Histol Histopathol (1997) 12: 185-194

Histology and Histopathology

Invited Review

Amplification of inhibitory mechanisms in cerebral ischemia: an alternative approach to neuronal protection

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Summary. The central nervous system consumes 20% of the cardiac output for normal function. The neurons are very sensitive to the effects of ischemia. Cessation of cerebral blood flow results in severe damage to neurons and other brain structures. This is secondary to a combination of energy loss, excessive excitation promoting intracellular calcium (Ca^{2+}) buildup, relative lack of inhibitory responses, generation of oxygen free radicals (especially during the reperfusion period) and several other destructive cascades. Medications that antagonize the effects of glutamate at post-synaptic receptors are either ineffective or have serious side-effects. Ca^{2+} entry blockers have shown disappointing results in clinical trials in patients with acute cerebral infarction. Data with protective effects of oxygen free radical scavengers in the post-ischemic period have shown conflicting results.

There is recent interest with the use of agents that increase cerebral inhibitory responses after an ischemic insult. Such agents are effective when used before, during or up to 4 hours after the ischemic insult. Many such medications have few side-effects and are in clinical use for other indications. This review will summarize inhibitory mechanisms that may be important in cerebral ischemia, and provide experimental evidence for their potential efficacy.

Key words: Adenosine, Calcium, GABA, Glutamate, Taurine

Introduction

Cerebrovascular disease continues to take a heavy toll on the human society. Despite a significant decline over the last 40 years (Kuller, 1989), it remains the third most common cause of death and the leading cause of chronic disability in North America (Kuller, 1989). In Canada, it accounts for 8% of hospital bed utilization and the direct cost of taking care of patients (hospital and nursing homes) is well over 4 billion dollars annually (Heart and Stroke Foundation of Canada, 1993). Cerebral ischemic damage also occurs in conditions where blood flow is transiently interrupted from systemic conditions including cardiac arrest, arrhythmia, severe hypotension, anesthetic accidents, carbon monoxide poisoning and drowning. In many such conditions there is «global» decrease in cerebral blood flow and the neuronal damage is selective an predominantly in the vulnerable regions of the brain (Rothman and Olney, 1986; Garcia and Anderson, 1989; White et al., 1993).

The mechanisms whereby brain cells, especially neurons, die during ischemia are not fully understood but are receiving increasing attention in recent years. A variety of parallel cascades of hemodynamic, biochemical and electrophysiological events combine to produce the neuronal damage (White et al., 1993; Lipton and Rosenberg, 1994). There is a rapid fall in the brain energy reserve with cessation of blood flow. This leads to disturbed ion homeostasis and tissue acidosis. An increased efflux of potassium (K^+) and influx of sodium (Na^+) and Ca^{2+} is characterized by cellular depolarization and release of massive amounts of excitatory aminoacids (EAAs), especially glutamate. There may also be deficient mechanisms for the reuptake of the EAAs (Lipton and Rosenberg, 1994). The increased glutamate activates at least 4 types of post-synaptic receptors. The ensuing additional increase in intracellular Ca2+ activates a variety of intracellular enzymes, including proteases, lipases and endonucleases that play a key role in cell death. Other events that contribute to the severity of the injury include lipid peroxidation, free radical generation (Kitagawa et al., 1990; Turelove et al., 1994a,b) and protein synthesis dysfunction. A variety of systemic factors, including the levels of serum glucose (Voll and Auer, 1988), endocrinological variations (Shuaib et al., 1994a) and ambient temperature changes (Busto et al., 1989; Shuaib et al., 1992d, 1995b) modulate the extent of neuronal damage. These mechanisms have been a subject of extensive reviews (Rothman and Olney, 1986; Garcia

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and Anderson, 1989; White et al., 1993; Lipton and Rosenberg, 1994).

Neuronal damages is not an «all or none phenomenon». The extent of the final damage depends on the severity and duration of the insult. The effects of the ischemic injury that begin in the «no-flow period», continue into the reperfusion period. This maturation of damage is seen within hours in most regions of the brain and may continue for many days to weeks. In monkeys with acute middle cerebral artery occlusion, there is an increase in the extent of damage for at least the initial 24 hours within the arterial distribution (Touzani et al., 1995). In the CA1 region of the hippocampus, however, morphological evidence of the damage may not be evident for up to 48 hours after the ischemic insult (Andiné et al., 1991; Kirino et al., 1992; Nakamura et al., 1993). In the substantia nigra reticulata, damage may not be severe for 7 days after the insult (Shuaib et al., 1992c). This delayed neuronal damage offers hope for intervention. The severity of this damage can be decreased with the use of glutamate antagonists (Lyden and Hedges, 1992; Shuaib et al., 1993a) endocrinological changes (Shuaib et al., 1994a,b) hypothermia (Busto et al., 1989; Shuaib et al., 1992a,e), hypoglycemia (Voll and Auer, 1988), GABA agonists (Lyden and Hedges, 1992; Shuaib et al., 1992b, 1993b, 1995a) and a variety of other manipulations. The protection seen with such agents depends on the type and duration of insult, the timings and route of infusion of the medications and several other physiological variables. Several reviews describe cerebral ischemia and pharmacological methods of neuronal protection (Garcia and Anderson, 1989; Choi and Rothman, 1990; Siesjö, 1992; Siesjo et al., 1995).

Recent studies have shown that neuroprotection may be evident if the medication is given several hours after the ischemic insult. The «window of protection» may be 4 hours in rodents, 6-8 hours in monkeys and possibly longer in humans (Heiss et al., 1992; Baron et al., 1995; Obrenovitch, 1996). The realization that medical therapy may prevent or limit the extent of neuronal injury when the medications are used hours after the ischemic insult have kindled excitement in clinical stroke experts (Baron et al., 1995). There are over a dozen clinical trials evaluating the efficacy of thrombolytic and neuroprotective agents in acute stroke (Wahlgren, 1995). Recent evidence suggests that thrombolytic therapy may improve neuronal outcome if used within the initial 3 hours of the insult (the NINDS RT-PA stroke study group, 1995). Glutamate antagonists, NO synthase inhibitors, free radical scavengers, GABA agonists and a host of other agents are currently being tested in acute ischemic stroke, subarachnoid hemorrhage and head injury (Wahlgren, 1995). Unfortunately to date none have been shown to have definite neuroprotection in clinical settings. We hope to get useful answers in the next 3-5 years.

Neuronal effects to ischemia has been studied in several anatomical regions of the brain, including the hippocampus, striatum, cerebral cortex, thalamus, substantia nigra reticulata and the cerebellum. The CA1 region of the hippocampus is very sensitive to the effects of ischemia and the damage develops days after the insult. It is the most studied region of the brain for understanding the mechanisms of ischemia. The hippocampus has at least four types of unique neuronal populations. Each neuronal type (granular cells in the dentate gyrus, CA1 and CA4 pyramidal neurons and interneurons) have specific synaptic connections that can be studied in hippocampal slice preparations. Stimulation of the granular cells results in glutamate-mediated excitation of the CA3 neurons which in turn excite the CA1 neurons. For the purpose of this review we will focus on the hippocampus which provides a very well studied, anatomically discrete entity, with distinctive neuronal cell types that can be analyzed individually (Thompson et al., 1993).

Table 1. Potential inhibitory mechanisms in cerebral ischemia.

PRESYNAPTIC	POSTSYNAPTIC
GABA (B receptor)	GABA (A receptor)
Calcium receptors blockers	Taurine
Adenosine	Serotonin
Sodium channel blockers	Norepinephrine
Insulin	Glutamate (metabotropic receptor)
Pre-conditioning	Adrenalectomy
Adrenalectomy	Others (NO??)
De-efferentation	

The glutamate agonists acting at pre-synaptic sites are metabotropic receptors and these increase K^+ conductance and may, in some instances, partially inhibit voltage-gated Ca^{2+} channels. It is now well established that the post-synaptic effects of glutamate agonists that affect K^+ or Ca^{2+} often occur through activation of the guanine nucleotide-binding proteins (G-proteins). However, demonstrating that pre-synaptic responses may be mediated through similar pathways has been difficult because of the inability to access the pre-synaptic metabotropic receptors. Most of the pre-synaptic metabotropic receptors cloned to date are from the G-protein-linked superfamily and their stimulation can be blocked by pertussis toxin which inactivates specific G-protein subtypes. Phosphorylation of G-proteins by activation of protein kinase C results in dysfunction of the pre- and post-synaptic actions of the agonists suggesting yet another possible role of G-proteins as a second messenger system in the pre-synaptic region (Thompson et al., 1993).

The main objective of this review is a focus on the inhibitory mechanisms that play a major role in neuronal protections from the effects of ischemia (Table 1). Several drugs that have inhibitory properties are safe and are in clinical use for other indications (for example in treatment of epilepsy). These therapies may have a useful role in neuroprotection. Chlormethiazole is currently undergoing clinical trials as a neuroprotective agent in acute ischemic stroke (Wahlgren, 1995).

Pre-synaptic mechanisms

The synaptic release of neurotransmitter is rigidly regulated in health. Excessive pre-synaptic release of glutamate is an important initiating event in neuronal injury during and after ischemia. The release of glutamate is initiated by a loss of high energy compounds and activation of Ca^{2+}, Na^{+} and K^{+} channels. During brief periods of ischemia, the concentration of glutamate can rise several fold (Benveniste et al., 1984). This increase has been observed in animals and in a human model of «stimulated ischemia» (Kanthan et al., 1995). With recirculation, there is an immediate fall in the extracellular concentration of glutamate. An attenuation of the glutamate release has also been shown to result in a decrease in the extent of morphological damage after an ischemic insult (Busto et al., 1989). This has best been studied with hypothermia (Busto et al., 1989), but has also recently been shown with hypo-thyroidism (Shuaib et al., 1994a). Stimulation of adenosine receptors and antagonism of N-type Ca^{2+} or pre-synaptic Na^{+} channels may also have similar effects. Other methods available to manipulate the pre-synaptic system include stimulation of the glutamatergic metabotropic receptors, acetylcholine system, ardrenergic and sertotenergic systems (especially in the CA1 regions of the hippocampus), preconditioning and «de-efferentiation» (Thompson et al., 1993).

Pre-synaptic Ca^{2+} channels

Calcium plays an important role in several intracellular processes. The release of most neuro-transmitters is dependent on entry of Ca^{2+} through specific channels. In the pre-synaptic regions, the N-type Ca^{2+} channel is heavily concentrated and is intimately involved in the regulation of glutamate release during ischemia. The abnormal glutamate release during ischemia and possibly in the next 24-48 hr can be manipulated by blocking the pre-synaptic N-type Ca^{2+} channels. This has been shown to have potent neuro-protective effects (Buchan et al., 1994).

A number of novel peptides have been discovered in the venom of the conus group of snails. These peptides have been shown to have extremely potent effects on the central nervous system of mammals (Valentino et al., 1993). The w-oncopeptides appear to have very potent effects on N-type Ca^{2+} channels. Synthetic compounds have also been developed, similar in characteristics to the parent compounds. SNX-111 is a synthetic oncopeptide that has been used in cerebral ischemia in focal and global models of ischemia. Its mechanism of action is not fully understood but was initially thought to be due to a decreased release of glutamate during ischemia. SNX-230 is a more potent inhibitor of glutamate release but does not appear to have neuro-protective effects. The protection seen at 6 and 24 hours would suggest that other mechanisms may be operating to explain neuroprotection with SNX-111. The delayed (24-48 hours post-ischemia) increase in Ca^{2+} in the CA1 region of the hippocampus has been linked to the development of delayed neuronal death. It is possible that SNX-111 may inhibit this delayed increase in Ca^{2+} in the hippocampus and therefore prevent neuronal damage (Buchan et al., 1994). The ability to prevent damage when used 24 hours after the insult has major health care implications. If safer agents are developed, it may be possible to treat patients who present with an acute ischemic stroke several hours after the insult.

Na^{+} channels blockers

With better understanding of Na^{+} channel biology, several new classes of drugs have been developed with specific properties localized to individual channel type. Use-dependent blockage of the Na^{+} channel has been shown to inhibit glutamate release. This class of drugs has been widely studied in epilepsy research and is clinically available to treat seizures. The compounds are safe and well tolerated by the patients (Harden, 1994). Because of their ability to prevent glutamate release, some compounds that act on the pre-synaptic Na^{+} receptors have been tested in brain ischemia. Lamotrigine and BM1003C87 have been recently studied in transient forebrain ischemia and has been shown to have neuroprotective effects when used before or after the insult (Meldrum et al., 1992; Shuaib et al., 1995c; Wiard et al., 1995). The medication was administered either orally or intraperitoneally. Medication was tolerated well and animals would show no side-effects. There is recent evidence that with many neuroprotective therapies, the protection evident at 7 days may not be evident if the animals are examined at delayed intervals after the insult. This suggests that the therapy may be «postponing» the damage and not offering «true protection». To test the neuroprotective effects of lamotrigine, we evaluated damage at 7 and 28 days after the insult. Protection was evident at both time points in the hippocampal and striatum. In addition to protecting the CA1 neurons, the use of such medication has been shown to enhance behavioral recovery. Microdialysis studies have shown that protection is a result of a decrease in the release of glutamate when the medication is used prior to the insult (Meldrum et al., 1992; Shuaib et al., 1995c). The mechanisms of protection when the medication is used after the insult are not fully understood but may be related to its ability to inhibit the post-ischemic fluctuations in glutamate levels.

Hypothermia

It has been known for a long time that profound hypothermia can protect the organism from the effects of ischemia. Hypothermia is routinely used in many surgical procedures to prevent ischemic damage to the heart, brain and other organs. It has recently become

apparent that neuronal protection does not require severe hypothermia but can be achieved with a mild decrease in the body temperature. This protection is best if the temperature is lowered prior to the insult (Ginsberg et al., 1992). Hypothermia induced after the insult may also be neuroprotective, especially if it is prolonged. Thus if the temperature is lowered 3 hours after the insult and maintained at the lower level for over 24 hours, it may offer protection (Colbourne and Corbett, 1994). The mechanisms for neuronal protection with hypothermia are not fully understood but are likely secondary to a combination of several factors (Ginsberg et al., 1992).

A major problem with early cerebral ischemia experiments was the extent of variability on neuronal damage with a given degree of arterial occlusion. In such early experiments scalp or brain temperature were rarely measured. The rectal temperature was considered sufficient for monitoring. Busto and colleagues demonstrated variability in cerebral and scalp temperatures in anesthetized animals with carotid occlusion (Busto et al., 1987). They subsequently showed that a decrease in temperature of 1-2 °C was associated with a significant neuronal protection. Later work from the same laboratory, using the in vivo microdialysis technique, showed that the release of glutamate during ischemia was dependent on the cerebral temperature. Glutamate release was significantly decreased with a small fall in CNS temperature. Conversely hyperthermia produced an increase in glutamate release during ischemia. The decreased release of glutamate under ischemic conditions is currently considered to be an important mechanism for neuronal protection during hypothermia (Busto et al., 1987). However, the decreased release of glutamate is not the only mechanism of protection since it cannot explain the decrease in neuronal damage when hypothermia is administered up to 3 hours after the insult.

We have evaluated the neuroprotective effects of hypothermia in a model of repetitive ischemia. If the temperature is lowered by 2-3 °C between the insults, there is significant neuronal protection. Protection was also evident if the hypothermia was initiated immediately after the third insult. A delay in the onset of hypothermia of more than 30 minutes would make the hypothermia ineffective (Shuaib et al., 1995b). We were cooling the animal for 3 hours. It is possible that longer duration hypothermia may have better effects.

A number of medications showing neuroprotective effects may produce hypothermia. If the temperature is maintained close to normal, the protection may be lost with such drugs (Buchan and Pulsinelli, 1990). Hypothermia may enhance the neuroprotective effects of glutamate antagonists (Shuaib et al., 1993a). This is only possible if the combination is given prior to the ischemic insult. With post-ischemic therapy, the combination of hypothermia and CGS-19755 (a glutamate receptor antagonist) were ineffective in preventing neuronal injury (McCrea et al., 1994).

Adenosine

There is evidence that adenosine may be neuroprotective during cerebral ischemia, and is thought to act by decreasing glutamate concentrations (Rudolphi et al., 1992). Adenosine is present in most regions of the brain with a concentration of between 50-300 nM in the unanesthetized rat. There are several types of adenosine receptors in the brain, the best characterized are A1 and A2 with A2 being further subdivided into A2a and A2b receptors. The formation of adenosine has been best studied in brain slice and cell culture. The release of adenosine is facilitated by an increase in extracellular glutamate and may result from stimulation of the NMDA and or AMPA receptors (Poli et al., 1991; Rudolphi et al., 1992).

Released adenosine interacts with cell surface receptors that are present on most neurons. There is a very high density of A1 receptors in the dendritic regions of the hippocampus, especially in the pars CA1. This region has a high concentration of NMDA glutamate receptors and is particularly sensitive to the effects of ischemia. A1 receptors are found in the pre- and post-synaptic regions of the neurons. Receptors are also found outside the synaptic region. They are particularly abundant on the dendrites of the hippocampus where they have been extensively studied. Other regions where there is a high density of adenosine receptors include the striatum (particularly the spiny, medium-sized neurons), and other dopamine-rich areas where they modify dopamine transmission.

Adenosine receptors density can vary with the availability of agonists. The long term presence of adenosine agonists can result in a down-regulation of receptors in most regions of the brain. Similarly, an up-regulation can be seen in the presence of antagonists. There is a decrease in adenosine receptor density in ischemia. This is most severe in the hippocampus and has been best studied in the forebrain ischemia model in gerbils.

Several independent mechanisms for the possible protection with adenosine have been described. Upon administration of agents that increase the extracellular levels of adenosine a decrease in the release of glutamate is seen (O'Regan et al., 1992; Simpson et al., 1992). This effect is secondary to stimulation of A1 receptors presumably on the pre-synaptic terminals. These receptors are linked via G-proteins to both K^+ and Ca^{2+} channels. A1 stimulation results in hyperpolarization of the membrane. Adenosine plays an important part in intracellular Ca^{2+} ion homeostasis. The opening of voltage-gated Ca^{2+} channels is inhibited by adenosine. It also inhibits the removal of magnesium from the NMD receptor thus preventing Ca^{2+} entry through this route during ischemia. The overall effect of adenosine during ischemia is therefore a limitation of excessive depolarizations by pre-synaptic, post-synaptic and non-synaptic mechanisms. NMR spectroscopy studies have shown that there is also possible metabolic protection by

a significantly reduced fall in ATP and creatinine phosphate in animals treated with an agonist of adenosine receptors (R-PIA). Also seen is a decrease in inorganic phosphates and acidosis which may have protective effects.

As a result of a very short half-life (3-6 seconds) and a poor penetrance of the blood-brain barrier, the effects of adenosine have not been studied in ischemia. Most of the work is ischemia with agonists or antagonists of adenosine have been conducted in small animals. Protective effects with agonists have been seen in a variety of focal and global models in gerbils, mice and rats. Additional indirect data in cell cultures of neurons and hippocampal slices have also shown confirmatory evidence for protection. The protective effects with R-phenylisopropyl adenosine (R-PIA) may be secondary to a decrease in the release of glutamate in the ischemia period or in the post-ischemic reperfusion period. Other potential protective mechanisms of R-PIA may include metabolic perturbations resulting in a decrease in the declining levels of ATP and creatinine phosphate and a decrease in the degree of acidosis seen with ischemia. Additionally, since most adenosine agonists can have hypothermic effects, hypothermia may be a possible mechanism seen in animals under conditions where temperature is not properly controlled. However, since R-PIA treatment of cells in culture results in protection it is unlikely that hypothermia is necessary for protection.

Most adenosine antagonists have been shown to increase the extent of neuronal damage with ischemia. These effects have been documented in rats, gerbils and hippocampal slice. The increase in damage may be a result of an increased release of glutamate or alterations in metabolic perturbations. Non-specific blockers, such as theophylline or caffeine, have shown conflicting results. Specific A1 receptor antagonists (dipropyl-cyclopentyl-xanthine [DPCPX]) increase the extent of brain damage (Rudolphi et al., 1992).

The extent of damage (or protection) during ischemia depends on acute or chronic administration of the adenosine antagonist. Theophylline produces an increase in neuronal damage during ischemia in rats and gerbils in both global and focal models of ischemia. Chronic caffeine use, however, results in significantly less damage in gerbils with 5 minute ischemia and rats with 10 minute ischemia, as compared to controls (Sutherland et al., 1991). This may be a consequence to an up-regulation of adenosine receptors.

Other possible pre-synaptic mechanisms

Neuronal protection during ischemia may be seen with potentiation of serotonergic and noradrenergic mechanisms. Similarly, pre-conditioning and de-efferention has also been shown to limit glutamate release and thus attenuate hippocampal damage. We have recently shown that animals made hypothyroid prior to ischemia have less neuronal damage (Shuaib et al., 1994a). This may be a result of a decrease in glutamate release (Shuaib et al., 1994a). A decreased glutamate release during ischemia may also be a possible mechanism of action with drugs that potentiate the effects of acetylcholine (Phillis et al., 1993) or GABA(B) receptors (Lal et al., 1995).

Post-synaptic mechanisms

While the pre-synaptic sites are attractive regions for manipulation of inhibitory responses, there are several post-synaptic mechanisms that either directly inhibit neurons or have modulatory effects that are neuro-protective during cerebral ischemia. Potentiation of such mechanisms has powerful neuroprotective effects.

GABA and cerebral ischemia

Gamma-aminobutyric acid (GABA) the major inhibitory neurotransmitter is present in virtually all regions of the brain and is synaptically active at approximately 40 percent of brain receptors (Bloom and Iverson, 1971). There are two major types of GABA receptors, designated GABA(A) and GABA(B) which are involved in a variety of physiological responses. Although activation of the two types of GABA receptors results in inhibition, their distribution, pharmacological responses and interactions with ions and nucleotides are very different (Francis and Pulsinelli, 1982; Onodera et al., 1987; Phillis and Walter, 1989). The GABA(A) receptors are in high concentration in the superficial lamina of the cerebral cortex, thalamic nuclei, striatum and the substantia nigra reticulata. The GABA(A) receptor is a complex protein with multiple recognition sites. GABA(B) receptors are concentrated in the superficial layers of the cerebral cortex, superior colliculus and the molecular layer of the cerebellum. The two receptors have separate mechanisms of action for the inhibitory response. The GABA(A) receptor conducts Cl-whereas the GABA(B) receptor is linked to K^+ and Ca^{2+} channels via G-proteins. Location of the receptor affects its sensitivity to ischemia. The GABA system in the basal ganglion is very sensitive to ischemia (Francis and Pulsinelli, 1982). In contrast, the GABAergic neurons in the hippocampus are more resistant to the effects of ischemia (Onodera et al., 1987). GABAergic medications have potent neuro-protective effects when used for up to 4 hours after the insult (Sternau et al., 1989; Shuaib et al., 1992b, 1993b, 1995a). We have tested several different classes of GABA potentiating medications in transient cerebral ischemia. Most medications have potent neuroprotective effects when used before or after the insult. Muscimol is protective in focal and global models of ischemia (Lyden and Hedges, 1992; Shuaib et al., 1993b). Combination with glutamate antagonists, enhances the neuro-protection. Neuroprotection is also seen with gamma vinyl GABA (Shuaib et al., 1992b), gabapentin (unpublished observations), chlormethiazole (Shuaib et al., 1995a) and baclofen (Lal et al., 1995). With most

GABAergic medications, significantly less damage is seen in the hippocampus, striatum, thalamus and the substantia nigra reticulata in the treated animals compared to the controls.

Glutamate metabotropic receptors

Pharmacological and molecular studies have demonstrated that there are two distinct classes of glutamate receptors (Schoepp and Conn, 1995). The ionotropic glutamate receptors are «ligand-gated internal ion channels» that have been shown to stimulate the entry of Na^+ and Ca^{2+} and have been studied extensively in cerebral ischemia. The metabotropic glutamate receptors are coupled to a variety of intracellular events through G-proteins. At present, 7 subtypes of metabotropic receptors (mGluR1-7) have been characterized and cloned. The receptors have 7 membrane-spanning segments (similar to most G-protein-linked receptors) but are much larger and have large extracellular and intracellular hydrophilic domains. The effects of stimulation of the metabotropic receptors are very complex. Stimulation of mGluR1 and mGluR5 receptors results in activation of phosphoinositide hydrolysis, formation of second messengers (inositol [IP3] and diacylglycerol [DAG]), activation of protein kinase C (PKC) and mobilization of intracellular Ca^{2+}. Stimulation of mGluR 2,3 and 4 have been shown to have stimulatory and inhibitory effects on formation of adenosine 3',5'-monophosphate (cAMP) levels. These effects are also dependent on the brain region where the mGluR receptor is being activated. Activation of mGluR receptors has also been shown to regulate guanosine 3',5'-monophosphate (cGMP) synthesis with effects on the signal transduction associated with nitric oxide synthetase and carbon monoxide. Other physiologic effects include release of arachidonic acid, modulation of voltage-gated and ion-gated channels (K^+ and Ca^{2+}) (Schoeppa and Conn, 1995).

Although the molecular biology of mGluR has rapidly advanced, a lack of pharmacological probes for specific subtypes has hindered examination of the effects of stimulation and inhibition on the effector system. Trans-1-aminocyclopentane-1,3-dicarboxylic acid (t-ACPD) is an agonist of mGluR with no specificity for any subtype. It has been used extensively to study mGluR. There are no experimental published data on the effects of t-ACPD in transient global ischemia. However, one report suggests potential neuroprotective effects in focal ischemia (Chiamulera et al., 1992). Other in vitro studies have shown protection under simulated ischemia in a variety of neuronal systems.

In rats, t-ACPD causes a depression of motor neurons by pre-synaptic-mediated mechanisms. Some of the pre-synaptic mechanisms involve Ca^{2+} channels and c-AMP. In addition, mild post-synaptic stimulatory effects are seen with t-ACPD (Chiamulera et al., 1992).

Recently, antagonists for mGluR have also been developed. [RS]-alpha-methyl-4-carboxyphenylglycine (MCPG) is the most specific antagonist thus far characterized. It has also been shown to specifically block the agonist effects of t-ACPD in vitro. Studies have shown to be particularly effective against the mGluR1 receptors. The drug has not been well studied in vivo and its effects during ischemia have not been evaluated (Schoepp and Conn, 1995).

Activation of mGluR receptors has been associated with neuroprotection in several regions. The injection of NMDA into the orbit results in severe damage. This can be decreased significantly by the injection of 1S-3R-ACPD into the orbit. In the whole animal, injection of 1SR-3RS-ACPD into the nucleus tractus solatrius produces vascular and cardiac responses similar to stimulation of the baroreceptors resulting in hypotension and bradycardia (Schoepp and Conn, 1995). In a focal middle cerebral artery occlusion model of ischemia in mice, t-ACPD (20mg/kg i.p.) when injected immediately after the occlusion resulted in significant protection in the striatum and cerebral cortex (Chiamulera et al., 1992). However, there were serious limitations with this study. Physiological changes were not monitored and there was no information on changes in cerebral temperature with the medication. The duration of ischemia was not mentioned nor were the methods for buffering stated (t-ACPD has a pH of 2.7). The results are, however, still very interesting and require further study in focal or global models of ischemia. Future studies should also look at the effects of mGluR agonists and antagonists on the release of glutamate during and after ischemia.

Taurine

Taurine is the most abundant neurotransmitter in the body (Lombardini, 1992). It can be synthesized from cysteine and is also consumed in the diet. Taurine is located in nerve endings in the brain, retina and striated muscles. It can be released by both electrical and chemical stimulation in a Ca^{2+}-dependent manner. Taurine has a depressant action on the post-synaptic cells. It also acts as a membrane-stabilizer linked to the Cl-ion channel. A high-affinity Na^+-dependent uptake system has also been demonstrated in both neurons and glial cells. The most convincing site for taurine as an inhibitory neurotransmitter is the inner plexiform layer of the retina.

During ischemia, there is excessive release of taurine from various regions of the brain (Lombardini, 1992). A similar release has also been demonstrated from the spinal cord in rabbits exposed to total occlusion of the distal aorta. The increase in extracellular taurine is seen almost immediately after the occlusion and persists for the duration of transient ischemia. The levels return to baseline within 20 minutes after flow is restored. In models of focal ischemia (modified Levine model) there was an increase in taurine 7 days after the insult in 7 of 8 animals exposed to hypoxia (8% oxygen) and unilateral carotid occlusion for 2.5 hours. The increase in taurine

may be a compensatory response to the release of glutamate or local edema. The release of taurine with cell swelling or NMDA receptor stimulants has been studied with microdialysis. The effects of ischemia on the tissue levels of EAAs and inhibitory aminoacids have been studied by Matsumoto et al. (1991) in the rat hippocampus in the 4-vessel rat model. K^+-induced release of glutamate and aspartate was reduced by 50% in the hippocampus 5 days after ischemia. A similar decrease in taurine or GABA was not seen, indicating a loss of pyramidal CA1 neurons but not small inhibitory interneurons. We have seen a progressive loss of GABA and taurine in the striatum days after ischemia in the gerbils (unpublished data). This loss may lead to excessive excitation of the substantia nigra reticulata producing delayed neuronal damage in this region. The damage can be prevented by GABA agonists or hypothermia (both protecting striatal small interneurons from the effects of ischemia).

The turtle brain shows resistance to the effects of ischemia and anoxia. The response of the turtle brain to ischemia has been studied by microdialysis. The surge in glutamate evident in the mammalian brains immediately after the insult was not evident. However, there was a massive release of taurine (approximately 24 times control). This increase in taurine (and other inhibitory amino acids) may account for decreased energy consumption observed in the anoxic turtle brain and may allow the animal to survive long periods of total anoxia (Lombardini, 1992).

There are no studies of taurine as a neuroprotective agent published to date. Taurine reduces reperfusion injury in the myocardium. This has been studied in animals and bypass patients. In isolated rat myocardium, taurine protects against the loss of mechanical function that results from the calcium paradox and prevents the loss of sarcolemmal ATPase activities. In isolated guinea pig heart, taurine protects against hypoxia-induced decline in contractile force and cardiac slow-action potentials. In humans, undergoing coronary bypass surgery, decreased lipid pre-oxidation and cell damage was seen in taurine treated patients compared to controls. These actions may be due to taurine's modulatory action on Ca^{2+} entry into hypoxic cells. Taurine has also been shown to have anti-oxidant properties. This could also account for the cardio-protective properties of the amino acid (Milei et al., 1995).

Serotonin

Neuronal reaction to stimulation by serotonin varies and is dependent upon the type of post-synaptic receptor (Bielenberg and Burkhardt, 1990). In the hippocampus there are several different types of serotonin receptors. The 5HT1a are located in the CA1 region and are predominantly inhibitory. Stimulation results in prolonged inhibition of pyramidal neurons. The rhape nuclei in the upper brainstem are the main efferents. Highly selective 5HT1a receptor agonists have been described and their pharmacology has been tested in rodents and larger animals (Bloom and Inverson, 1971; Zivin and Venditto, 1984; Bielenbreg and Burkhardt, 1990). These drugs have been shown to have potent neuroprotective effects with very few side-effects. Medications were effective when used both before or after the ischemic insult (Zivin and Venditto, 1984; Bielenberg and Burkhardt, 1990).

Norepinephrine

Similar to serotonin, the neuropharmacology of norepinephrine in the nervous system is very complex. The locus coeruleus innervates the CA1 region of the hippocampus and stimulation of these fibers results in inhibition of hippocampal pyramidal neurons. There are limited data on the role of norepinephrine system in ischemia (Koide et al., 1986; Bielenberg and Burkhardt, 1990). Ablation of the norepinephrine pathways may result in an increase in neuronal damage in the hippocampus during ischemic insult (Blomqvist et al., 1985). Conversely, drugs that enhance such mechanisms have neuroprotective properties (Matsumoto et al., 1993).

Other post-synaptic mechanisms

Other conditions that may potentiate inhibitory neuronal mechanisms include the use of insulin during ischemia (Shuaib et al., 1995d), adrenalectomy (Ravindran et al., 1994) and stimulation of acetylcholine receptors (Beley et al., 1991; Bertrand et al., 1992; Ishimaru et al., 1994). In vivo microdialysis studies have shown that there is an increase in the GABA levels in animals that are treated with insulin prior to ischemia (Shuaib et al., 1995d). This is independent of the hypoglycemic effects of insulin. Similarly, in animals that have had the adrenal glands removed 24 hours prior to the ischemic insult, a significant increase in GABA levels was recorded by in-vivo microdialysis (Ravindran et al., 1994). The increase in GABA may be a possible mechanism of neuronal protection in the two settings.

Concluding remarks

The inhibitory mechanisms discussed in this paper indicate that it may be possible to reduce or prevent neuronal death following cerebral ischemia. This offers hope for those undergoing or who have recently undergone an acute cerebral ischemic «attack». Several clinical trials which directly affect various pre- and post-synaptic inhibitory mechanisms are underway. These studies, in addition to basic research which is ongoing in the field, will help to further characterize the inhibitory effects of pre- and post-synaptic mechanisms on the outcome of the ischemic insult.

Acknowledgements. The author acknowledge the financial support of the Health Services Utilization and Research Commission and the Heart and Stroke Foundation of Saskatchewan.

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