, I, Carmeliet, P., MacKenzie, E.T., Vivien, D., and Buisson, A. (2001) *Nat. Med.,* **7**, 59-64.
[17] Liberatore, G.T., Samson, A., Bladin, C., Schleuning, W.D. and Medcalf, R.L. (2003) *Stroke,* **34**, 537-543.
[18] Buchan, A.M. (2001) *Update Intensive Care Emerg. Med.*, **37**, 236-251.
[19] Lee, J.M., Zipfel, G.J. and Choi, D.W. (1999) *Nature,* **399**(6738 Suppl), A7-A14.
[20] Pulsinelli, W.A., Brierley, J.B. and Plum, F. (1982) *Ann. Neurol.,* **11**, 491-498.
[21] Pulsinelli, W.A. and Buchan, A.M. (1988) *Stroke,* **19**, 913-914.
[22] Buchan, A.M. and Pulsinelli, W.A. (1990) *J. Neurosci.,* **10**, 311-316.
[23] Colbourne, F., Li, H., Buchan, A.M. and Clemens, J.A. (1999) *Stroke,* **30**, 662-668.
[24] Colbourne, F., Li, H. and Buchan, A.M. (1991) *J. Cereb Blood Flow Metab.,* **19**, 742-749.
[25] Pulsinelli, W.A. and Duffy, T.E. (1983) *J. Neurochem.,* **40**, 1500-1503.
[26] Buchan, A.M., Xue, D. and Slivka, A. (1992) *Stroke,* **23**, 273-279.
[27] Hata, R., Maeda, K., Hermann, D., Mies, G. and Hossmann, K-A. (2000) *J. Cereb Blood Flow Metab.,* **20**, 937-946.
[28] Huang, Z.G., Xue, D., Preston, E., Karbalai, H., and Buchan, A.M. (1999) *Can J. Neurol. Sci.,* **26**, 298-304.
[29] Barber, P.A., Auer, R.N., Buchan, A.M. and Sutherland, G.R. (2001) *Can J. Physiol. Pharmacol.,* **79**, 283-296.
[30] Colbourne, F., Corbett, D., Zhao, Z., Yang, J. and Buchan, A.M. (2000) *J. Cereb Blood Flow Metab.,* **20**, 1702-1708.
[31] Kristian, T., Katsura, K. and Siesjo, B.K. (1992) *Acta Physiol. Scand.*, **146**, 531-532.
[32] Buchan, A., Li, H. and Pulsinelli, W.A. (1991) J *Neurosci.,* **11**, 1049-1056.
[33] Xue, D., Huang, Z.G., Smith, K.E. and Buchan, A.M. (1992) *Brain Res.,* **587**,66-72.
[34] Valtysson, J., Hillered, L., Andine, P., Hagberg, H. and Persson, L.(1994) *Acta Neurochir. (Wien)*, **129**, 58-63.
[35] DeBow, S., Clark, D.L., Maclellan, C.L. and Colbourne, F. (2003) *Can J. Neurol. Sci.*, **30**, 368-374.
[36] Snider, B.J., Tee, L.Y., Canzoniero, L.M., Babcock, D.J. and Choi, D.W. (2002) *Eur. J. Neurosci.*, **15**, 419-428.
[37] Ikonomidou, C., Bosch, F., Miksa, M., Bittigau, P., Vockler, J., Dikranian, K., Tenkova, T.I., Stefovska, V., Turski, L. and Olney, J.W. (1999) *Science,* **283**, 70-74.
[38] Ikonomidou, C., Stefovska, V. and Turski, L. (2000) *Proc. Natl. Acad. Sci. USA,* **97**, 12885-12890.
[39] Cameron, H.A., McEwen, B.S. and Gould, E. (1995) *J. Neurosci.*, **15**, 4687-4692.
[40] Bernabeu, R. and Sharp, F.R. (2000) *J. Cereb Blood Flow Metab.,* **20**, 1669-1680.
[41] Cameron, H.A., Tanapat, P. and Gould, E. (1998) *Neuroscience,* **82**, 349-354.
[42] Arvidsson, A., Kokaia, Z. and Lindvall, O. (2001) *Eur. J. Neurosci.,* **14**, 10-18.
[43] Buchan, A.M. (1990) *Cerebrovasc Brain Metab. Rev.,* **2**,1-26.
[44] Gladstone, D.J., Black, S.E. and Hakim, A.M. (2002) *Stroke,* **33**, 2123-2136.
[45] Muir, K.W. and Grosset, D.G. (1999) *Stroke,* **30**,180-182.
[46] Dewar, D., Yam, P. and McCulloch, J. (1999) *Eur. J. Pharmacol.*, **375**, 41-50.
[47] Olney, J.W., Labruyere, J. and Price, M.T. (1989) *Science,* **244**, 1360-1362.
[48] Kidwell, C.S., Liebeskind, D.S., Starkman, S. and Saver, J.L. (2001) *Stroke*, **32**, 1349-1359.
[49] Davis, S.M., Albers, G.W., Diener, H.C., Lees, K.R. and Norris, J. (1997) *Lancet,* **349**, 32 .
[50] Davis, S.M., Lees, K.R., Albers, G.W., Diener, H.C., Markabi, S., Karlsson, G. and Norris, J. (2000) *Stroke*, **31**, 347-354.
[51] Albers, G.W., Goldstein, L.B., Hall, D., Lesko, L.M. and Aptiganel Acute Stroke Investigators. (2001) *JAMA,* **286**, 2673-2682.
[52] GAIN Investigators. (2000) *Stroke,* **31**, 358-365.
[53] STAIR Writing committee. (1999) *Stroke,* **30**, 2752-2758.
[54] Sacco, R.L., DeRosa, J.T., Haley, E.C. Jr, Levin, B., Ordronneau, P., Phillips, S.J., Rundek, T., Snipes, R.G., Thompson, J.L. and Glycine Antagonist in Neuroprotection Americas Investigators. (2001) *JAMA*, **285**, 1719-1728.
[55] Yang, Y., Li, Q., Ahmad, F. and Shuaib, A. (2000) *Neurosci. Lett.*, **285**, 119-122.
[56] Bradford, A. and Lees, K. (2000) *Curr. Control. Trials Cardiovasc Med.,* **1**, 184-190.
[57] Kornau, H.C., Schenker, L.T., Kennedy, M.B. and Seeburg, P.H. (1995) *Science,* **269**, 17.
[58] Takagi, N., Logan, R., Teves, L., Wallace, M.C. and Gurd, J.W. (2000) *J. Neurochem.*, **74**, 169-178.
[59] Sattler, R., Xiong, Z., Lu, W.Y., Hafner, M., MacDonald, J.F. and Tymianski, M. (1999) *Science*, **284**, 1845-1848.
[60] Aarts, M., Liu, Y., Liu, L., Besshoh, S., Arundine, M., Gurd, J.W., Wang, Y.T., Salter, M.W. and Tymianski, M. (2002) *Science,* **298**, 846-850.
[61] DeBow, S.B., Clark, D.L., MacLellan, C.L. and Colbourne, F. (2003) *Can. J. Neurol. Sci.*, **30**, 368-374.