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EFFECT OF PRETREATMENT WITH AN ADENOSINE ANALOGUE, N^6-CYCLOPENTYLADENOSINE (CPA), ON STROKE OUTCOME IN RATS

A Thesis

Submitted to the Graduate Faculty

in Partial Fulfilment of the Requirements

for the Degree of

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ABSTRACT

Adenosine A1 receptor agonists, such as N^6-cyclohexyladenosine (CHA), N^6-(R)-phenylisopropyladenosine (R-PIA), and N^6-cyclopentyladenosine (CPA), are neuroprotective when administered prior to a hypoxia-ischemia (H-I). Activation of A1 receptors may also mediate ischemic preconditioning. It was hypothesized, therefore, that a single administration of CPA prior to a H-I insult would provide neuroprotection against H-I induced damage in the rat hippocampus. This thesis examined the neuroprotective response to CPA when it was administered at varying doses and varying time points prior to a H-I insult. Male Wistar rats (n = 8) subjected to the Levine model of global H-I showed a 29.7% decrease in viable cells in the left hippocampus relative to the right (control) hippocampus (806.5 ± 43.1 vs. 567.0 ± 28.3). Administration of CPA (0.01, 0.1, 1.0, 3.0, 10.0 mg/kg; i.p.) 24 hours prior to a H-I insult significantly ($p < 0.05$) reduced cell loss in the hippocampus by 27.5%, 53.9%, 69.6%, 75.9%, and 95.6%, respectively. Administration of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 1.0 mg/kg, competitive adenosine A1 antagonist) 1 hour prior to CPA blocked this neuroprotective effect. Interpretation of the CPA dose-response curve generated an estimated ED_{50} of 0.0925 mg/kg. Administration of CPA (0.1 mg/kg) at varying time points (1, 3, 12, 24, and 48 hours) prior to a H-I insult produced a significant reduction in neuronal cell death (17.3%, 34.3%, 64.4%, 43.7%, and 48.3%, respectively). In addition, administration of CPA produced an immediate decrease in mean arterial blood pressure that lasted 90 minutes (CPA 0.01 mg/kg) or more than 120 minutes (CPA 0.1, 1.0, 3.0, and 10.0 mg/kg) but was not present 22 hours after administration of CPA (10.0 mg/kg). These results confirm the neuroprotective effect resulting from activation of adenosine A1 receptors before a H-I insult, against H-I induced damage and suggest a possible therapeutic application for adenosine A1 agonists for the prevention of stroke damage.

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ABBREVIATIONS

A1	adenosine receptor subtype 1
A2a	adenosine receptor subtype 2a
A2b	adenosine receptor subtype 2b
A3	adenosine receptor subtype 3
ADP	adenosine diphosphate
AMP	adenosine monophosphate
AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid
ANOVA	analysis of variance
ATP	adenosine triphosphate
BDNF	brain derived neur0trophic factor
CA1	region of Ammon's horn
CA2	region of Ammon's horn
CA3	region of Ammon's horn
CaM	calmodulin dependent kinase
c-AMP	cyclic-adenosine monophosphate
CHA	N^6-cyclohexyladenosine
CPA	N^6-cyclopentyladensoine
DAG	diacylglycerol
DNA	deoxyribonucleic acid
DPCPX	8-cyclopentyl-1,3-dipropylxanthine
ED_{50}	concentration producing 50% of the maximal effect

ER	endoplasmic reticulum
GABA	γ-amino-n-butyric acid
G_i	G-protein subtype
G_o	G-protein subtype
G_s	G-protein subtype
IB-MECA	N^6-(3-iodobenzyl)adenosine-5,-N-methyluronamide
IP_3	inositol-1,4,5-triphosphate
K_{ATP}	ATP-dependent potassium channel
MAPK	mitogen activated protein kinase
MCAO	middle cerebral artery occlusion
mGluR	metabotropic glutamate receptor
μM	micromolar
MnSOD	manganese-dependent superoxide dismutase
Na^+/K^+ ATPase	ATP-dependent sodium potassium pump
NBMPR	nitrobenzyl-6-thioinosine
NECA	N-methylcarboamidoadenosine
NFκB	nuclear factor kappa B
nM	nanomolar
NMDA	N-methyl-D-aspartate
PBS	phosphate buffered saline
PI	phosphotide inositol
PKA	protein kinase A

PKC	protein kinase C
PLA	phospholipase A
PLC	phospholipase C
ROS	reactive oxygen species
R-PIA	N^6-(R)-phenylisopropyladenosine
SAH	S-adenosylhomocysteine
SOD	superoxide dismutase
TIA	transient ischemic attack
t-PA	tissue plasminogen factor
VOCC	voltage operated calcium channel
VOSC	voltage operated sodium channel

1 GENERAL INTRODUCTION

1.1 Current Trends in Cardiovascular Disease

The social and economic costs associated with cardiovascular disease (heart disease and stroke) in Canada are continuing to rise each year (Heart and Stroke Foundation of Canada, 1994; 1999; Moore et al., 1997). Specifically, cerebral ischemia, a reduction of blood flow to the brain, is a leading cause of death in Canada (Heart and Stroke Foundation of Canada, 1997; 1999), the United States of America (American Heart Association, 1997) and worldwide (Heart and Stroke Foundation of Canada, 1999). Death as a result of cerebral ischemia occurs when areas of the brain are damaged and ultimately fail because of an inadequate blood supply to support normal neuronal functioning. In addition, cerebral ischemia is responsible for direct and indirect costs which exceed any other type of cardiovascular disease (Heart and Stroke Foundation of Canada, 1997). The inability of most families to care for impaired patients is why stroke patients account for an increasing proportion of people requiring permanent home care in Canada (Moore et al., 1997; Statistics Canada, 2000). Although the mortality rates associated with stroke have declined since the mid 1960s, the actual number of stroke cases increases each year and, with the projected rise in the elderly population, this increase is expected to continue for the next 15 years (Heart and Stroke Foundation of Canada, 1999). With these consistent rising trends, coupled with a lack of therapies available to stroke patients, stroke has become an important issue in Canadian and global health care. As a result, a considerable amount of research has been conducted to investigate potential methods of reducing the amount of damage that occurs as a result of cerebral ischemia.

Historically, stroke researchers have examined ways to reduce cerebral ischemic damage by focusing on post-insult treatments. A limitation to this track of investigation is the relatively short period of time (1 - 6 hours) that exists between the time of insult and onset of irreversible damage. This small time gap has been termed "the window of opportunity," as it is within this time that post-insult treatments have had notable results (Ginsberg and Pulsinelli, 1994; Sweeney, 1997; Hock, 1998). However, most people who experience a cerebral ischemic insult do not have access to medical attention before the window of opportunity has passed, as demonstrated by the fact that the majority of all deaths associated with stroke occurred before the person could reach a medical facility (American Heart Association, 1997). Clinical efforts to alleviate cellular damage during the window of opportunity have been met with limited success with the exception of tissue plasminogen activator (t-PA). Zivin and Miller (1999) reported that, in the USA, t-PA is approved for treatment of some stroke patients within the first three hours after symptom onset. In addition, the therapeutic window for the use of t-PA can be extended to 4 hours with a concomitant use of Argatroban, a thrombin inhibitor (Morris et al., 2001).

Prevention of a cerebral ischemic insult is ultimately an individual's responsibility because it requires life long attention to diet, physical activity and attitude. Moreover, other factors that an individual cannot control such as family history and previous heart attacks, contribute to an increased potential for a future cerebral ischemic insult. Thus, researchers have focused on pharmacological methods of alleviating neuronal death as a result of cerebral ischemia. Pharmacological treatment strategies for the prevention of neuronal

death include: N-methyl-D-aspartate (NMDA) receptor antagonists (Bao et al., 2001), calcium (Ca^{2+}) channel antagonists (Korenkov et al., 2000), γ-amino-butyric acid (GABA) agonists (del Zoppo et al., 1997), free radical scavengers (Mark et al., 1998), calpain inhibitors (Roberts-Lewis et al., 1994), and adenosine receptor agonists (reviewed in von Lubitz, 1999). Each treatment strategy warrants investigation; however, a combination of two or more of these treatments may evolve as the best treatment strategy. This investigation will focus on the properties of an adenosine receptor agonist.

1.2 Experimental Models of Ischemia

The pathophysiology of cerebral ischemia is complex. There are a variety of pathways that may lead to cell death (for review see section1.3 Mechanism of Cell Death). To investigate different aspects of these mechanisms, researchers can use animal models of cerebral ischemia. The utilization of animal models provides researchers with five principle benefits: (1) the ability to manipulate the duration and severity of a condition; (2) the ability to alter the timing of therapeutic interventions thus permitting the step-wise and time-dependent study of variables; (3) avoidance of ethical considerations associated with human experimentation; (4) animal (*in vivo*) models more closely reflect the human condition than cell line (*in vitro*) models; (5) a means of substantiating technological and therapeutic advances destined to be applied to humans (Tamura et al., 1997).

1.2.1 Animal Models of Ischemia

Several animal (*in vivo*) models have been developed to examine the effects of cerebral

ischemia on the brain. Models of global cerebral ischemia include: unilateral carotid artery occlusion (Levine Model), bilateral common carotid occlusion, and four-vessel occlusion (Pulsinelli Model). The most widely used model of focal cerebral ischemia is the middle cerebral artery occlusion (MCAO).

The bilateral common carotid artery occlusion exploits the absence of anastomoses between the vertebral and internal carotid circulation. To simulate cerebral ischemia, both common carotid arteries are occluded for 10 - 15 minutes which attenuates blood flow to the brain. However, people who experience cerebral ischemia do not experience occlusion of both common carotid arteries. Therefore, this model does not adequately reflect the human model of cerebral ischemia. The four-vessel occlusion model simulates cerebral ischemia through electrocoagulation of both vertebral arteries and occlusion of both common carotid arteries twenty-four hours later. The caveat of this procedure is that it produces inconsistent ischemia because of the difficulty of confirming complete electrocauterization of the vertebral arteries. Furthermore, similar to bilateral common carotid artery occlusion, this model does not adequately reproduce cerebral ischemia as experienced by humans (Tamura et al., 1997).

The MCAO model of focal ischemia is the most widely used model in the study of cerebral ischemia. The many versions of this model include: proximal MCAO model (Tamura model); intravascular thread model (Koizumi model); distal MCAO with bilateral common carotid artery occlusion model (Chen model); and photothrombosis model (Watson

model). MCAO models are popular because they produce cerebral ischemia in a way that is similar to human stroke. As well, this model produces consistent ischemia-induced infarct volumes. However, the MCAO model is technically difficult as it requires experienced skill to control the fine instruments used to produce the stroke.

All models of cerebral ischemia give rise to pathological changes characteristic of cerebral ischemia. But the Levine model targets those neurons which are located in particularly vulnerable areas of the brain, such as the hippocampus. In this study, the Levine model was used. It creates conditions similar to those experienced during periods of hypoxia and ischemia in humans (Ginsberg and Pulsinelli, 1994). This model employs a unilateral common carotid artery ligation followed by exposure to a hypoxic environment to produce neuronal damage (Rice et al., 1981). Both procedures are necessary to produce brain damage as the use of one procedure in the absence of the other does not produce histological damage (Miyasaka et al., 2000). Furthermore, aside from simulating parameters of human stroke, the Levine model is able to produce a condition whereby one hemisphere is hypoxic and relatively ischemic while the other hemisphere maintains normal blood flow and is only mildly hypoxic (Ginsberg and Busto, 1989). This condition allows for the investigation of pathological outcomes since one hemisphere can be compared to the other (Ginsberg, 1990, Miyasaka et al., 2000).

1.3 Mechanisms of Cell Death

1.3.1 General Consequences Leading to Cell Death

Cerebral ischemia is a reduction of blood flow to the brain (Tabers, 2001). It is usually a result of either a thrombosis (formation of a blood clot) in a cerebral blood vessel, an embolism (obstruction) of the aorta, carotid or vertebral blood vessels (Purves et al., 1997), or a result of a cerebral hemorrhage (bleeding for more than a few minutes, that compromises organ and tissue perfusion) (Rodnitzky, 1995). The interruption of cerebral blood flow is inherently accompanied by a lack of oxygen (hypoxia). However, the development of hypoxia is not always the result of an ischemic insult. Hypoxic conditions may also include the non-availability or reduced availability of oxygenated ambient air.

Cerebral ischemia initiates a cascade of events that lead to cell death (Figure 1). Cerebral ischemia results in a decrease in available glucose and oxygen which are necessary for aerobic respiration. Glucose is converted into pyruvate which drives the production of ATP via the Krebs cycle, while oxygen aids in the production of ATP by acting as an electron acceptor during oxidative phosphorylation. Prolonged depletion of glucose and oxygen leads to the fast depletion of modest energy stores (Gyulai, 1987). Ljunggren et al. (1974) showed in the rat that only 7 ½ minutes of brain ischemia led to a significant decrease in ATP. Shimizu et al. (1993) demonstrated using microdialysis that during the first 10 minutes of ischemia ATP was completely depleted. Because brain oxygen stores are small, they can only sustain production of ATP for a few seconds (Siesjö, 1975). This

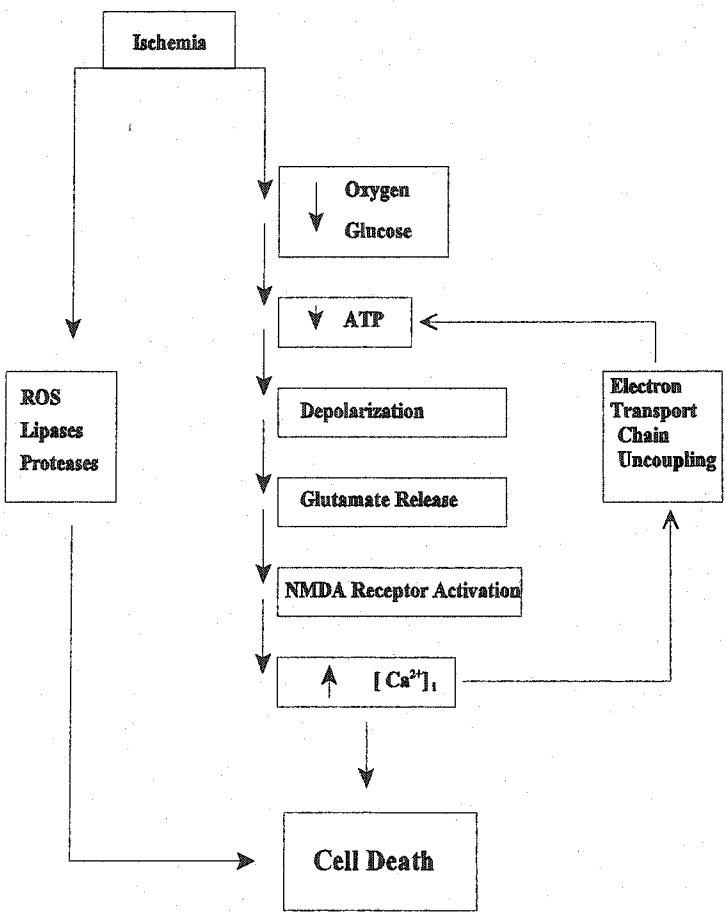

Figure 1. Summary of the potential events that may lead to cell death.

causes a prolonged lack of available chemical energy during hypoxic and ischemic conditions. Furthermore, mitochondrial ATP production does not occur in the absence of oxygen because ischemia inflicts damage to the mitochondria, manifesting as a decrease in mitochondrial respiration rates *in vivo* (Picklo et al., 1999) .

Without ATP, cells are no longer able to perform essential processes that require the expenditure of chemical energy and, as a result, are unable to maintain normal homeostatic ion concentrations (Zhu and Krnjevic, 1998). Brain cells need a constant supply of energy to maintain ionic equilibrium across neuronal membranes. Eventually, failure of the ATP dependent Na^+-K^+ pump allows the influx of Na^+, which is accompanied by the influx of Cl^- and H_2O, thereby causing cell swelling (Menzies et al., 1993; Pulsinelli, 1995). Cells swell as water is retained by the osmotic imbalance producing cytotoxic edema (Hatashita et al., 1990; Rosenberg, 1999). The inability of the nerve cells to maintain normal ion concentrations during ischemia and hypoxia gives rise to many processes which are linked to cell death including release of excitatory neurotransmitters, especialy glutamate, influx of calcium and formation of reactive oxygen species (Shaller et al., 1980; Johnston et al., 2001). Furthermore, an integral component of necrosis (cell lysis) is cell swelling, which results from the loss of selective permeability and membrane ion pump activity (Uchiyama, 1995). It is ultimately this inability to maintain normal ion concentrations that links different hypotheses of cell death.

1.3.2 Glutamate and its Contribution in Cell Death

Glutamate is an important excitatory neurotransmitter in the central nervous system. It acts pre and postsynaptically on ionotropic and metabotropic receptors (Table 1). The ionotropic receptors have been named according to selective agonists: (1) N-methyl-D-aspartate (NMDA), (2) kainate, and (3) γ-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA). These receptors all incorporate ion channels that are permeable to cations, although the relative permeability to Na^+ and Ca^{2+} varies according to the family. Activation of NMDA receptors causes the influx of both Na^+ and Ca^{2+}, whereas activation of AMPA and kainate receptors causes the influx of Na^+ and potentially Ca^{2+} depending upon the edited status of particular subunits of the receptor (Lynch and Guttman, 2002).

Metabotropic glutamate receptors (mGluR) are G-protein linked receptors that form a family of eight subtypes which have been subdivided into groups I, II, and III on the basis of sequence homology and pharmacological profile of activation (reviewed in Schoepp, 2001) (Table 1). Group I metabotropic glutamate receptors are G-protein-coupled to phospholipase C (PLC) (Conn and Pin, 1997) which stimulates the release of Ca^{2+} from the endoplasmic reticulum (Nakamura et al., 2000). Activation of group I glutamate receptors also causes astrocyte proliferation (Ciccarelli et al., 1997), glutamate release (Bezzi et al., 1998), and immediate-early gene transcription (Condorelli et al., 1993), Group II and III glutamate receptors are negatively coupled to adenylyl cyclase activity (Dingledine and McBain, 1999). Activatation of these receptors can negatively modulate

Table 1. Summary of glutamate receptor classification and their effectors. (IP_3: Inositol-1,4,5-triphosphate; c-AMP: cyclic adenosine monophosphate) (adapted from Ozawa et al., 1998).

Glutamate Receptors			
Ionotropic		**Ion Selectivity**	
NMDA		Na^+	Ca^{2+}
non-NMDA			
	AMPA	Na^+	(Ca^{2+})*
	Kainate	Na^+	(Ca^{2+})*

Metabotropic		**Second Messenger Systems**	
	Subtype	**Positive Coupling**	**Negative Coupling**
Group I			
	mGluR1	IP_3 c-AMP	
	mGluR5	IP_3	
Group II			
	mGluR2		c-AMP
	mGluR3		c-AMP
Group III			
	mGluR4		c-AMP
	mGluR6		c-AMP
	mGluR7		c-AMP
	mGluR8		c-AMP

* - dependent upon the edited status of particular subunits.

excitatory neurotransmitter output (Schoepp, 2001).

During and following cerebral ischemic attacks, the extracellular concentration of excitatory amino acids such as glutamate that are released from presynaptic cells is significantly increased in a Ca^{2+}-dependent fashion (Birnbaumer et al., 1994; Hicks and Conti, 1996; Levy et al., 1998). Shimizu et al. (1993) observed a 30-fold increase in extracellular glutamate concentrations following 10 - 30 minutes of ischemia. Numerous studies support the contention that excessive release of glutamate during ischemic conditions contributes to neuronal damage in the central nervous system (Choi, 1988a; Szatkowski and Atwell, 1994; Calabresi, 2000). In contrast, glutamate receptor antagonists have been shown to attenuate ischemia induced neuronal cell death (Alkan et al., 2001; Bao et al., 2001). In addition, in the MCAO model of focal ischemia, pre-treatment (30-min) and post-treatment (30-min) of the brain with the glutamate receptor antagonists MK-801 (Park et al., 1988) or CGS 19755 (Simon and Shiraishi, 1990) produced a decrease in neuronal cell loss in the rat. In addition, neurons lacking functional glutamate receptors have been shown to survive extended periods of ischemia (Peng et al., 1991; Tanaka et al., 2002).

The excitatory neurotransmitter glutamate has been linked to a sequence of events that ultimately leads to neuronal cell death (Figure 2). The incidence of glutamate induced excitotoxic cell death has been shown to be higher in regions of the brain such as the hippocampus that contain high concentrations of glutamate receptors (Kirino, 1982; Hsu

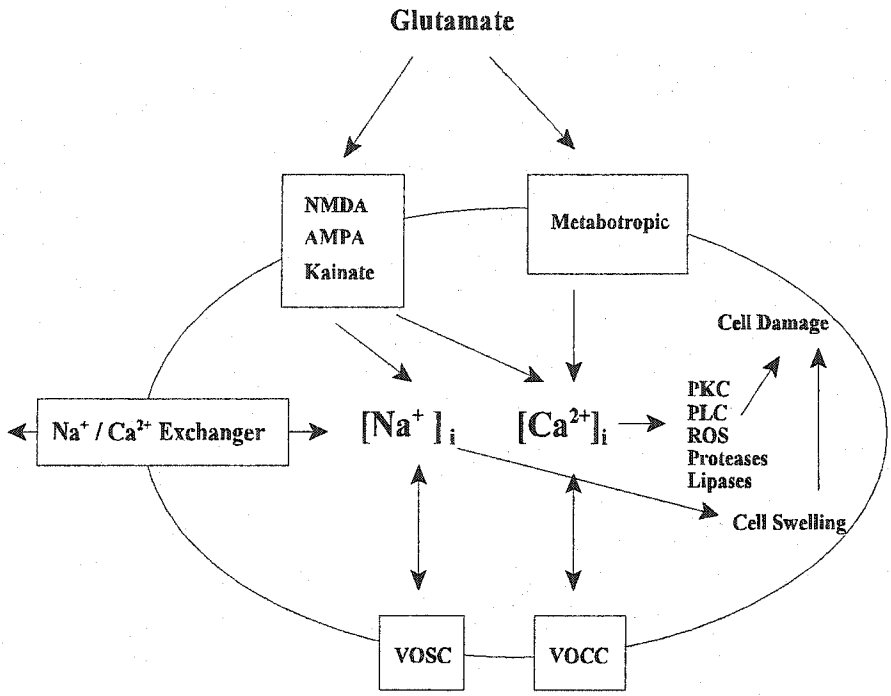

Figure 2. Pathways of glutamate induced cell damage. (AMPA: α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid; NMDA: N-methyl-D-aspartate; PKC: protein kinase C; PLC: phospholipase C; ROS: reactive oxygen species; VOSC: voltage - operated sodium channel; VOCC: voltage-operated calcium channel).

and Buzsaki, 1993; Hicks and Conti, 1996; Calabresi, 2000). During ischemic conditions, an increase in extracellular glutamate causes an increase in neuronal excitation and membrane depolarization via the influx of Na^+ ions (Rothman and Olney, 1986; Hicks and Conti, 1996). An increase in extracellular glutamate also causes an increase in the concentration of intracellular Ca^{2+} (Toms and Roberts, 1999; Sattler and Tymianski, 2001). For example, Dubinsky (1993) demonstrated that an application of glutamate significantly increased the concentration of intracellular Ca^{2+} in cultured hippocampal neurons. Randall and Thayer (1992) demonstrated the same results but added that the changes in Ca^{2+} concentrations that occur during glutamate induced neurotoxicity fluctuate post insult. Increasing levels of Na^+ inside the cell activates Na^+/K^+ ATPase pumps in an attempt to restore normal ion concentrations. However, activation of these pumps expends more of the already depleted stores of ATP, an event that exacerbates the destructive processes in the cell. Depolarization of the cell membrane triggers the opening of voltage-operated calcium channels (VOCC), resulting in a further influx of Ca^{2+} which adds to the existing influx from the activated NMDA and kainate receptors. Increased Ca^{2+} influx triggers more glutamate release from axon terminals and further membrane depolarization thus establishing a circular sequence of events that ultimately assists in cell death (Kermer et al., 1999). However, glutamate release is not the only deleterious effect brought about through excess calcium influx.

1.3.3 Calcium and its Contribution in Cell Death

There is normally a 10^4 : 1 extracellular to intracellular calcium gradient across neuronal

membranes (Carafoli, 1987; Clapham, 1995) because of poor membrane permeability to Ca^{2+} (Siesjö and Bengtsson, 1989). To maintain this Ca^{2+} gradient, the cell sequesters Ca^{2+} remaining intracellularly to mitochondria and the endoplasmic reticulum (Leist and Nicotera, 1999) and expends energy through different mechanisms to move unwanted Ca^{2+} out of the cell (Figure 3). The first mechanism is a Na^{+}/ Ca^{2+} exchanger. The second mechanism consists of several ATP dependent calcium pumps located on the plasma membrane, mitochondria and endoplasmic reticulum (Siesjö and Bengtsson, 1989) . At normal concentrations, Ca^{2+} fulfills functions in cell to cell communication and plays a role as a second messenger (reviewed in Brini and Carafoli, 2000). Ca^{2+} also elicits certain physiological responses such as long-term potentiation (Malenka et al., 1989) as well as regulation of neurite extension and regulation of the formation of synapses during development (Mattson et al., 1989).

In contrast to normal Ca^{2+} concentrations, excessive Ca^{2+} concentrations in the cell will eventually lead to cell death (Siesjö, 1992; Juurlink and Sweeney, 1997; Shuaib and Breker-Klassen, 1997; Ivanics et al., 2001). For example, Dubinsky (1993) showed that when cultured hippocampal neurons were subjected to application of an excitotoxic concentration of glutamate, the concentration of intracellular Ca^{2+} increased immediately and 85% of the neurons died after 24 hours. The increase in Ca^{2+} can be divided into three phases: (1) a triggering phase during which the neuron is exposed to glutamate and the intracellular concentration of Ca^{2+} increases to micromolar levels; (2) a latent phase during which Ca^{2+} concentrations return to baseline, and (3) a final phase that begins with

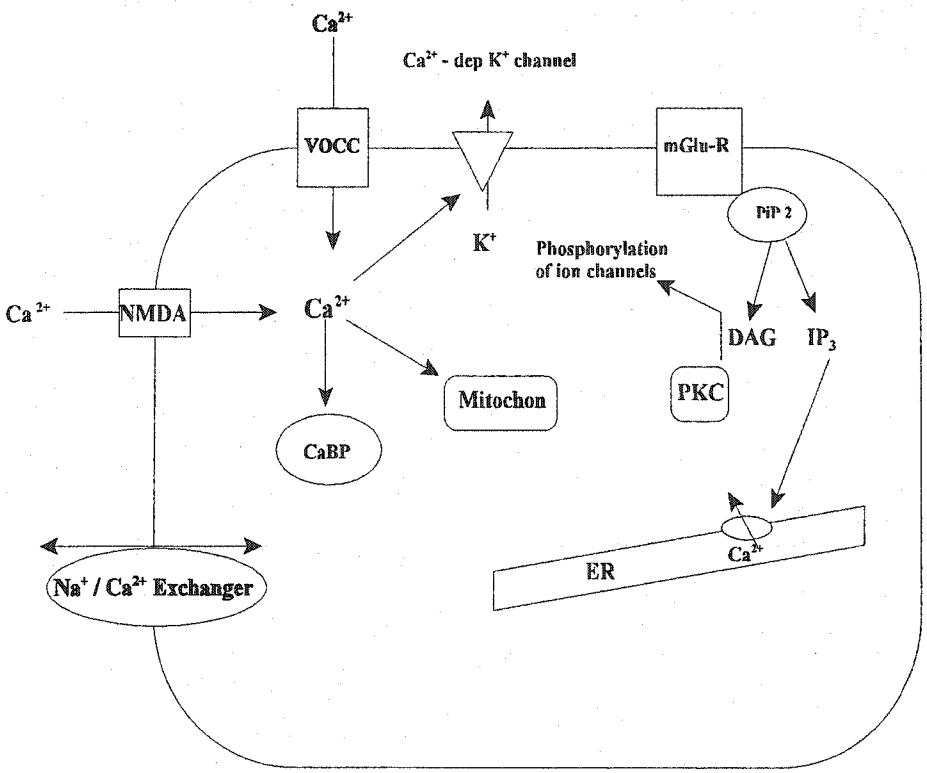

Figure 3. Receptors and pathways involved in the influx and extracellular release of calcium. (CaBP: calcium binding protein; DAG: diacylglycerol; ER: endoplasmic reticulum; IP_3: inositol-1,4,5-triphosphate; mGlu-R: metabotropic glutamate receptors; Mitochon: mitochondria; NMDA: N-methyl-D-aspartate; PIP_2: Inositol-4,5-biphosphate; PKC: protein kinase C; VOCC: voltage-operated calcium channels) (adapted from Disterhoft et al., 1994).

the gradual rise in the intracellular concentration of Ca^{2+} that reaches a plateau from which the neuron cannot recover (Randall and Thayer, 1992). Sustained high intracellular Ca^{2+} concentrations are also known to produce molecular and genetic changes which also lead to cell and membrane injury (Clapham, 1995; Brini and Carafoli, 2000; Cruzalegui and Bading, 2000). Prolonged concentrations of intracellular Ca^{2+} activated nucleases that resulted in cleaved DNA and a degradation of cell chromatin (Nicotera et al., 1994). Bading et al. (1993) showed that the initiation of gene transcription depended upon how Ca^{2+} enters the cell. In their study Ca^{2+} entry through VOCC and NMDA receptors initiated gene transcription. This excessive rise in Ca^{2+} also represents a stimulus which causes the over activation of lipases (lipid hydrolytic enzymes), proteases and endonucleases (Siesjö, 1992; Spira et al., 2001).

Protease and lipase activity can also initiate a cascade of destructive events within the nerve cell. Excessive protease activity will destroy parts of the supporting cytoskeleton and interfere with normal receptor function, membrane channels and ion transporters (Siesjö, 1992; Ouyang et al., 1999). It has been shown that extensive cytoskeleton and plasma membrane damage can disrupt the integrity of the cell, increase membrane permeability to ions and even macromolecules, compromise transport of essential cell products and induce signaling cascades finally mediating cell death (Zalewska, 1996). Moreover, proteases eventually give rise to oxygen free radicals, which are known to be noxious to cells (Siesjö, 1992). Of particular interest are a family of cytosolic proteases, calpains, that are optimally activated by calcium, under neutral pH conditions (Sorimashi

et al., 1994). As mentioned previously, the neuroprotective effects of calpain inhibition have been investigated. According to Goodman and Zagon (1986), tracking the initiation and degree of calpain proteolysis can be done by quantifying the breakdown of spectrin, a calpain substrate and cytoskeleton protein. In a model of global ischemia it was shown that hippocampal spectrin proteolysis precedes degeneration of CA1 cells (Roberts-Lewis et al., 1994). Similarly, Bartus et al. (1995a) demonstrated that following focal ischemia proteolysis of spectrin by calpain occurs prior to significant cell loss in the rat.

Additional support for the pathogenic role of calpain following brain ischemia is provided by pharmacological studies where calpain activity was inhibited (MacDonald et al., 2001). These studies show that drugs which inhibit calpain can significantly reduce the cell death of CA1 neurons that occurs following global ischemia (Bartus et al., 1995b). Calpain inhibitors administered to rats subjected to focal ischemia have produced similar results. Hong et al. (1994) demonstrated significant reduction of infarct volume with pre-ischemic administration of a calpain inhibitor while Bartus et al. (1994) demonstrated the same result with post-ischemia administration of a calpain inhibitor.

The over activation of lipases as seen in ischemia is also detrimental to neurons. An early event in ischemia is a Ca^{2+}-dependent increase in the activity of lipases (Kogure and Nakano, 1992). Lipase activity leads to the formation and accumulation of bioactive metabolites including free fatty acids, especially arachidonic acid, as well as, phosphitide inositol (PI), lysophospholipids (Kermer et al., 1999). Arachidonic acid forms metabolites

that exhibit second messenger activity, modifies synaptic activity and aids in the production of ROS whereas lysophospholipids and free fatty acids act as detergents thereby destroying the structural integrity of the cell membrane (Siesjö, 1992). There exists evidence to support the premise that elevations in free fatty acids contribute to the evolution of ischemic brain damage (Hara et al., 1991). Arachidonic acid concentrations increase following global ischemia and reach levels 10 to 20 times those observed in normal tissue (Nemoto et al., 1992). The accumulation of free fatty acids, in this case arachidonic acid, clearly precedes the onset of neuronal damage (Nemoto et al., 1992). The formation of ROS serves as yet another avenue to mediate cell death or at the very least exacerbate the present pathway towards cell death.

1.3.4 Reactive Oxygen Species and Their Contribution in Cell Death

ROS such as hydroxy radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) are by-products of cellular physiology generated by specific enzymes, auto-oxidation and energy transfer reactions (Matsuyama, 1997). Formation of ROS is linked to neuronal cell death (Bindokas et al., 1996; Patel et al., 1996; Tan et al., 1998) (Figure 4). ROS are highly reactive chemically and cause cellular damage by attacking lipids, proteins, and nucleic acids (reviewed in Chan, 2001). These highly reactive molecules can result in almost instantaneous peroxidation of cell membrane phopholipids as well as the oxidation of cellular proteins and nucleic acids (Chan et al., 1992; Chan, 1996; Tan et al., 1998). Damage to neurons, glia, and endothelial cells of the cerebral vessels was observed in rats whose parenchyma had been infused with ROS-generating solution (Chen et al., 1994).

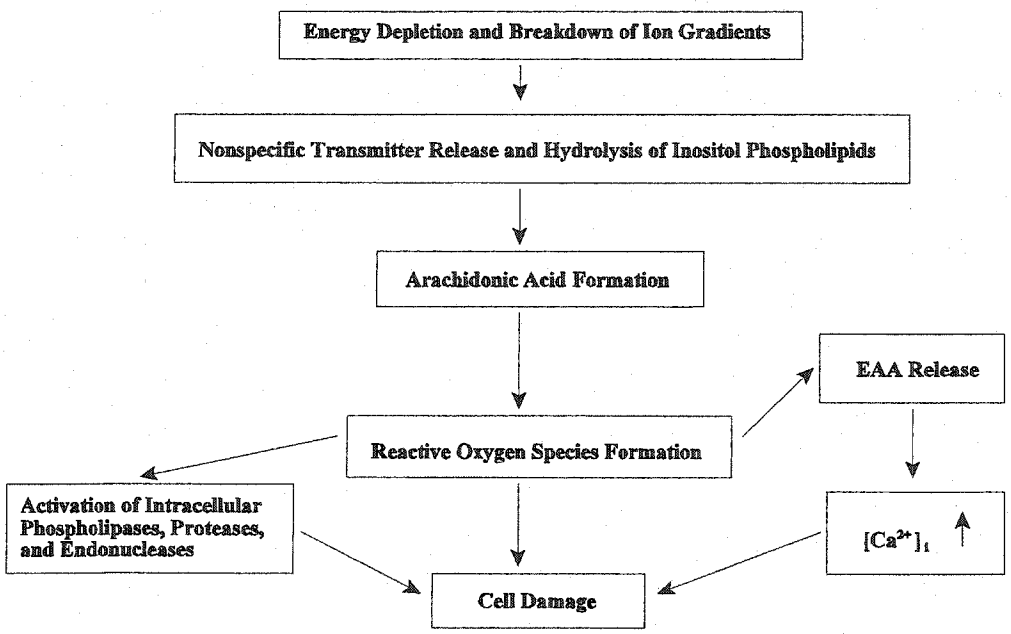

Figure 4. Pathways that contribute to the accumulation of reactive oxygen species (ROS) during ischemia (EAA: excitatory amino acids; $[Ca^{2+}]_i$: intracellular concentration of calcium) (adapted from Emerich and Bartus, 1999).

Although cells normally produce ROS, during cerebral ischemia (Peters et al., 1998; Li et al., 1999) and reperfusion (Ratan et al., 1994; Tan et al., 1999), production of ROS is increased. Under normal conditions, xanthine oxidase in the presence of oxygen oxidizes hypoxanthine to xanthine. A byproduct of this reaction is superoxide. Superoxide anions ($^{\circ}O_2^-$) are converted to $OH^{\cdot}$ and H_2O_2 (Matsuyama, 1997). However, during ischemic conditions there is a lack of available oxygen and an abundance of xanthine oxidase (Ratan et al., 1994). Upon reperfusion, oxygen becomes readily available thus driving the reaction to produce superoxide which results in the formation of more ROS thereby accentuating the destructive events described above (Sussmann et al., 1989; Halliwell, 1999).

Cells have enzymatic and non-enzymatic mechanisms to protect themselves from ROS mediated cell damage. Enzymatic neutralization can be achieved by superoxide dismutase (SOD), catalase and glutathione peroxidase (Mark et al., 1998), while the non-enzymatic methods use glutathione, α-tocopherol and ascorbic acid to neutralize ROS (Siesjö, 1992; Chen, 1996; Chan et al., 1998). However, during cerebral ischemia, the equilibrium between ROS production and the cell's defense mechanism is disrupted as a result of a substantial increase in ROS formation (Juurlink, 1999). This imbalance directs ROS mediated cell damage via lipid peroxidation of membrane bound polyunsaturated lipids (Sussmann et al., 1989). In addition, SOD levels in rats are unchanged at 24 hours following focal ischemia, suggesting that if ROS do participate in the pathogenesis of ischemic damage they may do so only during or following reperfusion (Michowiz et al., 1990).

1.3.5 Apoptotic Versus Necrotic Cell Death

Cell death is described as necrosis (cell lysis) or apoptosis (programmed cell death). Necrosis describes organ, tissue, or cellular death which has resulted from excessive physiological stresses and progressed in a random manner (Bosman et al., 1996). In contrast, apoptosis refers to a mechanism of cell death that is the result of a programmed cellular mechanism encoding for the death of certain cells (Kerr et al., 1972).

Each mode of cell death is characterized by unique and contrasting characteristics. The principal morphological characteristic associated with necrosis is cell swelling while cell shrinkage is observed in apoptosis (Lipton, 1999). The plasma membrane experiences enhanced permeability and compromised integrity during necrosis but not during apoptosis. Additionally, during necrosis, there is an overall destruction of cytoplasmic organelles which is not apparent in apoptosis (Uchiyama, 1995). Moreover, during necrosis, the destruction of cytoplasmic organelles is accompanied by nonspecific degradation of DNA and protein, as well as, the nonspecific hydrolysis of substrates (Schwartzman and Cidlowski, 1993; Li et al., 1995) while apoptosis is characterized by DNA cleavage (Dirnagl et al., 1999). Of particular interest is the requirement of ATP for the progression of apoptosis but not necrosis. For example, when cells were treated with a calcium ionophore in the presence of ATP supplying conditions apoptosis progressed. However, when the ATP supplying conditions were removed necrosis progressed (Egushi et al., 1997).

1.4 Relevant Anatomy in the Rat

1.4.1 Hippocampus

1.4.1.1 Regions of the Hippocampus

The regional anatomy of the hippocampus in rodents has been the subject of investigation for several decades (Blackstad, 1956; Swanson and Cowan, 1976; Swanson, 1981). Today, the overall territory of the rat hippocampus is described as occupying the majority of the ventroposterior and ventrolateral walls of the cerebral cortex. Within this defined area, the hippocampus has been subdivided into six distinct structures: (1) The entorhinal cortex, (2) the parasubiculum, (3) the presubiculum, (4) the subiculum proper, (5) Ammon's horn, and (6) the dentate gyrus. In addition to these six structures, the entorhinal cortex has been subdivided into the entorhinal cortex lateral, the entorhinal cortex intermediate, and the entorhinal cortex medial. As well, and most importantly to this investigation, Ammon's horn is subdivided into the CA1, CA2 and CA3 fields (Figure 5).

1.4.1.2 Cytoarchitecture of the Hippocampus

The cytoarchitecture of the hippocampus differs mainly in the appearance and the arrangement of cells within each region, as well as, between regions. For example, based on the size and distribution of cells, the entorhinal cortex has up to six distinct layers of cells which extend horizontally through each of the entorhinal subdivisions (lateral,

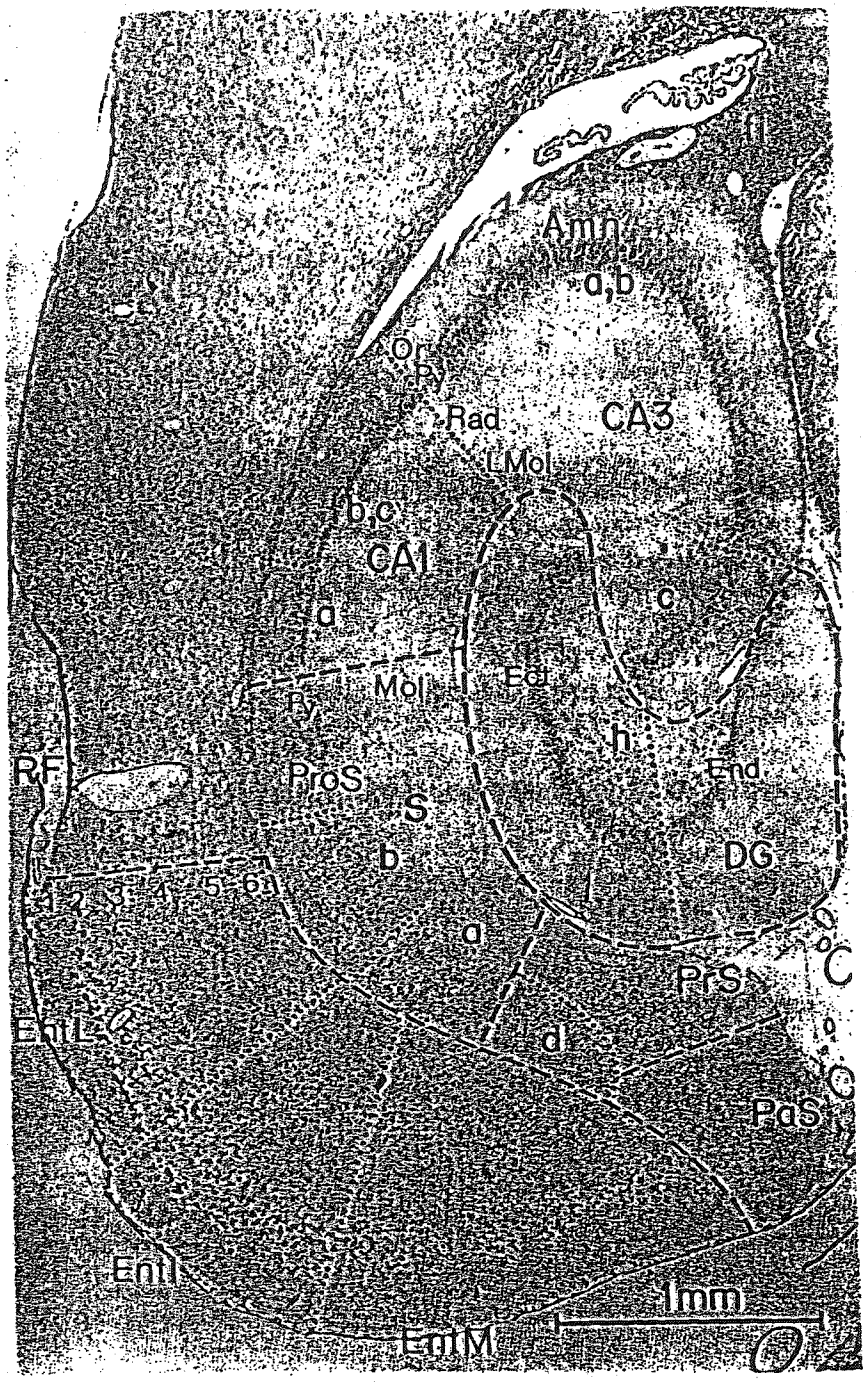

Figure 5. Relative location of brain regions associated with the hippocampus in rats. (Amn: Ammon's Horn; CA1, CA2, CA3: pyramidal cell feilds of Ammon's Horn; Ect: Ectal limb of the dentate gyrus; End: Endal limb of the dentate gyrus; Entl: Entorhinal cortex lateral; EntI: Entorhinal cortex intermediate; EntM: Entorhinal cortex medial; DG: Dentate Gyrus; h: hilus; PaS: Parasubiculum; PrS: Presubiculum; S: subiculum Proper) (taken from Bayer, 1985).

intermediate, mediate) (Hevner and Wong-Riley, 1992; Insausti et al., 1995). Layer one is characterized by the termination of axons and dendrites from outer layers of the brain. The larger stellate cells are concentrated in the second layer, while layer three is mainly comprised of smaller pyramidal cells. The majority of cells in the forth layer are large pyramidal cells. However, layers five and six have a mixture of both medium and small pyramidal cells (Insausti et al., 1995). Not only does the size of the cells change between these regions, the relative arrangement of these cells within and between these regions also changes. For example, the stellate cells in the second layer are arranged into tight pockets in the lateral and intermediate divisions of the entorhinal cortex but can be seen as a continuous layer in the medial division of the entorhinal cortex (Insausti et al., 1995). In addition, of the medium and small size cells located in layers five and six, the proportion of medium sized cells increases from the lateral to the medial divisions of the entorhinal cortex.

The subicular complex, which is comprised of the parasubiculum, the presubiculum, and the subiculum proper, contains three distinct cytoarchitechtural layers with each layer corresponding to a subdivision of the subicular complex. The parasubiculum is characterized by a layer of small pyramidal cells, whereas the presubiculum contains the same size pyramidal cells but they appear more densely packed than those of the parasubiculum (Naber et al., 2000). Both of these cell layers extend deeply into the entorhinal cortex where they superimpose upon the medium and small pyramidal cells of layers five and six of the entorhinal cortex (Harris et al., 2001). In contrast, the subiculum

proper is comprised mainly of a deep densely packed layer of pyramidal cells which becomes more narrow as it nears the presubiculum and CA1 of Ammon's horn (Naber et al., 2000).

Ammon's horn can be divided into three fields, CA1, CA2, and CA3. The principal cells of Ammon's horn are pyramidal cells which are organized 3-5 cells deep and collectively form the pyramidal cell layer (Frotscher, 1988). A main characteristic that defines the separation of these fields is the arrangement of the cells located in each region. In the CA1 region, the pyramidal cells are tightly organized while in the CA2 and CA3 regions the pyramidal cells are less densely packed. The use of light microscopy (400X) in this study clearly shows this feature and thus clearly defines where the CA1 region ends and the CA2 region begins. However, the distinction between the CA2 and CA3 regions is not as clearly defined as few cell morphology differences exist between these regions. In both regions, the cell bodies appear loosely packed and similar in size. Moreover, they appear the same in Nissl-stained preparations. Despite the similar appearance of cells located in CA2 and CA3, the location of these cells within the entirety of Ammon's Horn offers a means by which these regions can differentiated. In this study, pyramidal cells of the CA2 region were defined as those cells extending from the termination of CA1 to the beginning of the temporal arc of Ammon's horn (Figure 5).

The principal cells of the "U-shaped" dentate gyrus are granule cells (Bayer, 1985). They form the principal cell layer and appear to be stacked four and ten cells deep, depending

on the location within the dentate gyrus (Frotscher and Leranth, 1985). The granule cell layer is characterized as becoming thin towards the distal ends (ectal, endal) of its branches, while the thicker aspect is located in the apex of the dentate gyrus. Adjacent to this principal granule cell layer is the hilus of the dentate gyrus. It is characterized by the presence of polymorph, fusiform and modified pyramidal cells which are loosely packed and therefore it has been called the polymorphic cell layer. In addition, there is a third layer of cells that is found above the granule cell layer. This layer is called the molecular layer (Bayer, 1985).

1.4.1.3 Synaptic Organization Within the Hippocampus

The general synaptic organization within the hippocampus of rats is a circular progression of excitatory synapses which is referred to as the trisynaptic circuit (Figure 6). Although the progression of synapses is ultimately circular, there are unidirectional excitatory pathways which connect regions of the hippocampus (Frotscher et al., 1994) (Figure 6). The circular progression of synapses may allow for associative coding within the hippocampus for the purpose of memory formation (Vianna et al., 2000).

To describe the different connections within the trisynaptic circuit, it is easiest to start at the entorhinal cortex because this structure receives projections originating from several different cortical regions (Suzuki and Amaral, 1994a; 1994b). Projections from the entorhinal cortex to the dentate gyrus principally arise from the second and third layer (Rolls et al., 1998). Collectively, these projections form the perforant path. Projections

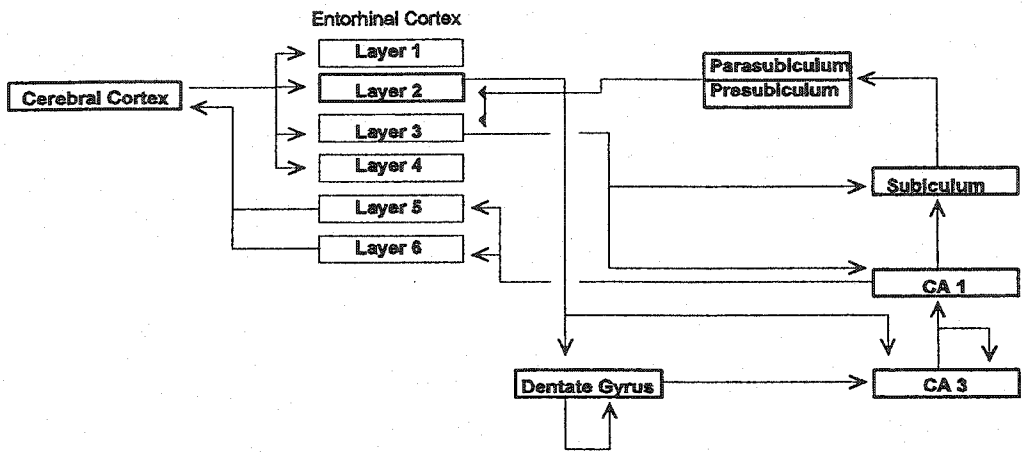

Figure 6. Summary of synaptic connections of the trisynaptic pathway in the hippocampus of rats.

that are part of the perforant path synapse in both the molecular layer and the granule layer of the dentate gyrus.

The granule cells of the dentate gyrus extend unmyelinated axons called mossy fibres to the polymorphic layer (also called the hilus) of the dentate. From here, the mossy fibres extend into the pyramidal cell layer of the CA3 region and terminate on the large "spines" of the apical and basal dendrites of the pyramidal cells (Frotscher et al., 1994). This presynaptic expansion of mossy fibres along with the postsynaptic expansion of the "spiny" dendrites provides a means through which one mossy fibre can have multiple synapses along one dendrite extension from a CA3 pyramidal cell. This multiple synapse connection provides a means of strongly influencing the pyramidal cells of the CA3 region (Li et al., 1994).

The CA3 pyramidal cells give rise to axons that form connections within the hippocampus and to corresponding regions of the contralateral hippocampus (Li et al., 1994). Connections to the contralateral hippocampus occurs via a large fibre bundle collectively called the fornix. Within the hippocampus, CA3 pyramidal cells project to the CA1 region through axons collectively called the Schaffer collaterals (Bernard and Wheal, 1994). Projections to CA2, and those projections back to CA3 are called the associative connections. Cells within the CA3 region that are located near the polymorphic layer of the dentate and cells from the CA2, also have projections leading back into the polymorphic layer (Figure 6).

All cells in the CA3 and CA2 region project to CA1 (Li et al., 1994). However, the transverse location of the cell will define the eventual area of connection to CA1 (Li et al., 1994). For example, cells of the CA3 region located proximally (near the hilus of the dentate gyrus) tend to send collaterals that connect with cells of the CA1 region located proximally to the subicular complex. In addition, cells of the CA3 region located closer to the CA2 region, as well as cells of the CA2 region tend to give rise to collaterals that connect to more temporal (lateral) cells of the CA1 region. Moreover, cells located in more septal areas of the CA3 layer will project collaterals to more septal cells of the CA1 region.

Cells in the CA1 region have two major projection paths. The first path is directed to the subiculum, while the second path terminates in the deep layers (layers five and six) of the entorhinal cortex (Harris et al., 2001) (Figure 6). Therefore, a main difference between CA3/CA2 and CA1 is that CA1 is the first field within the hippocampus to project back to the entorhinal cortex, thereby accomplishing the circular progression of connections of the trisynaptic circuit. Similar to the CA3 projections, CA1 projections have an organized pattern of distribution. For example, CA1 cells located proximally to the subiculum project to the distal aspect of the subiculum, whereas those cells located in the distal region of CA1 project to the proximal aspect of the subiculum. The projection path leading to different layers of the entorhinal cortex originates from CA1 regardless of the septotemporal level within CA1.

1.4.1.4 Vulnerability of the Hippocampus to Ischemia and Hypoxia

The hippocampus is particularly vulnerable to neuronal cell death as a result of ischemic and hypoxic conditions. In addition, individual regions of the hippocampus such as the CA1 and CA3 regions are more vulnerable to neuronal cell death than others, such as the CA2 region (Kirino et al., 1985; Hsu and Buzsaki, 1993). This selective vulnerability within the hippocampus can be attributed to relative distribution of neurotransmitter receptors. For example, because excessive concentrations of glutamate have been linked to cell death, the presence of glutamate receptors within the hippocampus make it vulnerable to ischemia-induced neuronal death (Hsu and Buzsaki, 1993; Schoepp and Conn, 1993). The predominance of glutamate receptors in the CA1 and CA3 regions makes these regions even more vulnerable to ischemia-induced neuronal death (Kirino, 1982; Hicks and Conti, 1996). In support of glutamate receptor-dependent vulnerability to cell death in the hippocampus, it has been shown that in regions where there are a lack of glutamate receptors there is better neuronal survival against ischemia (Peng et al., 1991; Tanaka et al., 2002).

1.4.2 Circulatory System in the Rat

1.4.2.1 Anatomy of the rat brain circulatory system.

The rat brain circulatory system (Figure 7) is similar to that of humans. In particular, the circle of Willis enables contralateral blood flow between left and right hemispheres of the brain (Klijn et al., 1997). The circle of Willis is directly supplied by the left and right

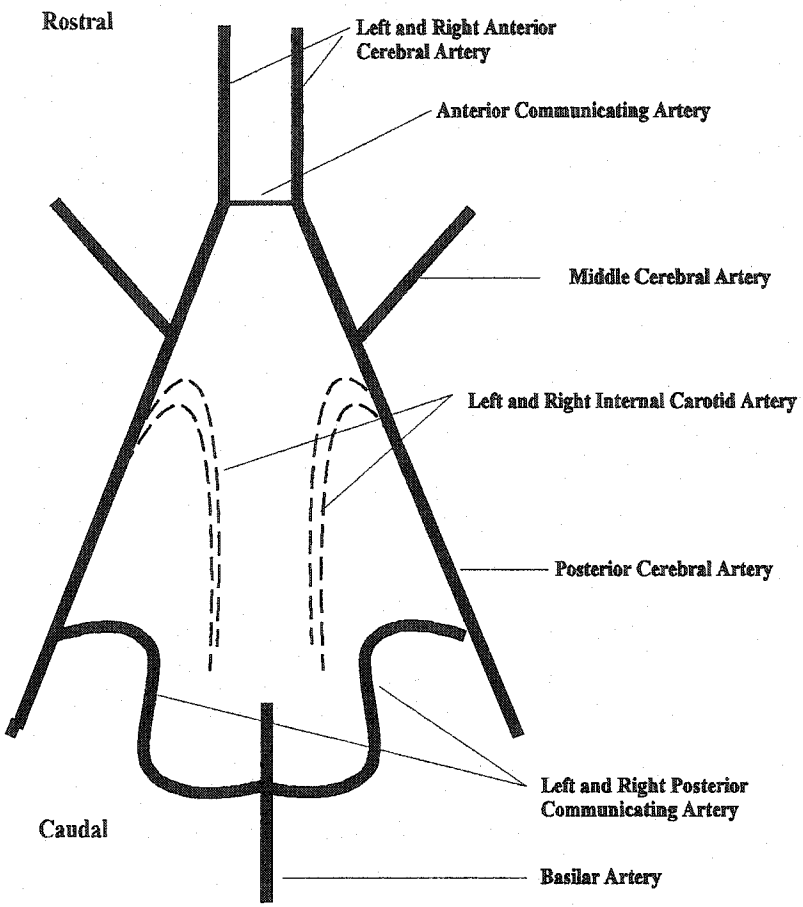

Figure 7. Schematic diagram of the arteries that contribute to the circle of Willis.

internal carotid arteries and is comprised of left and right posterior communicating arteries, an anterior communicating artery and left and right anterior and posterior cerebral arteries (Figure 7). The posterior communicating arteries anastomose with the basilar artery to supply the posterior aspects of the brain and both left and right cerebellar arteries (Schomer et al., 1994). The anterior communicating artery anastomoses to both the left and right anterior cerebral arteries (Powers, 1991).

1.4.2.2 Recruitment of Supporting Blood Vessels in the Brain

Occlusion of the common carotid artery produces diminished cerebral arterial pressure. In an effort to accommodate metabolic demands, collateral blood flow, via the circle of Willis, is increased with the recruitment of the communicating arteries (Powers, 1991). The anterior communicating artery and the ipsilateral posterior communicating artery are the primary collateral pathways via which the circle of Willis can redistribute blood flow to the deprived side of the brain (Schomer et al., 1994; Derdeyn et al., 1999). Use of this compensatory mechanism after unilateral carotid artery occlusion has been reported in both animal studies (Flaim et al., 1984; De Ley et al., 1985; Coyle and Panzenbeck, 1990) and human studies (van Everdingen et al., 1998; Hendrikse et al., 2001).

It is widely accepted that during and after times of cerebral ischemia there is recruitment of supporting blood vessels to maintain normal cerebral blood flow. However, there is debate regarding the interhemispheric hemodynamic changes associated with unilateral carotid artery occlusion. In studies where acute (five minute) occlusion of one common

carotid artery occurred, there was no interhemispheric difference in blood flow reported (De Ley et al., 1985; De Ley, 1990). However, in a study by Coyle and Panzenbeck (1990) where chronic occlusion of a common carotid artery occurred in rats, permanent occlusion of one carotid artery for either two days or six weeks produced blood flow that was 16 and 30 percent of control, respectively. Interestingly, they also reported that anastomoses between the basilar and carotid artery ipsilateral to the occluded side was significantly larger that on the contralateral side. In addition, Powers et al. (1987) showed in humans with chronic unilateral common carotid artery occlusion that although the posterior communicating arteries and the anterior communicating artery are recruited to improve perfusion to the ischemic brain region, the communicating arteries do not adequately re-establish normal blood flow until one week post insult.

1.5 Adenosine

Adenosine is a purine nucleoside consisting of an adenine base linked to a ribose sugar (Figure 8). In the mammalian body, adenosine acts predominantly as an inhibitory molecule while participating in the physiological activity of many mammalian tissues such as nervous and vascular tissue (Haas and Selback, 2000). Its many effects include vasodilation in the circulatory system, reduction of cellular metabolism in the nervous system and reduction of body temperature (Marangos and Boulanger, 1985; Daval et al., 1991; Dunwiddie and Masino, 2001).

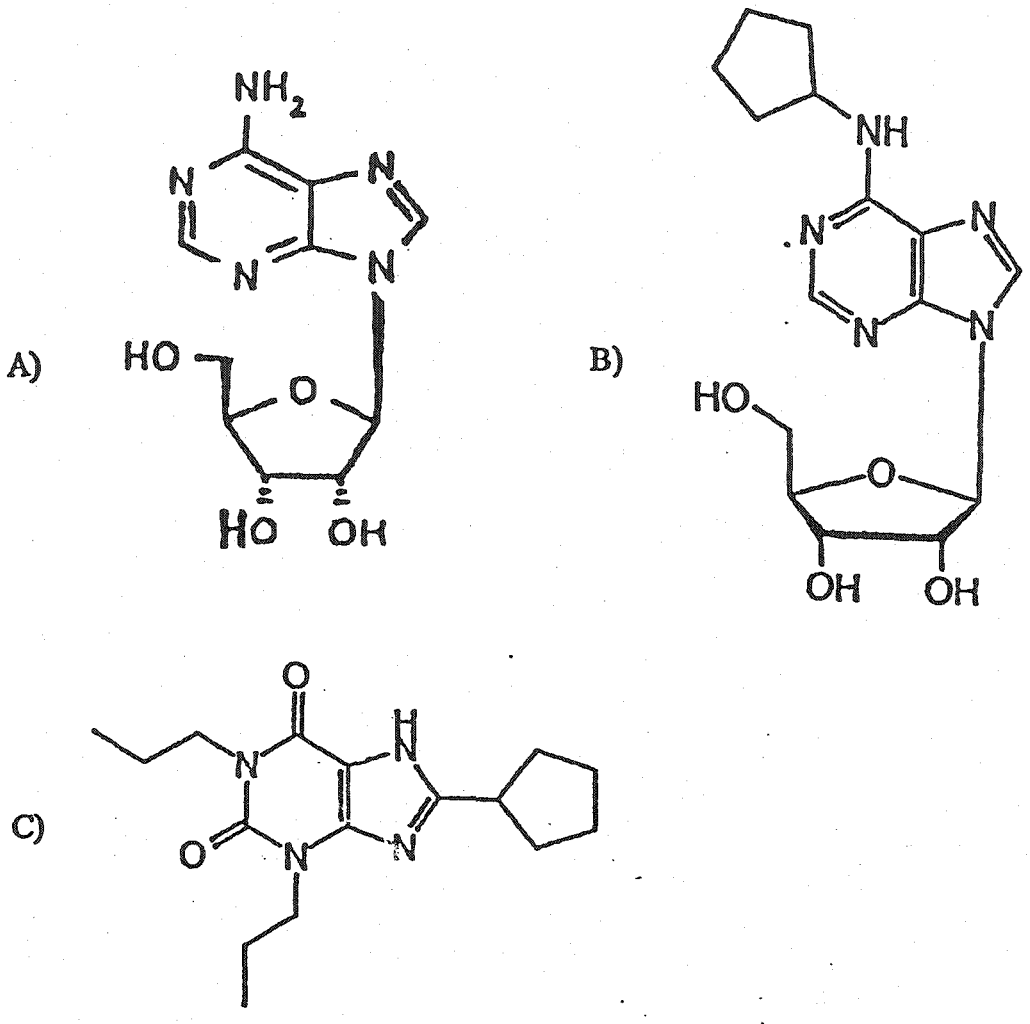

Figure 8. Chemical structure of A) Adenosine, B) N^6-cyclopentyladenosine (CPA), and C) 1,3-dipropyl-8-cyclopentyladenosine (DPCPX) (adapted from Fredholm et al., 2001).

1.5.1 Synthesis and Metabolism of Adenosine

Adenosine is formed in cerebral tissue through several pathways (Figure 9) and is present in the brain at extracellular concentrations of 40 - 60 nM during physiological conditions (Ballarin et al., 1991). The primary pathway of adenosine formation in the brain is through dephosphorylation of adenosine triphosphate (ATP) into adenosine monophosphate (AMP) followed by a second degradation into adenosine by the enzyme 5'-nucleotidase (Meghji, 1993; Zimmermann et al., 1998). The concentration of 5'-nucleotidase is particularly high in the hippocampus and on astrocyte membranes (Kreutzberg et al., 1976; Fredholm, 1997). Since this enzyme is found both intracellularly and extracellularly, adenosine can be formed both inside and outside the cell. However, it is not yet known whether adenosine formation mainly occurs intra or extracellularly (Cunha et al., 1994; Dunwiddie et al., 1997). But, it has been shown that the extracellular presence of adenosine during ischemia is greatly attributed to the cellular release of adenosine rather than its formation outside the cell by specific enzymes (Sinclair et al., 2000). Moreover, Whittingham (1990) showed that during metabolic stress induced by elevated electrical activity, hypoxia or ischemia, the bulk of adenosine originates from the intense degradation of ATP inside the cell. Another possible source of adenosine in the brain is through the hydrolysis of S-adenosylhomocysteine (SAH) by SAH hydrolase (Schrader et al., 1981). However, an SAH hydrolase inhibitor, adenosine-2,3-dialdehyde, does not significantly modify the adenosine outflow evoked by electrical stimulation or ischemia-like conditions (Latini et al., 1995).

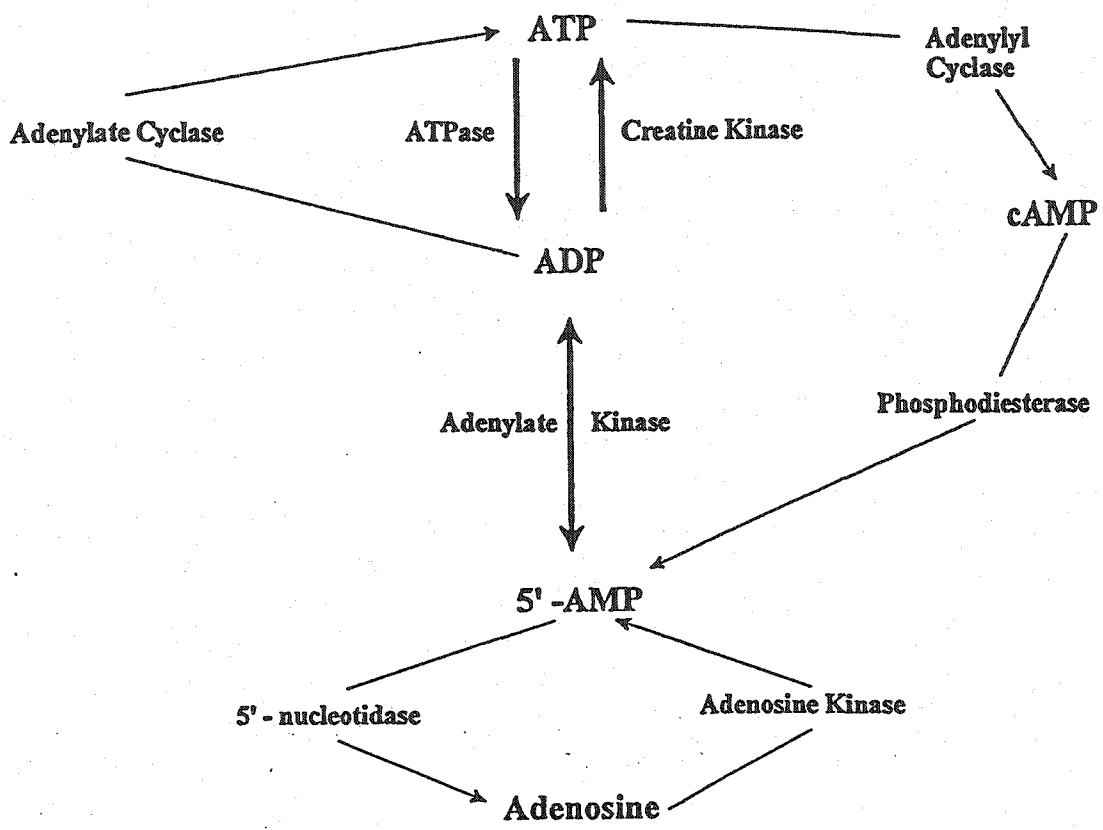

Figure 9. Central pathways leading to the formation of adenosine. (adapted from Dunwiddie and Masino, 2001)

As a hydrophilic nucleoside, adenosine does not readily cross the cell membrane by passive diffusion (Arch and Newsholme, 1978). Cellular release of adenosine occurs via nucleoside transporters, which are membrane proteins that mediate cross-membrane movement of purine and pyrimidine nucleosides (Baldwin et al., 1999; Sinclair et al., 2000). The transport of adenosine is mediated by two distinct families of nucleoside transporters: (1) concentrative and (2) equilibrative. The six concentrative transporters (N1-N6) are Na^+ dependent and couple the influx of adenosine to the influx of Na^+ whereas, the two equilibrative transporters (E1-E2), which have been shown to have broad distribution in the rat brain including on both neurons and astrocytes, move purine and pyrimidine nucleosides across the plasma membrane in a direction dictated by their concentration gradients (Geiger et al., 1997; Anderson et al., 1999a and 1999b). The equilibrative transporters are defined by their differential sensitivities to nitrobenzyl-6-thioinosine (NBMPR) (Cass et al., 1998). The "es" (equilibrative inhibitor-sensitive) transporter is blocked by a concentration of NBMPR lower than 1 nM, whereas, a concentration of NBMPR in excess of 1 μM is required to inhibit the "ei" (equilibrative inhibitor-insensitive) transporter (Cass et al., 1998). Although the role of neurons in regulating adenosine levels has been investigated, the role of astrocytes in regulating interstitial adenosine levels is not clearly defined. A recent study demonstrated that rat C6 glioma cells possess primarily equilibrative nucleoside transporters (ENT2) that function in adenosine uptake. However, intracellular metabolism prevented the release of adenosine from these cells during ATP-depleting conditions (Sinclair et al., 2000). In the central nervous system, the extracellular concentration of adenosine is efficiently controlled. It is

tightly regulated by rapid transport into cells via efficient equilibrative transporters (Parkinson et al., 1993; Anderson et al., 1999b). Once re-uptake has occurred, adenosine is either metabolized by adenosine kinase which establishes adenosine incorporation into a nucleotide pool, or is metabolized by adenosine deaminase which converts adenosine it inosine (reviewed in Arch and Newsholm, 1978).

1.5.2 Concentrations of Adenosine

During cerebral ischemia events there is an increase in extracellular adenosine in the brain (Hagberg et al., 1987; Phillis et al., 1987; Globus et al., 1988; Rudolphi et al., 1992a; Sweeney, 1997; Melani et al., 1999). This rise in the concentration of extracellular adenosine is not unique to nonhuman animal models. In patients who suffered either a transient ischemic attack (TIA) or a stroke, there was an adenosine surge (Pasini et al., 1999) . The adenosine surge did not dissipate quickly, returning to basal levels by day five for TIA patients and by day 15 for stroke patients. This increase in adenosine concentration represents an increase of up to 100 times the normal concentration (Bell et al., 1998; Parkinson et al., 2000). As well, exposure to hypoxic conditions also increased extracellular concentrations of adenosine (Dale et al., 2000; Kobayashi et al., 2000). Fowler (1993a, 1993b), demonstrated an increase in extracellular adenosine during hypoxia in rat hippocampal slices, while Pedata (1990) not only reported the same result but also showed that when rat brain slices were electrically stimulated the greatest increase in endogenous extracellular adenosine was seen in the hippocampus, with progressively less in the cortical and striatal regions. More specifically, there is an increase in adenosine

levels in the CA1 region of the hippocampus during hypoxia (Dale et al., 2000). This localized increase in adenosine concentrations in damaged areas has been suggested to act as an important homeostatic neuromodulator by down-regulating physiologic functions thereby conserving ATP (reviewed in Marangos, 1990).

1.5.3 Adenosine as a Neuroprotective Agent

There is an inverse relationship between the concentration of adenosine in the brain and the degree of ischemia-induced damage (Zhou et al., 1994). Different studies have examined the neuroprotective effect of adenosine receptor agonists both *in vitro* and *in vivo*. It has been shown that administration of adenosine significantly reduces hypoxia and hypoglycemia induced neuronal injury in cortical cells (Goldberg et al., 1988). Using hippocampal slices Lee and Lowenkopf (1993) showed that endogenous adenosine contributes to the neuroprotective effect by prolonging a delay to hypoxic depolarization. Consistent with this *in vitro* study, in an *in vivo* model of forebrain ischemia (von Lubitz et al., 1994a) and bilateral occlusion model (von Lubitz et al., 1999) it was shown that adenosine provided protection against mortality and neuronal damage in gerbils and significantly reduced mortality in the CA1 region of the hippocampus. These studies demonstrate that administration of some adenosine receptor agonists decreases the amount of nerve cell death resulting from cerebral ischemia, hypoxia or hypoglycemia. Additionally, it has been repeatedly shown that acute administration of adenosine receptor antagonists resulted in increased cell death (Sutherland et al., 1991; Héron et al., 1992; Daval and Nicolas, 1994; von Lubitz et al., 1994a, Zhou et al., 1994).

1.5.4 Adenosine Receptors

Both the central and peripheral effects of adenosine are mediated through activation of adenosine's four membrane bound metabotropic receptors: A1, A2a, A2b and A3 receptors (Burnstock 1989; Fredholm et al., 1994; Olah and Stiles, 1995; Fredholm et al., 1998; Ralevic and Burnstock, 1998). Although appropriately termed metabotropic receptors because of their association to a second messenger, such as cyclic adenosine monophosphate or inositol polyphosphate, these receptors can be grouped according to their influence on adenylyl cyclase. Both the A1 and A3 receptors stimulate phospholipase C (Linden, 1995; Abbracchio et al., 1995) and inhibit adenylyl cyclase (van Calker et al., 1978; Londos et al., 1980; Zhou et al., 1992; Fredholm et al., 1994) by interacting with G_i / G_o proteins (Jockers et al., 1994). In contrast to A1 and A3 receptors, the A2a and A2b receptors stimulate adenylyl cyclase (Fredholm et al., 1994) by interacting with G_s proteins (Palmer et al., 1995; Palmer and Stiles, 1997). Furthermore, adenosine receptors can be differentiated according to relative binding affinity for adenosine. The A1 receptor exhibits the greatest affinity for adenosine followed by A2a, A2b and A3 with affinities of 70, 150, 5100 and 6500 nM, respectively (Dunwiddie and Masino, 2001).

The adenosine receptor subtypes work independently and also work in concert with each other and other receptor subtypes. For example, an interaction between adenosine A1 and A2 receptors is mediated by protein kinase A (PKA) activity in the hippocampus, suppressing serotonin release (Okada et al., 2001). Additionally, agonists of metabotropic glutamate receptors are able to attenuate the inhibitory affects of adenosine A1 receptor

activation in hippocampal slices (Mendonca and Ribiero, 1997). O'Kane and Stone (1998) showed in rat hippocampal slices that activation of adenosine A1 receptors by CPA decreased population spike amplitude, whereas activation of A2a receptors had no effect. However, when the adenosine A1 and A2a receptors were activated together a significant attenuation of the inhibitory effect of CPA on population spike amplitude was observed.

1.5.4.1 Adenosine A1 Receptors

Of the four adenosine receptors, the A1 receptor is the most abundant and most widely distributed receptor in the mouse, gerbil and human (Fastbom et al., 1987; Parkinson and Fredholm, 1990; Parkinson et al., 1995). Although the A1 receptor is found in most parts of the brain, it is found in higher concentrations in the cerebellum, hippocampus and cerebral cortex (Fredholm and Jonzon, 1987; Daval et al., 1989; Saura et al., 1998). In the hippocampus, adenosine A1 receptors are more prominent in the CA1 and CA3 regions (Lee et al., 1983) as well as the CA2 region (Giraldez et al., 1998). They are located both pre and postsynaptically (Deckert and Jorgenson, 1988). They are strategically found on dendrites (Rivkees et al., 1995) and on axonal fibers of the hippocampus (Swanson et al., 1995) which upon activation result in decreasing neuronal activity (Dunwiddie, 1990) .

The treatment of cerebral ischemia with adenosine A1 receptor agonists has been supported by many studies (Rudolphi et al., 1992b; Jacobson et al., 1996; Sweeney, 1997; von Lubitz et al., 1999). It is accepted that there is an inverse relationship between the number of adenosine A1 receptors and the magnitude of cell death (Lee et al., 1983).

Over-expression of adenosine A1 receptors provides additional protection against ischemic injury (Matherne et al., 1997; Headrick et al., 1998). In various models of global and focal ischemia, acute application of adenosine A1 receptor agonists have been shown to be neuroprotective. Under global ischemia conditions, intra cerebral injections of N^6-(R)-phenylisopropyladenosine (R-PIA) (Evans et al., 1987) and N^6-cyclohexyladenosine (CHA) (von Lubitz et al, 1989), both A1 receptor agonists, reduced neuronal degeneration in the rat and gerbil, respectively. As well, von Lubitz et al. (1994a) showed that a systemic administration of CPA provided protection in a model of global ischemia. In focal models of stroke with permanent occlusion of the middle cerebral artery, CPA (Gotti et al., 1990) and R-PIA (Sheardown et al., 1995) were neuroprotective. In addition, a single administration of CPA protected cultured cerebellar neurons against hypoxia-induced death (Logan and Sweeney, 1997; Rigley et al., 2000). As well, animals treated with an acute administration of CPA demonstrated significant quantitative pyramidal cell preservation in the CA1 region of the hippocampus (von Lubitz et al., 1988). Furthermore, there is evidence that an acute administration of adenosine A1 receptor agonists, such as CPA, can inhibit excitatory amino acid efflux during a cerebral ischemic insult (Simpson et al., 1992; Héron et al., 1992). As well, chronic treatment with an adenosine A1 receptor selective antagonist did improve hippocampal CA1 recovery and mortality following 10 minutes of forebrain ischemia in gerbils and was not accompanied by receptor upregulation (von Lubitz et al., 1994a). Therefore, protection against cellular damage by CPA is thought to be mediated through the activation of adenosine A1 receptors on neurons (Logan and Sweeney, 1997).

Activation of adenosine A1 receptors causes decreased excitatory amino acid release from pre-synaptic neurons (Dolphin and Archer, 1983; Goda et al., 1998). Activation of A1 receptors with either CPA or NECA, A1 receptor agonists, significantly inhibited ischemia (10-min)-evoked release of both glutamate and aspartate (Simpson et al., 1992). Adenosine not only inhibits the spontaneous release of glutamate independently of PKA and PKC but also reverses the enhancement of exocytosis produced by PKA and PKC activators (Bouron, 1999). Activation of adenosine A1 receptors also causes hyperpolarization (Lee and Lowenkopf, 1993) and decreased Ca^{2+} influx through VOCC (Dolphin et al., 1986; Nogushi and Yamashita, 2000). This effect was completely reversed using a selective antagonist (8-cyclopentyltheophylline) and was mimicked by the selective adenosine A1 receptor agonist CHA. Not only does activation of adenosine A1 receptors inhibit the influx of Ca^{2+}, but it inhibits the influx through specific Ca^{2+} channels. Ambrosio et al. (1997) showed that CPA inhibited P/Q-types of Ca^{2+} channels and that this inhibitory effect was greatest in synaptosomes from the CA1 region of the hippocampus.

Adenosine predominantly inhibits neuronal activity. The postsynaptic effects of activating adenosine A1 receptors consist mainly of enhancing the outward potassium channel conductance (Dunwiddie, 1985; Fredholm et al., 1994) and the voltage-dependent chloride conductance (Mager et al., 1990). Together, these postsynaptic effects aid in stabilizing the postsynaptic membrane potential and decreasing NMDA receptor excitability (Schubert and Mager, 1991). The presynaptic hyperpolarization due to

adenosine A1 receptor activation results in the diminished release of excitatory amino acids (Phyllis, 1989). This diminished availability of glutamate reduces postsynaptic NMDA receptor activation which in turn reduces postsynaptic influx of Ca^{2+}. Consequently, the postsynaptic membrane potential does not increase sufficiently to activate VOCC (Schubert et al., 1994). As well, by stabilizing the postsynaptic membrane potential, the voltage-gated potassium currents whose operation constitutes a part of the protective hyperpolarizing sequence, will not be disrupted (Segal et al., 1984). Interestingly, Brundege and Dunwiddie (1996) described a process whereby an increase in activity in the hippocampal postsynaptic pyramidal neuron enhanced its own production of intracellular adenosine which, following transport to the extracellular space, acts as a retrograde messenger and inhibits the excitatory output of the same neuron.

1.5.4.2 Adenosine A2 Receptors

The A2 receptors are not as widely distributed as the A1 receptors. They are primarily located in the striatum and nucleus accumbens (Jarvis et al., 1989; Jarvis and Williams, 1989; Moreau and Huber, 1999) with modest amounts located in the cerebral cortex and hippocampus (Fredholm, 1997). Both subtypes of the A2 receptors are found on smooth muscle fibers and on endothelial cells of cerebral blood vessels (Kalaria and Harik, 1986). Of the A2 receptors, the A2b receptor is more widely distributed than the A2a receptor and is predominantly found on astrocytes (Hösli and Hösli, 1988).

In contrast to adenosine A1 receptors, the neuronal actions of adenosine A2 receptors are

excitatory (Ameri and Jurna, 1991; Sebastião and Ribeiro, 1996). A2 receptor activation, in particular the A2a subtype, enhances ischemia-evoked release of excitatory amino acids such as glutamate. For example, Simpson et al. (1992) demonstrated that both CPA and NECA inhibited ischemia-evoked release of excitatory amino acids at low concentrations but not at higher concentrations. They suggested that at low concentrations CPA and NECA preferentially activated A1 receptors to inhibit excitatory amino acid release while at higher concentrations these agonists coactivated A2 receptors which either offset or blocked the A1-mediated response. Additionally, CGS 21680, a selective A2a adenosine receptor agonist greatly enhanced extracellular glutamate release (Propoli et al., 1995).

Activation of adenosine A2a receptors produce a variety of effects in the body, such as stimulation of serotonin release in the hippocampus (Okada et al., 2001), facilitation of GABA release (Kirk and Richardson, 1995; Mori et al., 1996), and production of systemic vasodilation (Webb et al., 1991). Activation of adenosine A2a receptors was also responsible for hypoxia-induced dilation in the cerebral cortex (Coney and Marshall, 1998). It has also been suggested that stimulation of locomotor activity may be primarily an A2a effect (Ongini, 1997; El Yacoubi et al., 2000). Activation of the adenosine A2a receptor also produces a decrease in reactive oxygen species formation and participates in the induction of long-term potentiation (Sekino et al., 1991; Kessey et al., 1997). Activation of adenosine A2b receptors can lead to rise in intracellular Ca^{2+} (Feoktistov et al., 1994), is involved in control of vascular tone (Rubino et al., 1995), hepatic glucose balance (Harada et al., 2001), endothelial cell growth and gene expression (Grant et al.,

1999), and intestinal water secretion (Strohmeier et al., 1995).

Studies indicate that inhibition of adenosine A2a receptors reduces cerebral damage induced by global ischemia (Gao and Phyllis, 1994; von Lubitz et al., 1995a). A neuroprotective effect can be seen with the use of either A1 agonists and A2a antagonists which may be the result of decrease neurotransmitter release, particularly glutamate (von Lubitz et al., 1995a). Furthermore, CGS 15943, an A2 receptor antagonist, when administered intraperitoneally 15 minutes prior to a 5 minute period of global ischemia, reduced stroke injury and histopathological measurement of CA1 pyramidal neurons (Gao and Phyllis, 1994). In addition, adenosine A2a receptor may contribute to ischemic damage because mice lacking the adenosine A2a receptor showed reduced brain damage following focal ischemia (Chen et al., 1999). In contrast to this study, an administration of CGS 21680, an A2a selective agonist, following a 5 minute vessel-occlusion in gerbils provided significant protection against hippocampal CA1 neuronal loss (Sheardown and Knutsen, 1996).

1.5.4.3 Adenosine A3 Receptors

The presence of the A3 adenosine receptor has been demonstrated in the rat (Zhou et al., 1992), gerbil (Ji et al., 1994), and human (Salvatore et al., 1993) brain. Although they are widely distributed throughout the brain, they are found at densities significantly lower than other adenosine receptor subtypes (Ji et al., 1994). Additionally, Jacobson et al. (1993) observed a differential distribution of adenosine A3 receptors in the mouse central nervous

system with the highest density occurring in the cerebellum and striatum and lower densities occurring in the hippocampus and cortex. Adenosine A3 receptors have been shown to reside on microglia (Fiebech et al., 1996), on vascular smooth cells (Zhao et al., 1997) and on neurons (Dunwiddie et al., 1997).

Although the biological significance of the A3 receptor is ill-defined, it is known that activation of this receptor inhibits adenylyl cyclase (Zhou et al., 1992). Jacobson et al. (1993) first indicated a central effect of adenosine A3 receptor activation when IB-MECA, an adenosine A3 receptor agonist injected intraperitoneally, induced behaviorally depressant effects which were not reversed by A1 or A2 selective antagonists. Activation of adenosine A3 receptors produces a variety of results. Physiological responses to the activation of this receptor include induction of heart rate-dependent hypotension in rats (Fozard and Carruthers, 1993). As well, A3 receptor activation with IB-MECA produces a potentiation of Ca^{2+} current in a dissociated hippocampal pyramidal neuron preparation (Flemming and Mogul, 1997) and a dose-dependent increase in leukocyte adherence (Park et al., 1997). Moreover, in a rat hippocampal slice preparation, activation of A3 receptors increases electrical excitability (Flemming et al., 1997).

There is evidence which suggests that acute activation of adenosine A3 receptors can promote, while chronic activation, can attenuate neuroprotection during ischemic insults. Acute stimulation of adenosine A3 receptors with IB-MECA resulted in impaired postischemic cerebral blood flow, enhanced mortality and extensive hippocampal neuronal

damage in a gerbil model of global ischemia (von Lubitz et al., 1994b). However, in the same investigation, chronic administration with IB-MECA produced the opposite results in that postischemia cerebral blood flow, survival and neuronal recovery improved.

1.5.5 Therapeutically Harmful Effects of Adenosine.

Despite experimental evidence supporting a role for adenosine receptor-mediated neuroprotection, there are a number of factors which have limited the use of an adenosine A1 receptor agonists in ischemic or hypoxic brain injury. Activation of adenosine A1 receptors not only produces reduced damage as a result of cerebral ischemia but when A1 receptors on the heart are activated, it also produces bradycardia and hypotension (Sollevi, 1986; Williams, 1993). These two factors, of which the latter is the more serious, increase cerebral damage by decreasing the availability of glucose and oxygen to the brain thereby producing a condition which is similar to ischemia (Park et al., 1991; White et al., 1996). There is clear evidence indicating a relation between lowered blood pressure and an increased chance of a poor outcome after an ischemic insult (Zhu and Auer, 1995). Significant depression in arterial pressure is represented by a decrease in both the systolic and diastolic pressure which is affected by the dose and the rate of adenosine administration (Bulley and Whittnich, 1995). The decrease in arterial pressure appears to be mediated by reductions in cardiac output, resulting from very pronounced bradycardia (Cox et al., 1997).

1.5.6 Preconditioning as a Method of Neuroprotection

Although extended periods of cerebral ischemia or hypoxia result in neuronal damage, brief episodes of hypoxia or ischemia that produce little to no damage can provide both a neuroprotective effect and a cardioprotective effect against later more sustained periods of hypoxia or ischemia. This effect has been termed "preconditioning," and according to Stambaugh et al. (1997), in the heart this effect results from the activation of both adenosine A1 and A3 receptors. However, activating only adenosine A3 receptors in the heart during brief ischemia provided similar cardioprotection against injury during subsequent exposure to ischemia (Liang and Jacobson, 1998).

The preconditioning effect is not only apparent in the heart but also apparent in the brain (Pérez-Pinzón et al., 1997, Blondeau et al., 2000; Reshef et al., 2000). For example, preconditioning with sublethal (3-minute) ischemia protected hippocampal neurons against subsequent lethal (6-minute) cerebral ischemia (Heurteaux et al., 1995). Heurteaux et al. (1995) suggested that the mechanism of cerebral ischemic preconditioning involves the liberation of adenosine, stimulation of adenosine A1 receptors, and via these receptors opening of sulfonylurea-sensitive K^+-channels. In addition, there is support for adenosine-mediated cerebral ischemic preconditioning (Blondeau et al., 2000; Xu et al., 2002). Administration of R-PIA (Evans et al., 1987) or CHA (von Lubitz et al., 1989) prior to an ischemia insult protected neurons suggesting that adenosine plays a role in cerebral ischemic preconditioning. Furthermore, Campbell (1999) showed that activation of adenosine A1 receptors with CPA prior to a hypoxic-ischemia insult simulates ischemic

preconditioning. Inhibition of adenosine kinase has also been shown to improve ischemic preconditioning in the brain (Miller et al., 1996; Jiang et al., 1997).

Preconditioning has also been demonstrated against kainic acid-induced excitotoxicity (Plamondon et al., 1999). This finding supports the existence of bi-directional cross-tolerance between kainic acid excitotoxicity and global ischemia. Along with ischemic preconditioning and kainic acid induced preconditioning, chemical inhibition of oxidative phosphorylation or "chemical preconditioning" also provides neuroprotection. When hippocampal slices were pre-treated with 3-nitroproprionate, it induced long-lasting tolerance against hypoxia (Riepe et al., 1997). Kato et al. (1991) suggested that the considerable delay (6-24 hours) from the preconditioning stimulus until the onset of ischemic tolerance and the long duration (up to seven days) is consistent with a role for transcriptional changes in adaptation.

1.6 Hypothesis and Objectives:

A present lack of therapies available to high risk stroke patients combined with positive and negative results of adenosine based neuroprotective research and with the role of adenosine in preconditioning has led to the development of the following hypothesis: A single administration of N^6-cyclopentyladenosine (CPA), administered prior a hypoxic-ischemic insult, will precondition neurons in the rat hippocampus thereby providing neuroprotection during a subsequent hypoxic-ischemic insult.

In order to examine this hypothesis, four experimental objectives were developed as

follows:

1. To evaluate the effect of the Levine model of brain hypoxia-ischemia on subsequent damage to the rat hippocampus.

2. To establish a dose-response relationship and ED_{50} for CPA-induced neuroprotection *in vivo*.

3. To investigate the time-course of the neuroprotective response to CPA.

4. To examine and evaluate the hemodynamic effects immediately after and 24 hours after an administration of various doses of CPA.

2.0 MATERIALS AND METHODS

2.1 Animals

Male Wistar rats were obtained from Charles River Limited, St-Constant, Québec. Animals weighed 175-200 g on arrival and weighed 220-250 grams at time of dispatch. All animals were housed in groups of four animals per cage in a holding room in the North Barn at the Atlantic Veterinary College (AVC), Charlottetown, PE. The room was maintained on a 12 hour light / 12 hour dark cycle at a constant temperature of 22 ± 1°C. Standard rat chow and water were available to animals *ad libitum*. AVC Animal Resource staff performed daily monitoring and bi-weekly cage changes. Animal housing and treatment were conducted under protocols #99-033 and #00-027 of the University of Prince Edward Island (UPEI) Animal Care Committee, and in accordance with the guidelines set forth by the Canadian Counsil on Animal Care (CCAC). All animal experimentation was performed in the Duffy building of UPEI. Transportation of animals was arranged through and carried out by UPEI maintenance.

2.2 Drugs and Chemicals

N^6-cyclopentyladenosine (CPA) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) were acquired from Sigma-Aldrich Canada Limited, Oakville, Ontario. Somnotol® (sodium pentobarbital) was obtained by Dr. M. I. Sweeney-Nixon (Licenced by Health Canada, File # 9639-S-224) from Bimeda-MTC Animal Health, Cambridge, Ontario. Halothane (by prescription), betadine surgical scrub (phosphate free) and xylocaine 2% jelly were obtained from the Veterinary Teaching Hospital (VTH) at AVC, Charlottetown, PE.

Methanol and ethanol were obtained from Caledon Laboratories Limited, Georgetown, Ontario; formaldehyde, Anachemia Canada Incorporated, Montréal, Québec; glacial acetic acid, sodium chloride, potassium dihydrogen phosphate, Fisher Scientific, Nepean, Ontario; di-sodium hydrogen orthophosphate and potassium chloride, British Drug Houses, Dartmouth, Nova Scotia. Medical grade oxygen and 8 % oxygen were supplied by Praxair, Charlottetown, Prince Edward Island. All chemical solutions were prepared with distilled water or double distilled water with the exception of CPA and DPCPX which were dissolved in phosphate buffered saline.

2.3 Administration of CPA and DPCPX

The Levine model of ischemia-hypoxia was used in this investigation. To employ this model each animal was subjected to a five step procedure: 1) administration of CPA and/or DPCPX, 2) occlusion of the left common carotid artery, 3) exposure to an oxygen reduced medium, 4) cardiac puncture, perfusion and removal of brain, and 5) preparation of brain samples.

Treatment groups requiring administration of CPA and /or DPCPX received drugs via intra-peritoneal (i.p.) injection. The vehicle for both CPA and DPCPX was 0.9 % phosphate buffered saline (PBS, pH 7.4, containing 137 mM NaCl, 7.75 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl). The administration of DPCPX preceded administration of either CPA or vehicle by one hour. To administer the injection, animals were restrained by hand and a 1 ml, single use, syringe (Becton Dickenson, Rutherford, New Jersey) was

used to deliver the drug or drug vehicle to the right peritoneal cavity of the animal. The approximate volume of each injection was 1 ml / kg body weight. Animals were monitored after the injection to evaluate any behavioural changes.

2.4 Occlusion of Left Common Carotid Artery

Animals were initially anesthetized with halothane in a closed bell jar. They were subsequently maintained with halothane (2-3 % in O_2 at a flow rate of 2L / min) via anesthetic machine (Ohmeda, Helsinki, Finland) and halothane vaporizer (Benson Medical Industries Limited, Markham, Ontario). To maintain normal temperature, animals were placed on a heating pad (Fine Scientific Tools, Vancouver, British Columbia) set at 37.8 °C for the duration of the surgery. Before initiating the surgical procedure, the level of consciousness of the rat was assessed to verify that it had reached an adequate level of anesthesia for surgery. Both the ocular response and foot withdrawal techniques were used to determine the appropriate level of consciousness. Once adequately anesthetized, the neck of the rat was shaved with an electric razor (Jeri Redding Company, Brampton, Ontario) and sterilized with 70% ethanol and Betadine surgical scrub. A sagittal incision approximately 2 cm was made on the ventral aspect of the neck. The left common carotid artery was isolated from the surrounding tissue using 0.1 mm forceps (Fine Scientific Tools, Vancouver Canada, model 11251) and ligated with sterile surgical silk thread (2-0). The artery was returned to its original position and the incision was closed with an autoclip and 9 mm staples (Stoelting, Wood Dale, Illinois USA, model 205000) followed by a liberal application of xylocaine 2 % jelly to the exterior of the incision. Animals were

permitted to regain consciousness on the heating pad and then returned to their cage which was placed under a heating lamp (60 W) for 10 minutes.

2.5 Exposure to Oxygen Reduced (Hypoxic) Medium

Twenty-four hours following the ligation of the left common carotid artery, animals were exposed individually to an oxygen reduced (hypoxic) gas. Animals were placed in an air tight gassing box into which 8 % oxygen in balanced nitrogen (92 % N_2) was pumped for one hour at a flow rate of 2 L / minute. The gas was allowed to run for two minutes before starting to time for an hour. After gassing was complete, the animal was placed back in its cage and monitored for any behavioural changes. The sham procedure for this technique involved having the lid of the gassing box remain open for the duration of an hour thus keeping the animal exposed to ambient air.

2.6 Cardiac Puncture, Perfusion and Removal of Brain Sample

Seven days following exposure to the hypoxic gas, animals were anesthetized individually with Somnotol® (65 mg / kg; i.p.) using a 1 ml, single use, syringe (Becton Dickenson, Rutherford, New Jersey). Both the ocular reflex and the foot withdrawal techniques were used to assess the level of anesthesia. Once a surgical plane of deep anesthesia had been reached, the thoracic cavity was cut open thus exposing both the cardiac and pleural cavities. An 18 gauge, 3.75 cm needle was inserted and clamped with a hemostat into the left ventricle of the heart. A small incision was made on the lateral musculature of the right ventricle and the animal was subsequently transcardially perfused with approximately 250

ml of cold PBS (4 °C) at a rate of 10 ml / minute. Next, animals were perfused with approximately 100 ml of fixative (methanol, glacial acetic acid, formaldehyde; 8:1:1, FAM) at a rate of 10 ml / minute. After FAM perfusion, the animal was decapitated with sharp scissors. The brain was subsequently excised from the skull and placed into a vial containing FAM fixative. Vials were stored in a fridge at 4°C.

2.7 Preparation and Analysis of Brain Samples

A rodent brain matrix (Harvard Apparatus, Warren, Michigan) and single edged razor blades were used to obtain a 2 mm coronal section of the brain. Each 2 mm sample was taken 1 mm posterior to the optic chiasm. Each sample was marked by removing a small triangular wedge from the right hemisphere of the cerebral cortex. Subsequent sectioning and staining were performed at the Histopathology lab of Diagnostic Services, Department of Pathology and Microbiology, AVC. Hydration of the sample was performed in a Sakura Tissue-Tek VIP tissue processor (Sakura Finetek, Torrence, California). The prepared sample was embedded in paraffin using a Shandon embedding station (Shandon Manufacturing, Pittsburgh, Pennsylvania). Three 5 μm sections were made (250 μm - 350 μm apart) from the sample block using a Microm HH 335 E digital microtome (Microm, Walidorf, Germany). Serial sections were then stained with hematoxylin and eosin in a Sakura Tissue-Tek DRS staining chamber at AVC hitopathology.

Viable cells (those which appeared round with a clearly stained nucleus) in the CA1, CA2 and CA3 region of both the left and right side of the hippocampus were manually counted

under light microscopy (400 X) under a blind condition. Non-viable cells (those seen as shrunken mass displaying no clearly defined cell membrane or stained nucleus) were not counted. Viable cell counts of each region were added together to give a total viable cell count for the left and right hemispheres of the hippocampus. The difference between the total viable cell counts of the right and left hemispheres represented the amount of cellular damage due to hypoxia-ischemia. Mathematical representation of this calculated cell death as a percent is :

$$\frac{\text{Viable cells (right)} - \text{Viable cells (left)}}{\text{Viable cells (right)}} \times 100 = \text{percent cell damage}$$

The CA1, CA2 and CA3 regions of Ammon's horn can be separated based on the particular size and arrangement of cells found within each region. The CA1 region of the hippocampus begins ventral to the dorsal hippocampal commissure, lateral to the prosubiculum, and extends laterally to the midpoint of the most lateral extension. The pyramidal cell arrangement in the CA1 region consists of 3-5 layers of tightly packed cells. There is a clear destinction between the CA1 and CA2 regions. The CA2 region begins at the midpoint of the most lateral extension and extends to the first lateral bend. It is characterized by large pyramidal cells which are loosely packed. The CA3 region extends to and terminates at the hilus of the dentate gyrus. The CA2 and CA3 regions cannot be distinguished by their appearence. However, based on a stereotaxic atlas measurment, the CA2 and CA3 regions can be separated according to the directional pattern of cells.

2.8 Experimental Design

2.8.1 Dose-Response Relationship for CPA-Induced Neuroprotection

Animals were assigned to one of thirteen treatment groups (n = 6) (Table 2) and followed the procedure described in sections 2.3 - 2.7.

2.8.2 Time-Course of Neuroprotective Response to CPA

Sixty six animals were randomly assigned to one of eleven treatments (n = 6). Each treatment group followed the procedures outlined for the administration of drugs, surgery conditions and brain analysis. Animals in each treatment group received the same dose of CPA (0.1 mg/kg) with or without DPCPX (1.0 mg/kg). However, CPA was injected either 1, 3, 12, 24, or 48 hours prior to a hypoxia-ischemia insult. As in the previous experiment, DPCPX was administered one hour before CPA.

2.8.3 Hemodynamic Effects of CPA

Animals were randomly assigned to one of five treatment groups, each receiving one treatment dose of CPA (0.01; 0.1; 1.0; 3.0 or 10.0 mg/kg of body weight).

Phase 1: Chronic Femoral Cannulation.

Animals were initially anesthetized with halothane in a closed bell jar and maintained at 2-3% halothane while on a heating pad (37.8 °C). An incision (# 10 blade) was made along the upper medial aspect of the lower right limb. The incision extended

Table 2. The dose of CPA and DPCPX for each dose-response treatment group and control groups (n = 6).

	Dose (mg/kg)	
Treatments	**CPA**	**DPCPX**
Sham Surgery *	----	----
CPA Control	0.0	0.0
DPCPX Control	0.0	1.0
CPA Groups (dose-response)	0.01	0.0
	0.1	0.0
	1.0	0.0
	3.0	0.0
	10.0	0.0
CPA + DPCPX Groups	0.01	1.0
	0.1	1.0
	1.0	1.0
	3.0	1.0
	10.0	1.0

* Animals in the Sham Surgery group experienced surgery (halothane induced anesthesia, neck incision and closing of the incision with staples) without ligation of the left common carotid artery and followed by exposure to ambient air rather than exposure to reduced oxygen.

from the distal end of the femur to the sagittal aspect of the abdomen. The right femoral artery was isolated from the surrounding tissue using 0.1 mm forceps (Fine Scientific Tools) and subsequently cannulated with polyethylene tubing (PE 50; Intramedic, Becton Dickenson, Rutherford, New Jersey; model 427411). Care was taken to avoid damaging the sciatic nerve which runs parallel to the femoral artery. Tubing was filled with heparinized PBS (125 units in 10 ml of PBS) (Hepelean®, Organon Teknika, Toronto, Ontario), and secured within the artery using 2-0 silk thread. A hemostat was used to maneuver the free end of the cannula under the dermis to the nape of the neck where the cannula was exposed through the skin and sutured in place with sterile Maxon® 3-0 surgical thread (Sherwood Davis and Geck, St. Louis, Missouri). Tubing was plugged with a 23 gauge needle that was capped with hematocrit tube plaster and left exposed. Both the neck incision and the femoral incision were treated with 2% xylocaine jelly. Animals were allowed to regain consciousness on the heating pad and subsequently placed back into individual cages. Each day for one week, the cannula was flushed with a 23 gauge needle filled with heparinized PBS.

Phase 2: Blood Pressure Monitoring

One week following femoral cannulation, the exposed cannula was connected to a blood pressure monitoring system (Figure 10). This system included a blood pressure transducer (Harvard Apparatus, Saint Laurent, Quebec Canada, model 60-3002) and a universal oscillograph (Harvard Apparatus, Saint Laurent, Quebec Canada, model 50-

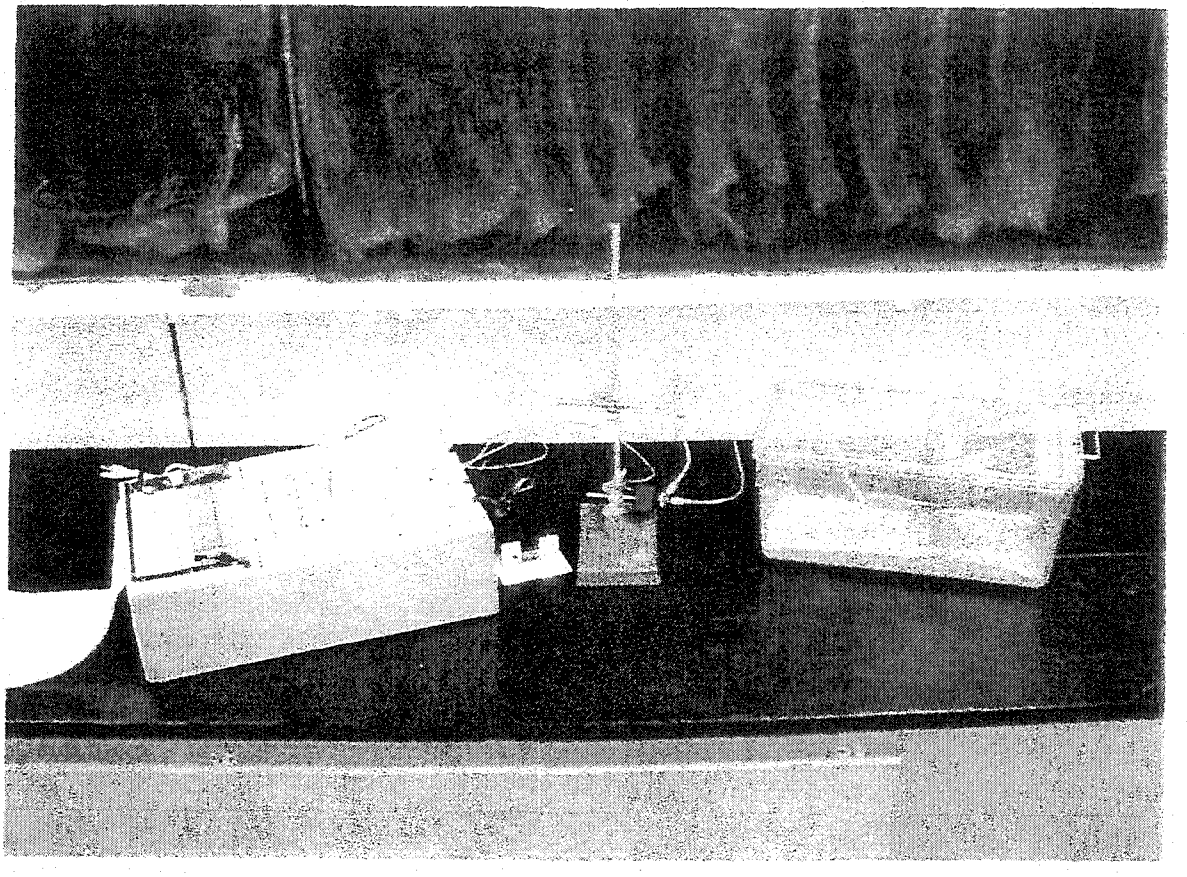

Figure 10. Photo of blood pressure monitoring system used in this investigation.

9315). Animals maintained consciousness in a cage throughout recordings. Blood pressure was monitored for 15 minutes to establish baseline pressure. Animals used to evaluate the immediate and long-term effect of CPA on arterial blood pressure received the injection 15 minutes into recording and 22 hours prior to recording, respectively. For both groups, blood pressure was monitored for 120 minutes or until baseline pressure was recovered. Animals were subsequently euthanized by CO_2 overdose.

2.8.4. Statistical Analysis

All data was tested and showed normal distribution. A Student unpaired 2-tailed t-test was used to compare surgery group to sham surgery group. One-way and 2-way ANOVA with post-hoc Tukey tests were used to examine trends between and within regions of the hippocampus. They were also used to examine differences within and between treatment regimes. A repeated measure function was added to the 2-way ANOVA analysis to examine changes in mean arterial blood pressure over time. For each test, $p < 0.05$, is considered statistically significant.

3.0 RESULTS

3.1 Effect of Hypoxia-Ischemia Model on the Hippocampus

The first objective of this thesis was to evaluate the effect of the Levine model of brain hypoxia-ischemia on subsequent damage to the rat hippocampus, as compared to damage in rats not subjected to hypoxia-ischemia (sham group). An estimate of hippocampal damage was achieved by comparing the number of viable cells in the hippocampus of the right (non-ligated) hemisphere to that in the left (surgically ligated) hemisphere.

In rats undergoing sham surgery, there were 801.1 ± 10.9 total viable cells counted in the combined CA1, CA2 and CA3 regions of the hippocampus in the right hemisphere, when 5 μm sections were counted (Table 3). There were differences in the number of viable cells in each region of the hippocampus ($F = 1885.25$; $p < 0.001$) (Table 3). The CA1 region had the greatest number of cells, with the CA3 region having half as many cells and the CA2 region having the smallest number of cells (Table 3). An identical pattern was seen in the brains of rats that were exposed to hypoxia-ischemia, with the CA1 containing twice as many neurons as the CA3 region, which was larger than the CA2 region, when the right hemisphere was evaluated ($F = 467.58$; $p < 0.001$) (Table 3). Thus, rats in the hypoxia-ischemia group had similar total viable cell counts in the right hemisphere as sham rats ($F = 0.47$; $p > 0.05$).

A sham ligation of the left common carotid artery did not produce damage to the left hippocampus, as confirmed by similar numbers of viable cells in the right and left

Table 3. Mean viable cell counts ± SE for the right hemisphere of the rat hippocampus for sham surgery animals and surgery animals (n = 8).

	CA1	CA2	CA3	Total
Number of Viable Cells in Right Hemisphere of Sham Surgery Animals	440.5 [a] ± 18.1	160.0 [b] ± 6.8	200.1 [c] ± 9.1	801.1 ± 10.9
Number of Viable Cells in Right Hemisphere of Surgery Animals	467.7 [a] ± 36.0	150.0 [b] ± 10.7	188.8 [c] ± 17.0	806.5 ± 34.1

a, b, c, $p < 0.001$; letters in a row not sharing a common superscript are statistically different.

hemispheres ($F = 0.47$; $p > 0.05$) (Table 4). However, in animals that were surgically ligated and exposed to hypoxia, total cell counts indicated significant damage in the left hippocampus relative to the right hemisphere ($F = 96.23$; $p < 0.0001$) (Table 5). Damage in the left hemisphere of animals whose left common carotid artery was ligated was different in the CA1, CA2 and CA3 regions of the hippocampus ($F = 8.79$; $p < 0.001$). A comparison of viable cell counts between the right and left hemispheres for the CA1, CA2, and CA3 regions of the hippocampus revealed that the CA3 region in the left hemisphere experienced the greatest decrease in viable cells (35.1 %), while the CA1 and CA2 regions of the left hippocampus experienced a decrease in viable cells of 27.4 % and 30.2 %, respectively.

The total number of damaged cells in the left hemisphere for sham surgery animals and ligated animals is presented in Figure 11. Animals whose common carotid artery was ligated had a greater number of damaged neurons in the left hemisphere of the hippocampus than animals in the sham surgery group ($p < 0001$). Difference in the left hemisphere of the hippocampus in sham surgery animals represents a 1.2 % decrease in the number of viable cells, whereas damage in the entire left hemisphere of the hippocampus in animals whose common carotid artery was ligated represents a 29.7 % decrease in the number of viable cells.

3.2 Effect of Different Doses of CPA on Cell Death in the Hippocampus

The second objective of this thesis was to evaluate the dose-response relationship of a

Table 4. Comparison of viable cell counts in left versus right hemispheres of the rat hippocampus after sham surgery. Values are mean viable cell counts ± SE (n = 8) except for last row where differences are expressed as percent.

	CA1	CA2	CA3	Total
Number of Viable Cells in Right Hemisphere of Hippocampus	440.5 [a] ± 18.1	160.0 [b] ± 6.8	200.1 [c] ± 9.1	800.6 ± 10.9
Number of Viable Cells in Left Hemisphere of Hippocampus	438.5 [a] ± 18.1	153.2 [b] ± 8.2	199.7 [c] ± 5.9	791.4 ± 10.8
Difference between Right and Left Viable Cell Counts	2.0 ± 1.2	6.8 ± 3.9	0.4 ± 3.8	9.2 ± 3.0
Percent Difference Between Right and Left Viable Cell Counts	0.5	4.3	0.2	1.2

a, b, c, $p < 0.001$; letters in a row not sharing a common superscript are statistically different.

Table 5. Comparison of viable cell counts in left versus right hemispheres of the rat hippocampus after Levine model surgery. Values are mean viable cell counts ± SE (n = 8) except for last row where differences are expressed as percent.

	CA1	CA2	CA3	Total
Number of Viable Cells in Right Hemisphere of Hippocampus	467.7 [a] ± 36.0	150.0 [b] ± 10.7	188.8 [c] ± 17.0	806.5 ± 34.1
Number of Viable Cells in Left Hemisphere of Hippocampus	339.5 * [a] ± 35.8	104.7 * [b] ± 16.0	122.6 * [c] ± 18.7	566.8 * ± 28.3
Difference Between Right and Left Viable Cell Counts	128.2 ± 8.8	45.3 ± 3.9	66.2 ± 6.9	239.7 ± 10.3
Percent Difference Between Right and Left Viable Cell Counts	27.4	30.2	35.1	29.7

* $p < 0.0001$; post hoc as compared to right hemisphere of hippocampus.
a, b, c, $p < 0.001$; letters in a row not sharing a common superscript are statistically different.

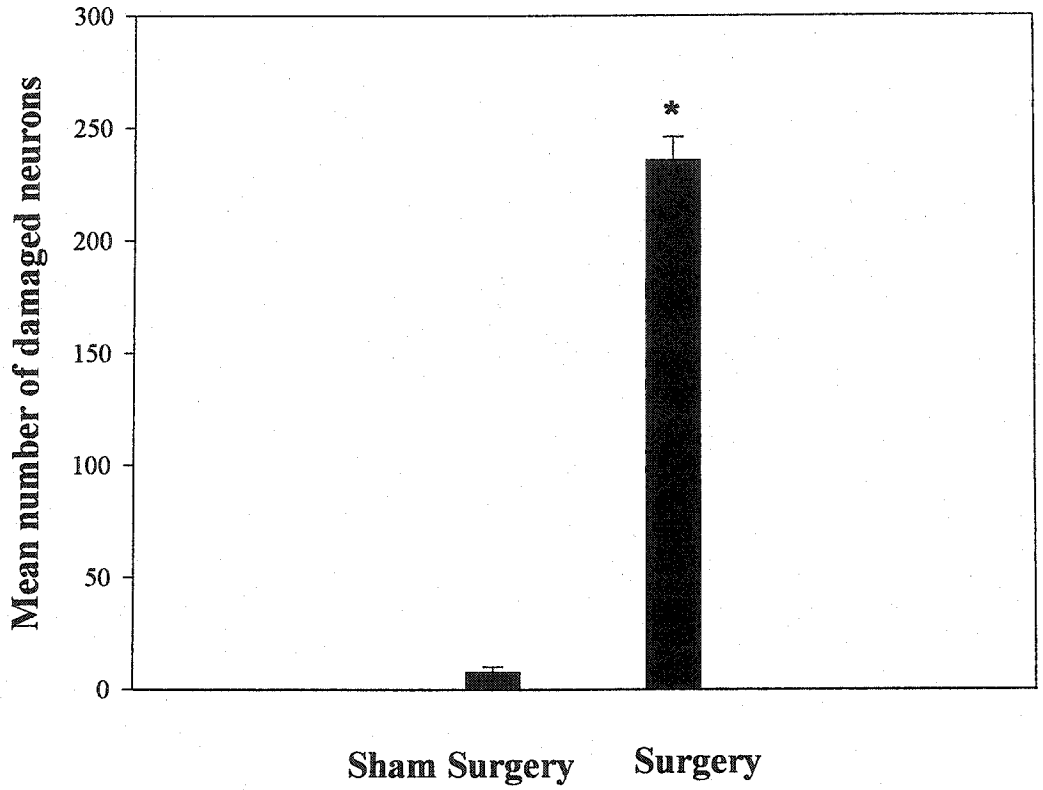

Figure 11 . Effect of surgery on the mean number of damaged cells ± SE (cell death; difference between left and right hemisphere) for sham surgery group (n=8) and surgery group (n=8). (* $p < 0.0001$; t-test as compared to sham surgery group).

single administration (i.p) of CPA at varying doses (0.01, 0.1, 1.0, 3.0, and 10.0 mg/kg), 24 hours prior to a hypoxia-ischemia insult, and in the presence or absence of DPCPX (1.0 mg/kg), on cell loss in the left hemisphere of the rat hippocampus. An estimate of hippocampal damage was achieved by comparing the number of viable cells in the hippocampus of the right (non-ligated) hemisphere to that in the left (surgically ligated) hemisphere. A CPA-mediated neuroprotective effect in this study is defined as a decrease in the number of damaged cells in the left hippocampus.

In the absence of CPA, animals that underwent a hypoxic-ischemic insult had damage in the left hemisphere of the hippocampus (Figure 12). This damage represents a 29.7 % loss of viable cells in the left hemisphere compared to the right hemisphere. However, a single administration of varying doses of CPA 24 hours prior to a hypoxic-ischemic insult was able to reduce the amount of damage seen in the left hemisphere ($F = 563.82$; $p < 0.0001$) (Figure 12). When compared to the number of damaged cells in the left hemisphere of animals receiving no CPA (0.0 mg/kg) and no DPCPX (0.0 mg/kg), the administration of CPA at doses of 0.01, 0.1, 1.0, 3.0, and 10.0 mg/kg, produced a reduction in the number of damaged cells in the left hemisphere of 27.5 %, 53.9 %, 69.6 %, 75,9 %, and 95.6 %, respectively (Figure 12). This dose-dependent reduction in the number of damaged cells by CPA generated an estimated dose of CPA which provided 50 % of the maximum amount of decrease in damaged cells (ED_{50}) of 0.0925 mg/kg of CPA (Figure 12).

When DPCPX was administered to animals not receiving CPA there was a similar amount

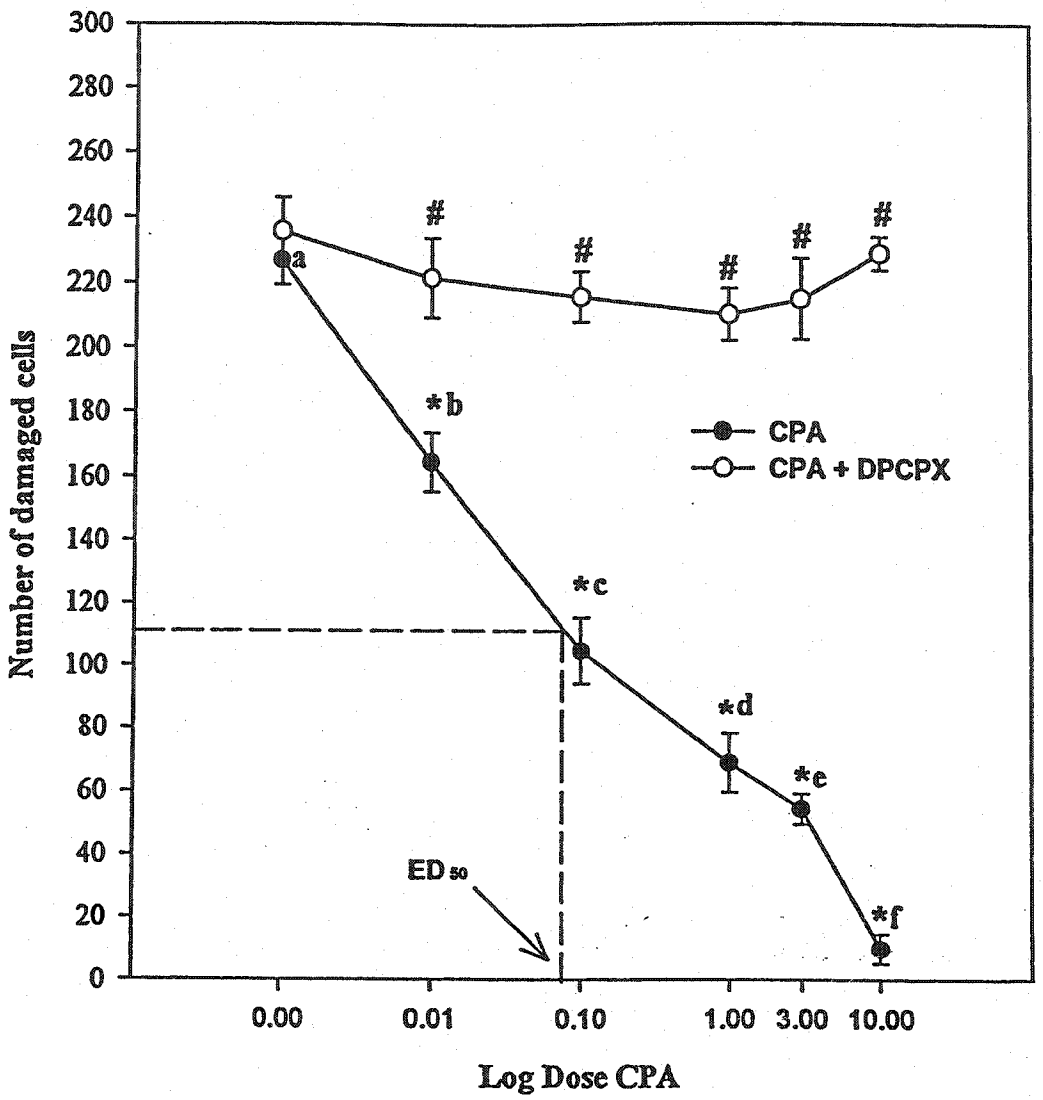

Figure 12. The effect of different doses of CPA in the absence and presence of DPCPX on the number of damaged cells in the hippocampus. Effect presented as the mean number of damaged cells ± SE (difference between left and right hemisphere) (n = 6). (* $p < 0.0001$; Post hoc analysis as compared to CPA 0.0 mg/kg; # $p < 0.0001$; Post hoc analysis as compared to corresponding CPA dose; a, b, c, d, e, f $p < 0.05$; data points not sharing a common letter superscript are statistically different).

of damage in the left hemisphere of the hippocampus compared to control animals (CPA 0.0 mg/kg and DPCPX 0.0 mg/kg) ($p > 0.05$) (Figure 12). Thus, DPCPX (1.0 mg/kg) did not significantly exacerbate damage in the left hippocampus of animals that were exposed to a hypoxic-ischemic insult. In addition, the administration of DPCPX (1.0 mg/kg) one hour prior to a single administration of CPA at doses of 0.01, 0.1, 1.0, 3.0, and 10.0 mg/kg blocked the CPA-mediated reduction of damaged cells in the left hippocampus ($p < 0.001$) (Figure 12). Furthermore, the amount of cell death in the left hemisphere of the hippocampus was similar for each group of animals receiving DPCPX, regardless of CPA dose ($F = 519.81$; $p > 0.05$). (ie. there was no parallel shift in the dose-response curve for CPA)

3.3 Effect of Different Administration Times of CPA on Hippocampal Cell Death

The third objective of this thesis was to investigate the neuroprotective response in the hippocampus of rats to various times of CPA administration (1, 3, 12, 24, 48 hours) prior to a hypoxic-ischemic insult. An estimate of hippocampal damage was achieved by comparing the number of viable cells in the hippocampus of the right (non-ligated) hemisphere to that in the left (surgically ligated) hemisphere.

CPA (0.1 mg/kg) was administered at varying times (1, 3, 12, 24, 48 hours) prior to a hypoxia-ischemia insult and a time-response curve was generated (Figure 13). In animals that received 0.0 mg/kg CPA (control), there were 226.4 ± 18.7 damaged cells in the left hemisphere of the hippocampus. However, a single administration of CPA at each time

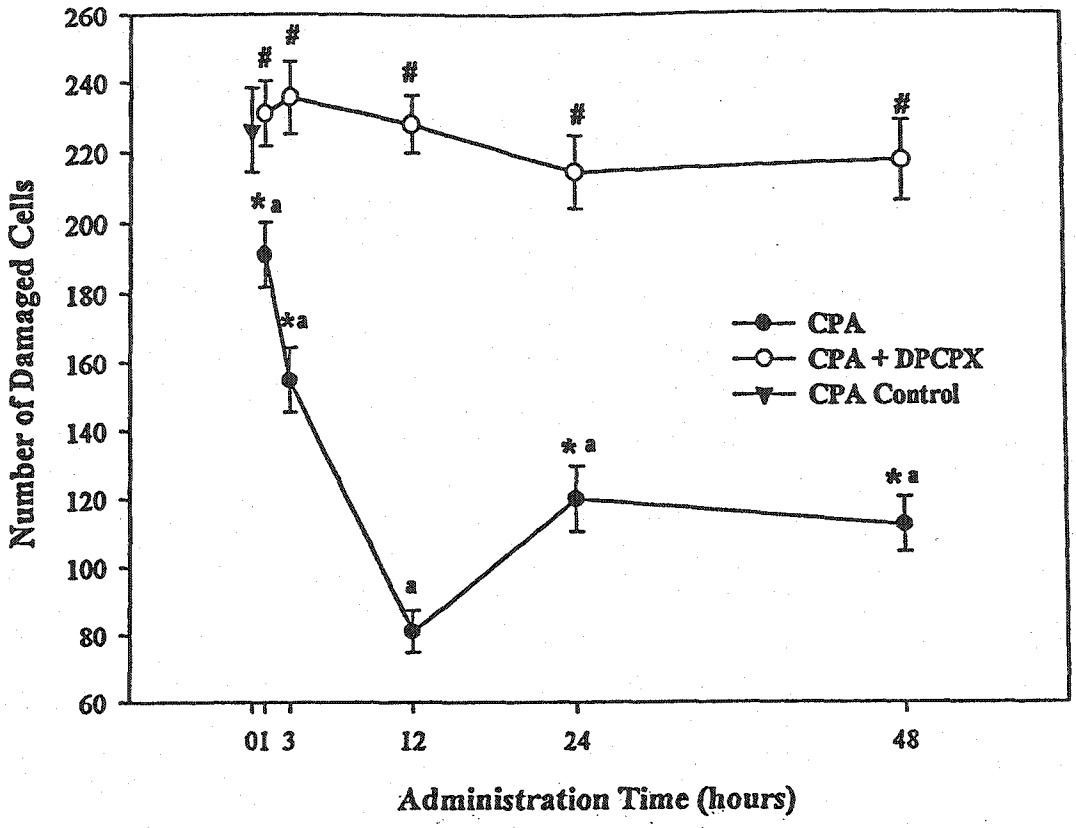

Figure 13. The effect of CPA (0.1 mg/kg) administered at varying time points prior to a hypoxia-ischemia insult, in the absence and presence of DPCPX (1.0 mg/kg) on the number of damaged cells in the left hippocampus relative to right (control) hippocampus. Values are the mean number of damaged cells ± SE (n = 6). (# $p < 0.0001$; Post hoc analysis as compared to corresponding CPA group; * $p < 0.001$; Post analysis as compared to CPA 12 hour; a $p < 0.001$; Post hoc analysis as compared to CPA control).

point prior to the hypoxia-ischemia insult reduced the number of damaged cells counted in the left hemisphere of the hippocampus.($p < 0.001$) (Figure 13). Thus, a single administration of CPA, up to 48 hours prior to a hypoxia-ischemia insult, is able to protect neurons in the hippocampus from hypoxia-ischemia-induced damage. CPA-mediated reduction of the number of damaged cells in the left hemisphere was different among time points. When the number of damaged cells in the left hemisphere of animals receiving CPA at different times prior to a hypoxia-ischemia insult were compared to the number of damaged cells counted in CPA control animals, the administration of CPA at 12 hours prior to insult produced the greatest reduction in the number of damaged cells (64.4 %). Using the same comparison, the administration of CPA at 1, 3, 24, and 48 hours prior to insult produced a reduction in the number of damaged cells of 17.3 %, 34.3 %, 43.7 %, and 48.3 %, respectively, that were significantly less than to the reduction seen at the 12 hour time point ($p < 0.001$) (Figure 13).

The administration of DPCPX (1.0 mg/kg) one hour before an administration of CPA at 1, 3, 12, 24, and 48 hours prior to insult produced no change in the numbers of damaged cells in the left hemisphere of the rat hippocampus relative to control ($p > 0.05$) or to any other point ($F = 2.56$; $p > 0.05$) (Figure 13). But, the number of damaged cells in the left hemisphere that is reported for animals receiving DPCPX is not similar to the number of damaged cells in the left hemisphere reported for animals receiving only CPA ($p < 0.0001$) (Figure 13). Thus, administration of DPCPX (1.0 mg/kg) one hour before an administration of CPA (0.1 mg/kg) blocked CPA-mediated reduction in the number of

damaged cells in the left hemisphere of the hippocampus.

3.4 Immediate Effect of CPA on Arterial Blood Pressure in the Conscious Rat.

A fourth objective of this thesis was to examine the hemodynamic effects of CPA on arterial pressure. Therefore, conscious rats were given a single administration (i.p.) of various doses of CPA (0.01, 0.1, 1.0, 3.0, and 10.0 mg/kg) or vehicle (saline) and the immediate effect on mean arterial pressure (MAP) was recorded for two hours (Figure 14a).

When CPA was administered, it reduced MAP in a conscious rat ($F = 26.89$; $p < 0.0001$) (Figure 14a). This effect is dose-dependent because the CPA-mediated decrease in MAP was greatest for 10.0 mg/kg CPA and became less pronounced with decreasing doses of CPA. In support of a dose related effect for CPA-mediated decrease in MAP, area under the curve showed that there was an increase in the amplitude of MAP reduction over the recorded time as the dose of CPA increased (Figure 14b). CPA (10.0 mg/kg) reduced MAP in the conscious rat by 61.5 % compared to baseline MAP pressure in rats that received vehicle ($p < 0.0001$), whereas CPA (0.01 mg/kg) reduced MAP by 32.3 % compared to baseline MAP pressure in rats that received vehicle ($p < 0.0001$) (Figure 14a). Other doses of CPA (0.1, 1.0, and 3.0 mg/kg) decreased MAP in a conscious rat by 51.6 %, 56.2 % and 61.1 %, respectively (Figure 14a).

When the CPA-mediated decrease in MAP was analysed over time, the maximum decrease

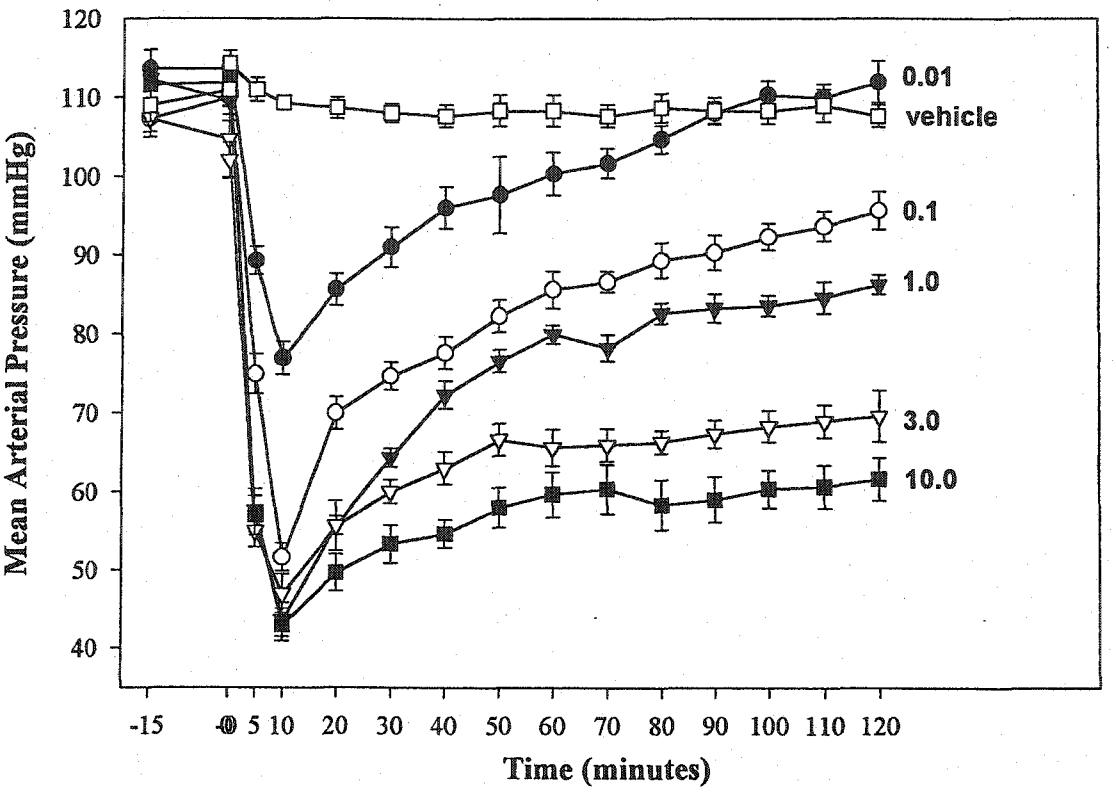

Figure 14a. Mean arterial pressure ± SE in the rat before and after administration of vehicle (saline) and CPA (0.01, 0.1, 1.0, 3.0, 10.0 mg/kg) (n = 3).

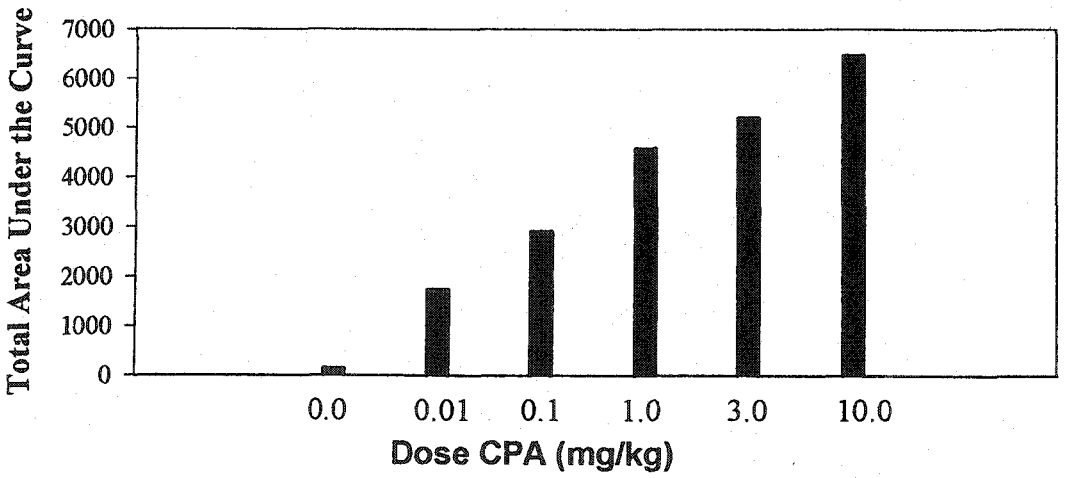

Figure 14b. The effect of CPA (0.01, 0.1, 1.0, 3.0, 10.0 mg/kg) mediated decrease in mean arterial pressure presented as total area under the curve (n = 3).

in MAP for each dose of CPA occurred 10 minutes after administration (Figure 14a). As well, only those animals receiving CPA (0.01 mg/kg) were able to re-establish baseline MAP within the test time. MAP in animals receiving CPA (0.01 mg/kg) remained significantly different to baseline until 100 minutes after administration (Figure 14a).

3.5 Long-Term Effect of CPA on Arterial Pressure in the Conscious Rat

The final objective of this thesis was to examine the hemodynamic effect that CPA may produce in conscious rats 22 hours after administration. Therefore, MAP was recorded for 120 minutes in animals that received a single i.p injection of CPA (10.0 mg/kg) or vehicle (saline) 22 hours prior to recording.

In animals that received CPA (10.0 mg/kg) 22 hours before recording MAP, the MAP 22 hours later was similar to the MAP of animals receiving vehicle 22 hours before recording ($F = 3.02$; $p > 0.05$) (Figure 15). However, during the recording time animals in the CPA (10.0 mg/kg) group and animals in the vehicle group experienced a decrease in MAP over time ($F = 6.25$; $p < 0.0001$). The highest recorded MAP for both groups occurred when recording commenced (Time = 0 minutes) with the first decrease in MAP occurring five minutes later and remaining lower than the MAP at Time = 0 minutes for the duration of the recording time (Figure 15). In addition, the decrease in MAP over time for both groups was similar at each time point ($p > 0.05$). Thus, the decrease in MAP over time in both groups may be the result of the single high MAP recording at the beginning of the recording session.

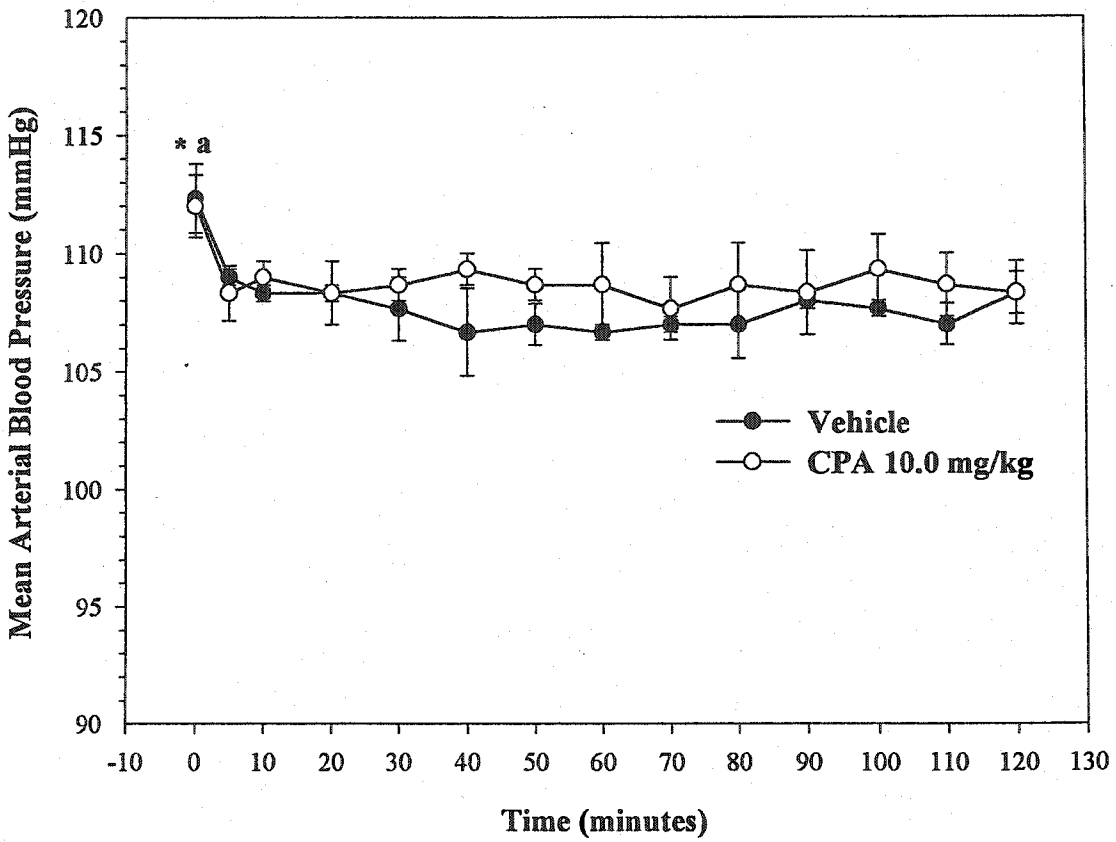

Figure 15. Mean arterial blood pressure ± SE in the rat (n=3) 22-24 hours after a single administration of vehicle (saline) or CPA 10.0 mg/kg.

4.0 DISCUSSION

The results presented in this thesis suggest that a single administration of CPA prior to a hypoxia-ischemia insult protects neurons in the hippocampus of male rats from damage resulting from a subsequent insult. The greatest amount of protection occurs when CPA is administered 12 hours prior to hypoxia-ischemia. In addition, an administration of CPA causes considerable hypotension in the rat but this effect is not evident 22 hours after administration.

4.1 Model of Hypoxia-Ischemia Cell Damage

The Levine model of global hypoxia-ischemia was used in this investigation. The Levine model in rats is characterized by a ligation to the left common carotid artery followed one day later by exposure to a hypoxic environment. Application of this model in this study produced neuronal damage (29.7 %) in the left hemisphere of animals whose left common carotid artery was ligated compared to sham animals (1.2 %). These results suggest that ligation of one common carotid artery and subsequent exposure to a hypoxic environment induces neuronal cell death in the hippocampus of male rats. Ligation of the common carotid artery reduces blood flow to the brain (Miyasaka et al., 2000) which results in a decrease in available glucose and oxygen in the brain (Ginsberg, 1990). Glucose and oxygen are used for the production of chemical energy such as ATP in neurons. Therefore, the lack of glucose and oxygen to the brain and the subsequent lack of ATP production in the brain during an ischemic insult creates a scenario whereby the neuron is not able to maintain normal homeostatic ion gradients. Inability to maintain normal

homeostatic processes can lead to cell swelling, an increase in intracellular Ca^{2+}, and an inability to regulate propagation of action potentials in the cell. The eventual result is cell death (for review see section1.3.1).

Cell damage in the hippocampus produced by the Levine model was not similar between the CA1, CA2 and CA3 regions suggesting that some regions are more vulnerable to hypoxia-ischemia induced damage. It is generally thought that the CA1 and CA3 regions of the hippocampus are the most vulnerable regions to this type of insult (Hsu and Buzsaki, 1993) because of the high concentration of glutamate receptors in these regions (Mori and Mashina, 1995). However, although the CA3 experienced the greatest amount of cell damage, the results suggest that the CA2 region may be just as vulnerable as the CA1 region. This disparity may be due to the difficulty in separating cells found in the CA2 and CA3 regions as they exhibit similar morphological characteristics (Frotscher, 1988). In this study the CA2 region was consistently defined from the termination of the CA1 region to the temporal arc of Ammon's Horn. It is also important to note that two methods were used in this study to attenuate any effect that a change in the regional representation of the hippocampus may have on subsequent cell counts. First, a rat brain matrix was used to consistently select sections of the hippocampus within 1 mm of the optic chiasm. Thus, sections of the hippocampus that were analysed in this study were harvested from a similar point in each rat brain. Second, serial sections of the hippocampus were analysed to acquire a mean viable cell count for each region and the entire hippocampus. The similar cell counts of the right (control) hemisphere in both sham

surgery animals and ligated animals confirm that these two methods have adequately addressed this concern. In addition, our decision to use the Levine model in this investigation was based on the fact that this model produces neuronal cell damage in the whole hippocampus and not on the premise that there exists selective vulnerability between regions of the hippocampus (Hsu and Buzsaki, 1993).

Opponents of the Levine model have suggested that a main caveat of the Levine model is that the brain is not truly ischemic after ligation so that cell death is the result of hypoxia and thus does not accurately reflect conditions of stroke. However, according to Ginsberg and Busto (1989), cell death produced by the Levine model is attributable to the combination of ischemia and the subsequent exposure to a hypoxic environment. There exists two pieces of evidence to support this conclusion. First, by itself hypoxia greater than 20 mmHg does not initiate neuronal cell death (Rie et al., 1980; MacMillan, 1989; Baldelli et al., 1993; Pearigen et al., 1996), but it does increase neuronal cell death in the presence of cerebral ischemia (Miyamoto and Auer, 2000). Second, permanent occlusion of one common carotid artery does produce a decrease in cerebral blood flow to the brain hemisphere that is ipsilateral to the occlusion (Miyasaka et al., 2000). In addition, in animals (Coyle and Penzenbeck, 1990), and humans (van Evendinger et al., 1998; Derdeyn et al., 1999; Hendrikse et al., 2001), with chronic unilateral carotid artery occlusion that was similar to our model, contralateral blood flow via the circle of Willis to the ischemic hemisphere does not adequately re-establish normal blood flow. Ironically, the circle of Willis which exists to enable blood supply between left and right brain hemispheres is

commonly incomplete in the general population and is much more frequent among patients who have had strokes (Barnett et al., 1998).

A general caution of the use of animal models is the tendency of researchers to directly link results from animal models to human conditions. It is important to explain that animal model experimentation, although useful in determining specific outcomes, may not offer a exact similarity to human conditions and thus parallel outcomes of experimentation. Therefore, researchers must be cautious when making the link of results acquired through animal experimentation to a human application.

A general concern of ischemic models is maintenance of normal core and brain temperature. This is particularly important when we consider that a decrease of only a few degrees Celsius in the brain can dramatically reduce ischemic damage (Busto et al., 1987; Corbett et al., 1997; Colbourne et al., 1999). It is generally accepted that hypothermic conditions give rise to neuroprotection via depression of the cerebral metabolic rate of oxygen consumption (Bering, 1961; Hagerdal et al., 1975). This is especially true when mild hypothermia is experienced post insult (Corbett et al., 1997; Colbourne et al., 1999). This phenomenon makes it difficult to evaluate the amount of neuronal cell death produced by ischemic models because a decrease in brain temperature may reduce the amount of cell death reported. Therefore, we used a heating pad during surgery and a heating lamp after surgery in an effort to maintain normal brain temperature. However, monitoring core body temperature does not provide an accurate estimate of brain

temperature (Busto et al., 1987). It is extremely difficult to accurately measure brain temperature without inserting temperature probes into the brain and even the use of tympanic temperature probes or temporalis muscle probes do not adequately measure brain temperature (reviewed in Corbett and Nurse, 1998). Therefore, cell death reported in this study may not represent the maximum amount of cell death that can be produced by this model, but this model does produce a means by which we can evaluate a potential neuroprotective effect in the hippocampus. In addition, the amount of hippocampal cell death reported in this study is similar to the amount of cell death reported in Campbell (1999). As well, different groups of animals that had their left common carotid artery ligated in the absence of CPA had similar amounts of cell death in the hippocampus.

4.2 CPA-Induced Neuroprotection

The results of this investigation show that CPA, when administered as a single dose 24 hours prior to a hypoxic-ischemic insult, reduced neuronal cell damage in the hippocampus of male rats. Moreover, CPA reduced neuronal cell damage in a dose-dependent fashion. The results support previous studies which show that endogenous adenosine (Rudolphi et al., 1992a; Rudolphi et al., 1992b), adenosine uptake inhibitors (Parkinson et al., 1994), and activation of adenosine A1 receptors by CPA both *in vitro* and *in vivo* (von Lubitz et al., 1988; Gotti et al., 1990; von Lubitz et al., 1994a; Campbell, 1999) reduces neuronal cell loss in the hippocampus induced by models of cerebral ischemia. Furthermore, administration of the competitive adenosine A1 receptor antagonist, DPCPX, blocked CPA-induced neuroprotection in the hippocampus in the current study, indicating that the

neuroprotective effect can be attributed to activation of adenosine A1 receptors. This is consistent with previous reports that activation of adenosine A1 receptors with A1 agonists such as R-PIA (Evans et al., 1987; Sheardown et al., 1995), and CHA (von Lubitz et al., 1989) prior to an ischemic insult provides neuroprotection.

Activation of adenosine A1 receptors has been shown to induce neuroprotective mechanisms such as a decrease in excitatory amino acid release from presynaptic neurons (Goda et al., 1998) and subsequent decrease in glutamate receptor activation (Schubert et al., 1994), a decrease in Ca^{2+} influx through VOCC (Dolphin et al., 1986; Ambrosio et al., 1997), hyperpolarization through outward K^+ conductance (Dunwiddie et al., 1985) by activation of K_{ATP} channels (Heurteaux et al., 1995), and inward Cl^- currents (Mager et al., 1990). Overall, activation of these receptors serves to down-regulate normal physiological function initially via hyperpolarization of the cell (Fredholm et al., 1994). Hyperpolarization causes a decrease in the cell's resting membrane potential (Schubert et al., 1994) which makes it difficult to reach depolarization threshold causing less frequent depolarization of the cell and ultimately less neuronal death (for review see section 1.3.2).

In support of our contention that the neuroprotective effect reported in this study was the result of adenosine A1 receptor activation, an administration of DPCPX (1.0 mg/kg), a competitive adenosine A1 antagonist, eliminated the reduction of damaged cells in the left hippocampus of animals that had their left common carotid ligated and received CPA. The administration of DPCPX (1.0 mg/kg) one hour prior to administration of CPA blocked

the CPA-induced reduction of damaged cells to a level where the number of damaged cells was similar to controls. It is important to note that the concentration of DPCPX used in this study was high compared to the reported K_i for adenosine A1 receptors (0.46 nM) and A2 receptors (340 nM) in rat whole brain membranes (Bruns et al., 1987; Lohse et al., 1987). But, DPCPX (1.0 mg/kg) has been used in another study that examined the role of adenosine receptors in neuroprotection (Xu et al., 2002). DPCPX is normally considered a competitive A1 antagonist. However, if DPCPX were competing against CPA for only A1 receptor binding sites, there would be a parallel right shift in the CPA dose-response curve. But, there was no shift in the dose-response curve in this study. There may be two explanations for this result. First, the dose of DPCPX may have been so high that it blocked the total neuroprotective effect of endogenous adenosine and CPA. However, administration of DPCPX in the presence and absence of CPA did not increase the number of damaged cells compared to control animals that were ligated and received no DPCPX or CPA. Second, the high dose of DPCPX may have inhibited both A1 and A2 adenosine receptors. It is generally accepted that inhibiting A1 receptors causes a decrease in neuroprotection while inhibiting A2 receptors causes an increase in neuroprotection (von Lubitz et al., 1995a; reviewed in von Lubitz, 1999). Inhibition of A1 receptors decreases the receptor's ability to suppress membrane excitability while inhibition of A2 receptors suppresses the receptor's ability to stimulate membrane excitability (Fredholm et al., 1994). Therefore, one would expect a greater decrease in neuroprotection with only A1 inhibition as opposed to inhibition of both A1 and A2 receptors. However, the results of this study may be explained by the relative density of these receptors in the hippocampus.

Inhibition of A1 receptors may outweigh the effect of A2 inhibition because A1 receptors are found in higher densities than A2 receptors in the hippocampus (Fredholm and Jonzon, 1987). Thus, the hippocampus may be more susceptible to the inhibition of A1 receptors compared to he inhibition of A2 receptors. Nevertheless, in this study an administration of DPCPX one hour before administration of CPA eliminated CPA induced reduction of nerve cell death.

The administration of CPA at varying doses 24 hours before a hypoxic-ischemic insult and the viable cells were counted to generate a CPA dose-response curve from which an ED_{50} was calculated. The resulting ED_{50} of 0.0925 mg/kg for this investigation is not similar to the dose of CPA (3.0 mg /kg) that produced 75 % reduction of hippocampal cell death in gerbils that underwent two vessel occlusion (Knutsen et al., 1999). It is important to note that even a 100 fold increase in the concentration of CPA was not able to completely alleviate a loss of cells in the left hemisphere suggesting that adenosine A1 receptor activation may not provide complete protection against hypoxia-ischemia induced cell damage.

4.3 Time Course of CPA-Induced Neuroprotection

Few studies have examined the potential of CPA to protect neurons when it is administered before a hypoxia-ischemia insult. In those studies, the administration times examined were relatively close to the time of insult. For example, Heurteaux et al. (1995) examined the neuroprotective effect of CPA administered 15 minutes prior to insult.

Therefore, this study investigated the neuroprotective effect of a single administration of CPA (0.1 mg/kg) at varying time points (1, 3, 12, 24, 48 hours) prior to a hypoxia-ischemia insult. As expected, CPA reduced neuronal damage in the hippocampus of male rats at each tested time point while the addition of DPCPX (1.0 mg/kg) eliminated this effect. However, the observation that the greatest amount of neuroprotection occurred when CPA was administered 12 hours prior to insult was unexpected. This is surprising because CPA has a relatively short half life (8.2 ± 0.4 minutes; i.v. infusion) (Mathôt et al., 1993). But, adenosine A1 receptors are metabotropic receptors and activation of these receptors can produce both rapid metabolic changes and more delayed molecular changes (Linden, 2001).

The possible mechanisms by which activation of adenosine A1 receptors produces sustained neuroprotection against hypoxic-ischemic insults may be linked to the receptor's "downstream" effects (Figure 16). It is generally accepted that activation of adenosine A1 receptors is coupled to G_i and G_o G-protein subtypes (Palmer and Stiles, 1997). These proteins induce many effects which include G_i - mediated inhibition of c-AMP production, opening of K_{ATP} channels and enhancement of phospholipase C (PLC) activity which initiates inositol triphosphate (IP_3) / diacylglycerol (DAG) activity (Akbar et al., 1994; Freund et al., 1994; Jockers et al., 1994; Gerwins and Fredholm, 1995). Activation of G_o subunit by adenosine A1 receptors has been shown to inhibit L-type Ca^{2+} currents (Sweeney and Dolphin, 1995; Nogushi and Yamashita, 2000).

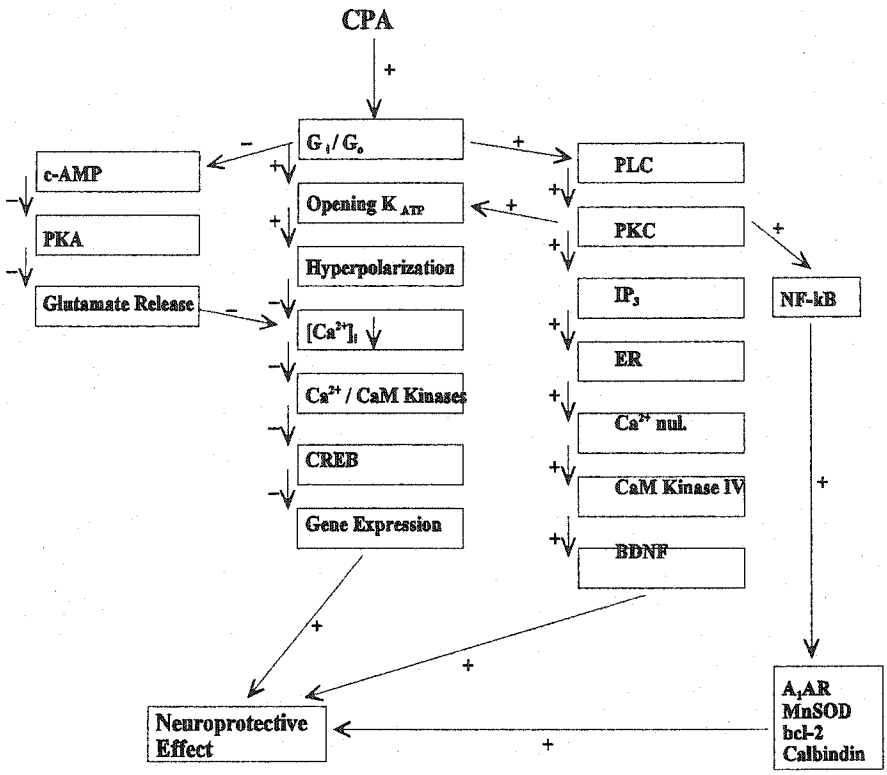

Figure 16. Summary of CPA initiated pathways that may potentially lead to immediate and sustained neuroprotection. (+ stimulation or activation; - inhibition; A_1AR, adenosine A1 receptor; BDNF, brain derived neurotrophic factor; $[Ca^{2+}]_i$, intracellular calcium; Ca^{2+} nul., nuclear calcium; CaM kinase, Ca^{2+}/calmodulin-dependent protein kinase; CREB, c-AMP response element binding protein; c-AMP, cyclic adenosine monophosphate; ER, endoplasmic reticulum; G_i/G_o, G-protein subunits; IP_3, inositol-1,4,5-triphosphate; K_{ATP}, ATP-dependent K^+ channels; MnSOD, manganese-dependent superoxide dismutase; NF-κB, nuclear factor kappa B; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C).

The activation of G_i and G_o subtypes may initiate three interconnected pathways that ultimately serve to protect cells from hypoxia-ischemia induced damage (Figure 16). One pathway may begin through G_i - mediated inhibition of c-AMP formation. Activation of A1 receptors inhibits c-AMP (Fredholm et al., 2001). Therefore, it is possible that if A1 activation inhibits c-AMP, then events down-stream such as glutamate induced excitotoxicity may be arrested. In support of this hypothesis, Thompson et al. (1993) showed that inhibition of c-AMP blocked PKA mediated release of glutamate. In addition, Bouron (1999) showed that adenosine not only inhibits the spontaneous release of glutamate independently of PKA but it also reverses the enhancement of exocytosis produced by PKA.

PKA has also been shown to induce cytosolic c-AMP response element binding protein (CREB) mediated transcription via phosphorylation of the CREB activating site (Gonzalez and Montminy, 1992). Thus, activation of adenosine A1 receptors can inhibit CREB-mediated transcription by inhibiting PKA activity (Figure 16). However, cytosolic CREB-mediated transcription can also be activated through an increase in the intracellular concentration of Ca^{2+} (Negulescu et al., 1994) resulting in CREB-mediated production of early-immediate genes, such as c-fos, that have been linked to cell death (Bito et al., 1996; Das et al., 1999). Therefore, by attenuating an intracellular increase in the concentration of Ca^{2+} via inhibition of VOCCs and NMDA receptors, CPA may inhibit cell damaging CREB-mediated gene expression.

Inhibition of CREB-mediated transcription via inhibition of PKA and the subsequent control of cytosolic Ca^{2+} is not the only possible avenue which may begin to explain the sustained neuroprotection that was observed in this study. As mentioned earlier, through activation of adenosine A1 receptors, G-protein subunits mediate the activation and inhibition of a variety of pathways. As well as inhibiting c-AMP, G_i proteins have been shown to induce the opening of K_{ATP} channels (Figure 16). This effect is important because the protection signal in delayed protection from ischemic preconditioning conditions has been shown to be mediated by opening of K_{ATP} in the heart (Hoag et al., 1997; Bernardo et al., 1999; Fryer et al., 1999), in the brain (Heurteaux et al., 1993; 1995; Haddad and Jiang 1994), and in cultured neurons (Lauritzen et al., 1997). In support of these findings, it was shown that an administration of glibenclamide, a specific K_{ATP} channel blocker, attenuated the R-PIA induced delayed protection of culture neurons against ischemic conditions (Reshef et al., 1998). Opening of K_{ATP} channels in neurons results in repolarization, thereby reducing membrane excitability. This effect down-regulates neuronal metabolic and energy consuming activity which results in protection against ischemic or hypoxic damage (Wann, 1993). As well, via its hyperpolarizing effect, K_{ATP} channels are able to inhibit an increase of intracellular Ca^{2+} through VOCC. By inhibiting an increase in Ca^{2+}, the cell experiences inhibition of CREB-mediated gene expression via the pathway described above. In addition, it has been suggested that K_{ATP} channels produce sustained neuroprotection by hyperpolarizing the cell which results in decreased glutamate release and consequently a reduction in excitotoxicity (Heurteaux et al., 1995) (Figure 16).

The ability of adenosine A1 receptor activation to protect cells and the importance of K_{ATP} channels in sustained neuroprotection has been confirmed in various studies (Reshef et al., 1996; 1997; 1999; 2000). Reshef et al. (1996) and (1997) showed that activation of adenosine A1 receptors in primary rat neuronal cultures produced a wide window of protection which appeared immediately and lasted up to 72 hours. Moreover, they concluded that the opening of K_{ATP} channels was a mandatory step in adenosine A1 receptor mediated neuroprotection (Reshef et al., 1999; 2000). Their results suggest that the "window of opportunity" may reflect a combined operation of several different adenosine-induced mechanisms, all of which are dependent on opening of K_{ATP} channels, but each maturing at different time points (Reshef et al., 2000). If this is true, then the sustained CPA-induced neuroprotective effect reported in this study may be the result of such a paradigm.

Other potential explanations for the sustained neuroprotection observed in this study may include G_i-mediated activation of phospholipase C (PLC) and the subsequent activation of protein kinase C (PKC) (Figure 16). Once activated, PLC separates to form both phospholipid-derived second messenger DAG and IP_3. An essential role of DAG as a second messenger is to activate PKC. PKC has been suggested to play an important role in sustained neuroprotection (Reshef et al., 2000) and cardioprotection (Hoag et al., 1997) against ischemic conditions. PKC can induce the opening of K_{ATP} channels (Hu et al., 1999; Reshef et al., 2000) via activation of mitogen activated protein kinase (MAPK) (Baines et al., 1999). As discussed above, opening of K_{ATP} channels is important to

adenosine-induced neuroprotection. Additionally, it has been shown that PKC phosphorylates proteins which activate systems such as Na^+/Ca^{2+} exchangers and Ca^{2+}-ATPase which leads to a protective decrease in intracellular Ca^{2+} (Iwasa and Hosey, 1984). Therefore, the PKC-mediated effect on K_{ATP} channels provides another means by which activation of adenosine A1 receptors generate a neuroprotective effect.

PLC may also provide neuroprotection via activation of IP_3 (Figure 16). IP_3 acts on the endoplasmic reticulum to slightly increase the concentration of cytosolic Ca^{2+} which is diverted into the nucleus (reviewed in Bootman et al., 2000). Upon entering the nucleus, Ca^{2+} has been shown to activate CaM kinase IV which can enhance expression of brain-derived neurotrophic factor (BDNF) (Greenberg et al., 1992). BDNF has been shown to protect cultured hippocampal and cortical neurons against injury induced by glucose deprivation (Cheng and Mattson, 1994). These results suggest that PLC can mediate the inhibition of potentially damaging gene expression through activation of PKC and the enhancement of potentially beneficial gene expression through activation of IP_3 (Figure 16).

PLC seems to mediate yet a third avenue which may explain sustained neuroprotection by acute activation of adenosine A1 receptors (Figure 16). Activation of PLC has been shown to induce the activation of the transcription factor nuclear factor-κB (NF-κB) via activation of PKC activity (Guo and Xu, 2001). NF-κB plays an integral role in neuronal survival (reviewed in Mattson et al., 2000) and can increase adenosine A1 receptor

expression *in vitro* (Zhongzhen et al., 1998). NF-κB has been shown to improve brain tolerance (ischemic, epileptic, polyunsaturated fatty acid-induced preconditioning) to neuronal cell death (Blondeau et al., 2001). NF-κB has also been shown to play an integral role in the induction of neuroprotective gene products such as manganese-dependent superoxide dismutase (MnSOD) (Wong et al., 1989; Mattson et al., 1997; Keller et al., 1998; Majima et al.,1998) and Bcl-2 (Martinow et al., 1994; Linnick et al., 1995; Toyoda et al., 1997; Guo and Xu, 2001). The neuroprotective property of Bcl-2 is associated with its ability to suppress accumulation of ROS (Hockenbery et al., 1993), and its ability to act on membranes to suppress lipid peroxidation (Bruce-Keller et al., 1997). In addition to enhanced antioxidant defense and Bcl-2 mediated anti-apoptotic defense, NF-κB may provide neuroprotection by enhancing the expression of calbindin. The concentration of calbindin has been shown to increase in hippocampal neurons (Cheng et al., 1994), and astrocytes (Mattson et al., 1995) after administration of a NF-κB activator. Calbindin, which can bind with up to four Ca^{2+} ions protects cells by controlling the concentration of cytosolic Ca^{2+}.

The main argument which connects possible mechanisms of neuroprotection is that the immediate metabolic changes that occur in the neuron after A1 activation would probably not maintain sustained neuroprotection. Therefore, the mechanism(s) underlying sustained neuroprotection must involve intracellular molecular changes giving rise to enhanced expression of "life-promoting" proteins, or giving rise to the inhibition of "death-promoting" proteins, or differing development times for neuroprotective pathways. Each

discussed mechanism seems to recruit one or both of these events. Thus, it could be the combination of all these mechanisms and their varying times of development and duration which gives rise to the sustained neuroprotection observed in this investigation. Nevertheless, because we observed sustained neuroprotection that was longer than anticipated metabolic changes, there must exist a mechanism(s) by which a single administration of CPA provides relatively sustained neuroprotection.

4.4 Hemodynamic Effects of CPA

The existence of some deleterious side effects associated with the administration of CPA, such as hypotension, has been the main concern with regard to its possible clinical application (White et al., 1996). Although CPA has been shown to decrease blood pressure (Mathôt et al., 1994), no one has investigated the effect of different doses of CPA (i.p.) on blood pressure in the rat nor has anyone investigated the long term hypotensive effects of a single dose CPA administration. We observed that administration of different doses of CPA reduced MAP in the awake rat in a dose-dependent fashion. The decrease in MAP is most likely the result of adenosine A1 receptor activation, as it is known that activation of this subtype of adenosine receptor produces hypotension (Williams, 1993). Hypotension in the rat is primarily a result of adenosine A1 receptor mediated bradycardia and a subsequent decrease in cardiac blood volume output (Mathôt et al., 1994). In addition, at higher doses of CPA, activation of A2 receptors causes peripheral vasodilation which contributes to the hypotensive condition (Daval et al., 1991).

CPA-mediated hypotension in the rat is important to this study because it has been previously shown that a brief period of mild ischemia can protect the brain against future episodes of ischemia (Heurteaux et al., 1995). This phenomenon is called ischemic preconditioning. Ischemic preconditioning can produce rapid and delayed neuroprotection in the brain (Kitagawa et al., 1990; Mori et al., 2000; Shimizu et al., 2001). Rapid neuroprotection is evident one day later (Stagliano et al., 1999) while delayed neuroprotection is evident up to seven days after the initial ischemic episode (Nakamura et al., 2002). In addition, it is important to mention that this study incorporated an i.p. administration of CPA. Therefore, CPA would have elicited an A1 receptor effect on the heart before in crossed the blood brain barrier and entered the cervical circulation. Activation of A1 receptors on the heart causes bradycardia and reduced cardiac output thereby causing a reduction in blood pressure (Mathôt et al., 1994). This reduction in blood pressure may simulate cerebral ischemic conditions which would elicit an immediate release of endogenous adenosine in the brain (Melani et al., 1999) thereby initiating neuroprotective events. The rapid decrease in MAP after an i.p. injection of CPA observed in this study supports this theory. Moreover, the arrival of CPA in the brain during and after the metabolism of endogenous adenosine may create an additive neuroprotective effect by reactivating A1 receptors in the brain. Collectively, CPA-mediated activation of A1 receptors on the heart and the subsequent activation of A1 receptors in the brain produce a neuroprotective response. The development and duration of this response may be the result of the pathways previously described .

The neuroprotective effect measured in this study may also be the result of only ischemia preconditioning. This theory may be true because the method of injection was systemic which would elicit primary and secondary effects. The primary effect is the hypotensive response produced through activation of adenosine A1 receptors on the heart. The reduction in mean arterial pressure has been shown to mimic ischemic preconditioning. The secondary neuroprotective effect may be activation of adenosine A1 receptors in the brain. However, given that this investigation did not directly inject CPA into the brain, this study more strongly supports the neuroprotective effect as being the result of primary actions on the heart which produces a decrease in mean arterial pressure resulting in ischemic preconditioning of neurons.

To distinguish whether or not the neuroprotective response observed in this study is the result of ischemic preconditioning, one would have to mimic the CPA-induced reduction in MAP with the use of a hypotensive agent such as nitroprusside, determine if there is a subsequent decrease in cerebral blood pressure and evaluate any resulting neuroprotective effect in the hippocampus. If there was a reduction in the amount of nerve cell death in the hippocampus, then perhaps the neuroprotective effect in this study is a result of a hypotensive condition and thus ischemic preconditioning. If no neuroprotective effect was observed, one must conclude that even though CPA causes hypotension, hypotension does not contribute to the neuroprotective effect. Furthermore, co-administration of an adenosine A1 antagonist that does not cross the blood brain barrier with CPA would eliminate the primary hypotensive effect. Any neuroprotective effect measured subsequent

to this treatment may suggest a role for CPA induced neuroprotective via activation of adenosine A1 receptors in the brain.

4.5 Relevance and Application of Investigation

The importance of this investigation is emphasized by the increasing number of people experiencing cardiovascular disease, as well as a lack of available therapies for this disease (Heart and Stroke Foundation of Canada, 1999). Existing pharmacological interventions such as t-PA are aimed at reducing neuronal damage after an insult has occurred (Zivin and Miller, 1999); a time which presents only a small window for effective treatment. Although t-PA has produced acceptable results against some types of stroke (Ringleb et al., 2002), its use is limited to a narrow window of administration after an insult. Thus, investigations have been initiated to examine pharmacological interventions that are aimed at preventing stroke induced neuronal damage. Specifically, endogenous adenosine is neuroprotective (von Lubitz et al., 1994a) and so researchers have focused on mimicking this effect with different adenosine receptor agonists (Evans et al., 1987; Héron et al., 1994; Sheardown et al., 1995; Logan and Sweeney, 1997; von Lubitz et al., 1999). However, none of these investigations have examined long pre-insult administration. Thus, because of an absence of information on this particular condition and because CPA has been shown to produce a neuroprotective effect (von Lubitz et al., 1994a), this study examined the idea that an administration of CPA prior to a hypoxic-ischemic insult would provide sustained neuroprotection

The results of this investigation are relevant both biologically and therapeutically. The biological relevance of this study concerns the current theory of neuroprotection. Our results support earlier investigations which suggested that endogenous adenosine and activation of adenosine A1 receptors are important elements for the formation of neuroprotection against hypoxic-ischemic insults (Heurteaux et al., 1995; Jacobson et al., 1996; Logan and Sweeney, 1997; Reshef et al., 2000). As well, this investigation supports the hypothesis that activation of adenosine A1 receptors can be effective before, and not only during, a cerebral ischemia insult. The most interesting biological result from this investigation is that A1 receptors can be activated 48 hours prior to an insult and still provide significant neuroprotection to hippocampal neurons. This result suggests that the adenosine A1 receptor is not only capable of producing immediate changes to produce neuroprotection but it can also elicit activation of a longer lasting neuroprotective mechanism(s). However, the overall mechanisms of sustained neuroprotection are still under investigation. Ultimately, activation of adenosine A1 receptors both on the heart and in the brain by CPA may chemically precondition neurons to future episodes of hypoxia-ischemia. But, more studies need to be done to clarify these means. Additionally, activation of A1 receptors and the subsequent long term effects may provide insight into the cellular machinery that allows animals such as the fresh water turtle (*Chrysemys picta belli*) to withstand long periods of anoxia (Buck and Bickler, 1995). Ultimately, by expanding our understanding of the role of adenosine coordinated cellular mechanisms, we not only contribute to knowledge about brain physiology but also to the development of preventative strategies.

The results presented in the thesis cautiously support the use of adenosine A1 agonists as a pharmacological based preventative strategy against stroke induced neuronal damage. As discussed earlier, the results of this study were obtained through animal experimentation. Although these results are intriguing, it is still difficult to show a strong correlation to the effect that CPA may have in humans. Additionally, poor outcomes associated with chronic exposure to A1 agonists (von Lubitz et al., 1994a) and severe hemodynamic effects (Mathôt et al., 1994) may limit this application. With the recent advent of adenosine derivatives, such as deoxyribose analogues of CPA, which produce reduced hemodynamic effects (Lorenzen et al., 1997), A1 agonists may yet prove useful given the means of the neuroprotective can be isolated as non-hypotension induced.

4.6 Summary, Conclusions and Future Considerations

The results of this investigation support the hypothesis that a single administration of CPA, when administered up to 48 hours prior to a hypoxic-ischemic insult, preconditions vulnerable neurons for immediate and sustained neuroprotection against future hypoxic-ischemic induced neuronal damage. The means by which CPA initiates neuroprotection may be the combined effect of simulated cerebral ischemic preconditioning via activation of A1 receptors on the heart and the eventual initiation of protective molecular changes via activation of A1 receptors in the brain. However, further experimentation must isolate the particular mechanism. Regardless of the mechanism(s) by which CPA induced neuroprotection in this study, this investigation offers support for the critical role of

endogenous adenosine in the development of neuroprotection and the potential of adenosine based pharmacotherapeutics against hypoxia-ischemia induced neuronal cell death. However, the CPA induced hypotensive effects remain a concern. Considering the severity of CPA-mediated hypotension observed and that the MAP returned to basal levels after 24 hours only with the minimum dose of CPA, investigations into adenosine analogues with less hypotensive effects are warranted.

Future directions in this research must establish the mechanism(s) by which CPA is able to provide neuroprotection. To accomplish this objective, researchers must establish which changes occur in the cell after acute activation of adenosine A1 receptors, the results of these changes and the means by which these changes occur. As well, researchers must investigate further the possibility that a CPA-mediated hypotensive effect contributes to the observed neuroprotective effect. This study recommends the use of systemic adenosine A1 antagonists or the direct injection into the brain of CPA to qualify the mechanism of CPA induced neuroprotection. The discovery of this mechanism(s) would be invaluable to understanding how neurons become tolerant to hypoxic and ischemic conditions.

5.0 REFERENCES

Abbracchio, M.P.; Branbila, R.; Ceruti, S.; Kim, H.O.; von Lubitz, D.K.J.E.; Jacobson, K.A. and Cattaberi, F. 1995 G-protein-dependent activation of phopholipase C by adenosine A3 receptors in the rat brain. Mol. Pharmacol. 48:1038-1045.

Akbar, M.; Okajima, F.; Tomura, H.; Shimegi, S. and Kondo, Y. 1994 A single species of adenosine A1 receptor expressed in Chinese hamster ovary cells not only inhibits c-AMP accumulation but also stimulates phospholipase C and arachidonate release. Mol. Pharmacol. 45:1036-1042.

Alkan, T.; Kahveci, N.; Buyukuysal, L.; Korfali, E. and Ozluk, K. 2001 Neuroprotective effects of MK-801 and hypothermia used alone and in combination in hypoxic-ischemia brain injury in neonatal rats. Arch Physiol. Biochem. 109:135-144.

Ambrosio, A.; Malva, J.O.; Carvalho, A.P. and Carvalho, C.M. 1997 Inhibition of N-, P/Q and other types of calcium channels in rat hippocampal nerve terminals by adenosine A1 receptor. Eur. J. Pharmacol. 340:301-310.

Ameri, A. and Jurna, I. 1991 Adenosine A1 and non-A1 receptors: intracellular analysis of the action of adenosine agonists and antagonists in rat hippocampal neurons. Brain Res. 546:69-78.

American Heart Association. 1997 National health and nutrition examination survey III (NHANES III) 1988-1994.

Anderson, C.M.; Baldwin, S.A.; Young, J.D. and Cass, C.E. 1999a Distribution of mRNA encoding a nitrobenzylthioinosine-insensitive nucleoside transporter (ENT2) in rat brain. Bran Res. Mol. Brain Res. 70:293-297.

Anderson, C.M.; Xiong, W.; Geiger, J.D.; Young, J.D.; Cass, C.E.; Baldwin, S.A. and Parkinson, F.E. 1999b Distribution of equilibrative nitrobenzylthioinosine sensitive nucleoside transporters (ENT1) in brain. J. Neurochem. 73:867-873.

Arch, J.R.S. and Newsholme, E.A. 1978 The control of the metabolism and the hormonal control of adenosine. Essays in Biochem. 14:82-123.

Bading, H.; Ginty, D.D. and Greenberg, M.E. 1993 Regulation of gene expression in hippocampal neurons by distinct calcium signalling pathways. Science 260:181-186.

Baines, C.P.; Cohen, M.V. and Downey, J.M. 1999 Signal transduction in ischemic preconditioning: role of kinase induced mitochondrial K(ATP) channels. J. Cardiovasc. Electrophysiol. 10:741-754.

Baldelli, R.J.; Green, F.H.Y. and Auer, R.N. 1993 Sulfide toxicity: the role of mechanical ventilation and hypotension in determining survival rate and brain necrosis. J. Appl. Physiol. 75:1348-1353.

Baldwin, S.A.; Mackay, J.R.; Cass, C.E. and Young, J.D. 1999 Nucleoside transporters: molecular biology and implications for therapeutic development. Mol. Med. Today 5:216-224.

Ballarin, M.; Fredholm, B.B.; Mahy, N. and Ambrosia, S. 1991 Extracellular levels of adenosine and its metabolites in the striatum of awake rats: inhibition of uptake and metabolism. Acta. Physiol. Scand. 142:97-103.

Bao, W.L.; Williams, A.J.; Faden, A.I. and Tortella, F.C. 2001 Selective mGluR5 receptor antagonists or agonists provides neuroprotection in a rat model of focal cerebral ischemia. Brain Res. 922:173-179.

Barnett, H.J.M.; Mohr, Y.P.; Stein, B.M. and Yatsu, F.M. (eds) 1998 Stroke: Pathophysiology, diagnosis and management. Churchill Livingston, New York. 263.

Bartus, R.T.; Dean, R.L.; Cavanaugh, H.; Eveleth, D.; Carriero, D.L. and Lynch, G. 1995a Time-related neuronal changes following middle cerebral artery occlusion: implications for therapeutic intervention and the role of calpain. J. Cereb. Blood Flow Metab. 15:969-979.

Bartus, R.T.; Elliot, P.J.; Hayward, N.H.; Dean, R.L.; Harbeson, S.; Straub, J.A.; Li, Z. and Powers, J.C. 1995b Calpain as a novel target for treating acute neurodegenerative disorders. Neurol. Res. 17:249-258.

Bartus, R.T; Baker, K.L.; Heiser, A.D.; Sawyer, A.D.; Dean, R.L.; Elliot, P.J. and Straub, J.A. 1994 Postischemic administration of AK275, a calpain inhibitor, provides substantial protection against focal ischemia brain damage. J. Cereb. Blood Flow Metab. 14:537-544.

Bayer, S.H. 1985 Hippocampal region. In: The Rat Nervous System (Vol 1). Forebrain and Midbrain. Paxinos, G. (ed). Academic Press, Toronto, 335-352.

Bell, M.J.; Kochanek, P.M.; Carcillo, J.A.; Mi, J.; Schoding, J.K.; Wisniewski, S.R.; Clark, R.S.; Dixon, C.E.; Marion, D.W. and Jackson, E. 1998 Intersticial adenosine, inosine and hypoxanthine are increased after experimental traumatic brain injury in the rat. J. Neurotra. 3:163-170.

Bering, E.A. 1961 Effect of body temperature change on cerebral oxygen consumption in the intact monkey. Am. J. Physiol. 200:417-419.

Bernard, C. and Wheal, H.V. 1994 Model of local connectivity patterns in CA3 and CA1 areas of the hippocampus. Hippocampus 4:497-529.

Bernardo, N.L.; D'Angelo, M.; Okubo, S.; Joy, A. and Kukreja, C. 1999 Delayed ischemic preconditioning is mediated by opening of ATP-sensitive potassium channels in rabbit heart. Am. J. Phys. 276:H1323-H1330.

Bezzi, P.; Carmignoto, G.; Pasti, L.; Vesce, S.; Rossi, D.; Rizzini, B.L.; Pozzan, T. and Volterra, A. 1998 Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 391:281-285.

Bindokas, V.P.; Jordán, J.; Lee, C.C. and Miller, R.J. 1996 Superoxide production in rat hippocampal neurons: Selective imaging with hydroethidine. J. Neurosci. 16:1324-1336.

Birnbaumer, L.; Campbell, K.P.; Catterall, W.A.; Harpold, M.M.; Holfman, F.; Horne, W.A.; Mori, Y.; Schwartzs, A.; Snutch, T.P.; Tanabe, T. and Tsien, R.W. 1994 The naming of voltage-gated calcium channels. Neuron 13:505-506.

Bito, H.; Deisseroth, K. and Tsien, R.W. 1996 CREB phosphorylation and dephosphorylation: a calcium and stimulus duration-dependent switch for hippocampal gene expression. Cell 87:1203-1214.

Blackstad, T.W. 1956 Commisural connections of the hippocampal region of the rat with spacial reference to their mode of termination. J. Comp. Neurol. 105:517-537.

Blondeau, N.; Widmann, C.; Lazdunski, M. and Heurteaux, C. 2001 Activation of the nuclear factor-κB is a key event in brain tolerance. J. Neurosci. 21:4668-4677.

Blondeau, N.; Plamondon, H.; Richelme, C.; Heurteaux, C. and Lazdunski, M. 2000 K(ATP) channel openers, adenosine agonists and epileptic preconditioning are stress signals inducing hippocampal neuroprotection. Neurosci. 100:465-474.

Bootman, M.D.; Thomas, D.; Tovey, S.C.; Berridge, M.J. and Lipp, P. 2000 Nuclear calcium signalling. Cell Mol. Life Sci. 57:371-378.

Bosman, F.T.; Visser, B.C. and van Oeveren, J. 1996 Apoptosis: Pathophysiology of programmed cell death. Pathol. Res. Pract. 192:676-683.

Bouron, A. 1999 Adenosine suppresses protein kinase A- and C-induced enhancement of glutamate release in the hippocampus. Eur. J. Pharmacol. 11:4446-4450.

Brini, M. and Carafoli, E. 2000 Calcium signalling: A historical account, recent developments and future perspectives. Cell Mol. Life Sci. 57:354-370.

Bruce-Keller, A.J.; Begly, J.G.; Fu, W.; Butterfield, D.A.; Bredesen, D.E.; Hutchins, J.B.; Hensley, K. and Mattson, M.P. 1997 Bcl-2 protects isolated plasma and mitochondrial membranes against lipid peroxidation induced by hydrogen peroxide and amyloid β-peptide. J. Neurochem. 70:31-39.

Brundege, J.M. and Dunwiddie, T.V. 1996 Modulation of excitatory synaptic transmission by adenosine release from single hippocampal pyramidal neurons. J. Neurosci. 16:5603-5612.

Bruns, R.F.; Fergus, J.H.; Badger, E.W.; Bristol, J.A.; Santay, L.A.; Hartman, J.D.; Hays, S.J. and Huang, C.C. 1987 Binding of the A1-selective adenosine agonist 8-cyclopentyl-1,3-dipropylxanthine in the rat brain membranes. Naunyn Schmiedebergs Arch. Pharmacol. 335:59-63.

Buck, L.T. and Bickler, P.E. 1995 Role of adenosine in NMDA modulation in the cerebral cortex of an anoxia-tolerant turtle. J. Exp. Biol. 198:1621-1628.

Bulley, S.R. and Wittnich, C. 1995 Adenosine infusion: a rational approach towards induced hypotension. Can. J. Cardiol. 4:327-334.

Burnstock, G. 1989 Purine receptors. In: Adenosine Receptors in the Nervous System. Robeiro, J.A. (ed). Taylor and Francis, New York. 1-14.

Busto, R.; Dietrich, W.D.; Globus, Y.-T.; Valdés, I.; Scheinberg, P. and Ginsberg, M.D. 1987 Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. J. Cereb. Blood Flow Metab. 7:729-738.

Calabresi, P.; Picconi, B.; Saulle, E.; Centonze, D.; Hainworth, A.H. and Bernardi, G. 2000 Is pharmacological neuroprotection dependent on reduced glutamate release? Stroke 31:766-773.

Campbell, D.S. 1999 An acute administration of N^6-cyclopentyladenosine (CPA) 24 hours before a cerebral hypoxia-ischemia insult reduces the amount of nerve cell death in the rat hippocampus. Honours Thesis. University of Prince Edward Island, Department of Biology.

Carafoli, E. 1987 Intracellular calcium homeostasis. Annu. Rev. Biochem. 56:395-433.

Cass, C.E.;Young, J.D. and Baldwin, S.A. 1998 Recent advances in the molecular biology of nucleoside transporters of mammalian cells. Biochem. Cell Biol. 76:761-770.

Chan, P.H. 2001 Reactive oxygen radicals in signalling and damage in the ischemic brain. J. Cereb. Blood Flow Metab. 21:2-14.

Chan, P.H.; Kawase, M.; Murakani, K.; Chen, S.; Li, Y.; Calagui, B.; Reola, L.; Carlson, E. and Epstein, C.J. 1998 Over expression of SOD1 in transgenic rats protects vulnerable neurons against ischemic damage after global cerebral ischemia and reperfusion. J. Neurosci. 18:8292-8299.

Chan, P.H. 1996 Role of oxidants in ischemia brain damage. Stroke 27:1124-1129.

Chan, P.H.; Chen, S.; Imaizumi, S.; Chu, L.; Kelleher, J.A.; Gregory, G.A. and Chan, T. 1992 New insights into the role of oxygen radicals in cerebral ischemia. In: Neurochemical Correlates of Ischemia.. Bazan, M.G.; Braquet, P. and Ginsberg, M. (eds) Plenum Press. New York. 277-294.

Chen, J.F.; Huang, Z.H.; Ma, J.Y.; Zhu, J.M. and Moratella, R. 1999 A2a adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. J. Neurosci. 19:9192-2000.

Chen, P.K.; Imaizumi, S. and Chan, T. 1996 Role of oxidants in ischemic brain damage. Stroke 27:1124-1129.

Chen, P.K.; Chan, S.; Imaizumi, S.; Chus, L.; Kellerher, J.A.; Gregory, G.A. and Chan, T. 1994 New insights into the role of oxygen radicals in cerebral ischemia. In: Neurochemical Correlates of Ischemia. Bazan, M.G.; Braquet, P. and Ginsberg, M. (eds) Plenum, Press. New York. 183-218.

Cheng, B. and Mattson, M.P. 1994 NT-3 and BDNF protect hippocampal, septal, and cortical neurons against metabolic compromise. Brain Res. 640:56-67.

Cheng, B.; Christakos, S. and Mattson, M.P. 1994 Tumor necrosis factor protects neurons against excitotoxic/metabolic insults and promotes maintenance of calcium homeostasis. Neuron 73:502-512.

Choi, D.W. 1988a Glutamate neurotoxicity and diseases of the nervous system. Neuron 1:623-634.

Ciccarelli, R.; Sureda, F.X.; Casabona, G.; Dilorio, P.; Caruso, A.; Spinella, F.; Condorelli, D.F.; Nicoletti, F. and Caciagli, F. 1997 Opposite influence of that metabotropic glutamate receptor subtypes mGlu-3 and -5 on astrocyte proliferation in culture. Glia 21:390-398.

Clapham, D.E 1995 Calcium signalling. Cell 80:259-268.

Colbourne, F.; Li, H. and Buchan, A.M. 1999 Infatigable CA1 sector neuroprotection with mild hypothermia induced 6 hours after severe forebrain ischemia in rats. J. Cereb. Blood Flow Metab. 19:742-749.

Coney, A.M. and Marshall, J.M. 1998 Role of adenosine and its metabolites in the vasodilation induced in the cerebral cortex of the rat by systemic hypoxia. J. Physiol. 2:507-518.

Condorelli, D.F.; Dell'Albani, P.; Amico, C.; Kaczmarek, L.; Nicoletti, F.; Lukasiuk, K. and Stella, A.M. 1993 Induction of primary response genes by excitatory amino acid receptor agonists in primary astroglial cultures. J. Neurochem. 60:877-885.

Conn, P.J. and Pin, J.P. 1997 Pharmacology and function of metabotropic glutamate receptors. Annu. Rev. Pharmacol. Toxicol. 37:205-237.

Corbett, D. and Nurse, S. 1998 The problem of assessing effective neuroprotection in experimental ischemia. Prog. Neurobiol. 54:531-548.

Corbett, D.; Nurse, S. and Colbourne, F. 1997 Hypothermic neuroprotection. A global ischemia study using 1-20 month-old gerbils. Stroke 28:2238-2242.

Coyle, P. and Panzenbeck, M.J. 1990 Collateral development after carotid artery occlusion in Fischer 344 rats. Stroke 21:316-321.

Cox, B.F.; Kenneth, L.; Clark, M.H.; Perrone, G.E.; Welzel, B.D.; Greenland, D.J.; Colussi, D.J. and Merkel, L.A. 1997 Cardiovascular and metabolic effects of adenosine A1-receptor agonists in streptozotocin-treated rats. J. Cardiovasc. Pharmacol. 29:417-426.

Cruzalegui, F.H.; and Bading, H 2000 Calcium-regulated protein kinase cascades and their transcription factor targets. Cell. Mol. Life Sci. 57:402-408.

Cunha, R.A.; Milusheva, E.; Vizi, E.S.; Ribeiro, J.A. and Sebastião, A.M. 1994 Excitatory and inhibitory effects of A1 and A2a adenosine receptor activation on the electrically evoked [^{3}H] acetylcholine release from different areas of the rat hippocampus. J. Neurochem. 63:207-214.

Dale, N.; Pearson, T. and Frenguelli, B.G. 2000 Direct measurement of adenosine release during hypoxia in the CA1 region of the rat hippocampal slice. J. Physiol. 256:143-155.

Das, D.K.; Engleman, R.M. and Maulik, N. 1999 Oxygen free radical signalling in ischemic preconditioning. Ann. N.Y. Acad. Sci. 874:49-65.

Daval, J.-L. and Nicolas, F. 1994 Opposite effects of cyclohexyladenosine and theophylline on hypoxic damage in cultured neurons. Neurosci. Lett. 175:114-116.

Daval, J.-L.; Nehlig, A. and Nicolas, F. 1991 Physiological and pharmacological properties of adenosine: Therapeutic implications. Life Sci. 49:1435-1453.

Daval, J.-L.; von Lubitz, D.K.J.E.; Deckert, J.; Redmond, D. and Marangos, P.J. 1989 Protective effect of cyclohexyladenosine on adenosine A1 receptors, guanine nucleotide and forskolin binding sites following transient brain ischemia: A quantitative autoradiographic study. Brain Res. 491:212-226.

Deckert, J. and Jorgenson, M.B. 1988 Evidence for pre- and post-synaptic localization of A1 receptors in the CA1 region of the rat hippocampus: A quantitative autoradiographic study. Brain Res. 446:161-164.

De Ley, G. 1990 Hemodynamic adaptations in proximal cerebrovascular occlusion. Verh. Acad. Geneeskd. Belg. 52:413-454.

De Ley, G.; Nishimyumuremyi, J-.B. and Leusen, I. 1985 Hemispheric blood flow in the rat after unilateral common carotid occlusion: Evolution with time. Stroke 16:69-73.

del Zoppo, G.L.; Wagner, S. and Tagaya, M. 1997 Trends and future developments in the pharmacological treatment of acute ischemic stroke. Drugs 54:9-38.

Derdeyn, C.P.; Videen, T.O.; Fritch, S.; Carpenter, D.A.; Grubb, R.L. and Powers, W.J. 1999 Compensatory mechanisms for chronic cerebral hypoperfusion in patients with carotid occlusion. Stroke 30:1019-1024.

Dingledine, R. and McBain, C.J. 1999 Glutamate and aspartate. In: Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th ed. Seigel, G.T.; Agranoff, B.W.; Albers, R.W.; Fisher, S.K. and Uhler, M.D. (eds) Lippincott-Raven. New York, N.Y. 315-333.

Dirnagl, U.; Iadecola, C. and Moskowshi, M.A. 1999 Pathobiology of ischemic stroke: An integrated view. Trends Neurosci. 22:391-397.

Disterhoft, J.F.; Moyer, J.R. and Thompson, L.T. 1994 The calcium rational in aging and Alzheimer's disease. Ann. NY Acad. Sci. 747:382-406.

Dolphin, A.C.; Forda, S.R. and Scott, R.H. 1986 Calcium-dependent currents in cultured rat dorsal root ganglion neurons are inhibited by an adenosine analogue. J. Physiol. 373:47-61.

Dolphin, A.C. and Archer, E.R. 1983 An adenosine agonist inhibits and a cyclic AMP analogue enhances the release of glutamate but not GABA from slices of rat dentate gyrus. Neurosci. Lett. 43:49-54.

Dubinsky, J.M. 1993 Intracellular calcium levels during period of delayed excitotoxicity. J. Neurosci. 13:623-631.

Dunwiddie, T.V. and Masino, S.A. 2001 The role and regulation of adenosine in the central nervous system. Annu. Rev. Neurosci. 24:31-55.

Dunwiddie, T.V.; Diao, L. and Proctor, W.R. 1997 Adenine nucleotides undergo rapid, quantitative conversion to adenosine in the extracellular space in the rat hippocampus. J. Neurosci. 17:7673-7682.

Dunwiddie, T.V. 1990 Electrophysiological aspects of adenosine receptor function. In: Adenosine and Adenosine Receptors. Williams, M. (ed) Humana Press. New Jersey. 143-172.

Dunwiddie, T.V. 1985 The physiological role of adenosine in the central nervous system. Int. Rev. Neurobiol. 27:63-139.

Egushi, Y.; Shimizu, S. and Tsujimoto, Y. 1997 Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Res. 57:1835-1840.

El Yacoubi, M.; Ledent, C.; Ménard, J.F.; Parmentier, M. and Costentin, J. 2000 The stimulant effects of caffeine on locomotor behaviour in mice are mediated thrpugh its blockade of adenosine A2a receptors. Br. J. Pharmacol. 129:1465-1473.

Emerich, D.F. and Bartus, R.T. 1999 Intracellular events associated with cerebral ischemia. In: Stoke Therapy: Basic Preclinical and Clinical Directions. Miller, P. (ed) Wiley-Liss, Incorporated. New York. 199-207.

Evans D.I.; Jones, R.S. and Woodhall, G. 2001 Differential actions of PKA and PKC in the regulation of glutamate release by group III mGluRs in the entorhinal cortex. J. Neurophys. 85:571-579.

Evans, M.C.; Swan, J.H. and Meldrum, B.S. 1987 An adenosine analogue, 2-chloroadenosine, protects against long term development of ischemic cell loss in the rat hippocampus. Neurosci. Lett. 83:287-292.

Fastbom, J.; Pazos, A. and Palacios, J.M. 1987 The distribution of adenosine A1 receptors and 5'-nucleotidase in the brain of some commonly used experimental animals. Neurosci. 22:813-826.

Feoktistov, I.; Murray, J.J. and Biaggioni, I. 1994 Positive modulation of intracellular Ca^{2+} by adenosine A2b, prostocyclin and prostaglandin E2 receptors via cholera toxin-sensitive mechanism in human erythroleukemia cells. Mol. Pharmacol. 45:1160-1167.

Fiebech, B.L.; Biber, K.; Gyufko, K.; Berger, M.; Bauer, J. and van Calker, D. 1996 Adenosine A2b receptors mediate an increase in interleukin-6 mRNA and IL-6 protein synthesis in human astroglial cells. J. Neurochem. 66:1426-1431.

Flaim, S. F.; Nellis, S.H.; Toggart, E.J.; Drexler, H.; Kanda, K. and Newman, E.D. 1984 Multiple simultaneous determination of hemodynamics and flow distribution in the rat. J. Pharmacol. Meth. 11:1-39.

Flemming, K. M. and Mogul, D.J. 1997 Adenosine A3 receptors potentiate hippocampal calcium current by a PKA-dependent/PKC-independent pathway. Neuropharmacol. 36:353-362.

Flemming, K.M.; Ji, T. and Mogul, D.J. 1997 Adenosine A3 receptor activation increases electrical excitability of hippocampal pyramidal neurons. Ann. N.Y. Acad. Sci. 603:497-499.

Fowler, J.C. 1993a Changes in extracellular adenosine levels and population spike amplitude during graded hypoxia in the rat hippocampal slice. Naunyn-Schmiedebergs Arch. Pharmacol. 347:73-78.

Fowler, J.C. 1993b Purine release and inhibition of synaptic transmission during hypoxia and hypoglycemia in rat hippocampal slices. Neurosci. Lett. 157:83-86.

Fozard, M. and Carruthers, H. 1993 Adenosine A3 receptor agonists mediate hypotension in the angiotension supported circulation in the pithed rat. Br. J. Pharmacol. 109:3-5.

Fredholm, B.B.; Ijzerman, A.P.; Jacobson, K.A.; Klotz, K.N. and Linden, J. 2001 International Union of Pharmacology XXV. Nomenclature and classification of adenosine receptors. Pharmacol. Rev. 53:527-552.

Fredholm, B.B.; Ijzerman, A.P.; Jacobson, K.A.; Linden, J.; Schwabe, U. and Stiles, G.L. 1998 Adenosine receptors In: The IUPHAR Compendium of Receptor Characterization and Classification. IUPHAR Media Ltd., London. 48-57.

Fredholm, B.B. 1997 Adenosine and neuroprotection. In: Neuroprotective Agents and Cerebral Ischemia. Academic Press, London. 259-280.

Fredholm, B.B.; Abbrachio, M.P.; Burnstock, G.; Daly, J.D.; Harden, T.K.; Jacobson, K.A.; Leff, P. and Williams, M. 1994 Nomenclature and classification of purinireceptors. Pharmacol. Rev. 46:143-156.

Fredholm, B.B. and Jonzon, B. 1987 Adenosine receptors: agonists and antagonists. In: Role of Adenosine in Cerebral Metabolism and Blood Flow. Stefanovich, V. and Okyayuz-Baklouti, I. (eds) VNU Press, Utrecht. 17-45.

Freund, S.; Ungerer, M. and Lohse, M.J. 1994 A1 adenosine receptors expressed in CHO-cells couple to adenylyl cyclase and to phospholipase C. Naunyn-Schmiedeberg Arch Pharmacol. 350:49-56.

Frotscher, M.; Soriano, E. and Misgeld, U. 1994 Divergence of hippocampal mossy fibers. Synapse 16:148-160.

Frotscher, M. 1988 Neuronal elements in the hippocampus and synaptic connections. In: Neurotransmission in the Hippocampus. Kugler, P.; Misgeld, U. and Zilles, K. (eds). Springer, Paris. 1-19.

Frotscher, M. and Leranth, C. 1985 Cholinergic innervation of the rat hippocampus as revealed by choline acetyltransferase immunohistochemistry: A combined light and electron microscopy study. J. Comp. Neurol. 239:237-246.

Fryer, R.M.; Hsu, A.K.; Eells, J.T.; Nagase, H. and Gross, G.J. 1999 Opioid-induced second window of cardioprotection; potential role of K_{ATP} channels. Circ. Res. 84:846-851.

Gao, Y. and Phyllis, J.W. 1994 CGS15943, an adenosine A2 antagonist, reduces cerebral ischemia injury in Mongolian gerbils. Life Sci. 55:61-65.

Geiger, J. D.; Parkinson, F.E. and Kowalick, E.A. 1997 Regulators of endogenous adenosine levels as therapeutic targets. In: Purigenic Approaches in Experimental Therapeutics. Jacobson, K.A and Jarvis, M.F. (eds). Wiley-Liss. New York. 126-137.

Gerwins, P. and Fredholm, B.B. 1995 Activation of adenosine A1 and bradykinin receptors increase protein kinase C and phopholipase D activity in smooth muscle cells. Naunyn-Schmiedeberg Arch. Pharmacol. 351:186-193.

Gilraldez, L.; Zanetti, F.; Antonelli, M.C.; Rodriguez, G. and Girardi, E. 1998 CNS adenosine receptors are altered after the administration of convulsant 3-mercurtopropionic acid and cyclopentyladenosine: An autoradiographic study. Neurochem. Res. 23:175-181.

Ginsberg, M.D. and Pulsinelli, W.A. 1994 The ischemic penumbra, injury thresholds and the therapeutic window for acute stroke. Ann. Neurol. 36:553-554.

Ginsberg, M.D. 1990 Models of cerebral ischemia in the rodent. In: Cerebral Ischemia and Resuscitation. Schurr, A. and Rigor, B.M. (eds) CRC Press, Florida. 1-25.

Ginsberg, M.D. and Busto, R. 1989 Rodent models in cerebral ischemia. Stroke 20:1627-1642.

Globus, M.Y.; Busto, R.; Diethrich, D.W.; Martinez, E.; Valdes, I. And Ginsberg, M.D. 1988 Effect of ischemia on the *in vivo* release of striatal dopamine, glutamate and γ-aminobutyric acid studied in intracerebral microdialysis. J. Neurochem. 51:1455-1464.

Goda, H.; Ooboshi, H.; Nakane, H.; Ibayashi, S.; Sadoshima, S. and Fujishima, M. 1998 Modulation of ischemia invoked release of excitatory and inhibitory amino acids by adenosine A1 receptor agonist. Eur. J. Pharmacol. 357:149-155.

Goldberg, M.P.; Monyer, H.; Weiss, J.H. and Choi, D.W. 1988 Adenosine reduces cortical neuronal injury induced by oxygen or glucose deprivation *in vitro*. Neurosci. Lett. 89:323-327.

Gonzalez, G.A. and Montminy, M.R. 1992 Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59:675-680.

Goodman, S.R. and Zagon, I.S. 1986 The neural cell spectrin skeleton: a review. Am. J. Physiol. 250:C347-C360.

Gotti, B.; Benavides, J.; MacKenzie, E.T. and Scalton, B. 1990 The pharmacology of focal cortical ischemia in the mouse. Brain Res. 522:290-307.

Grant, M.B.; Tarnuzzer, R.W.; Caballero, S.; Ozeck, M.J.; Davis, M.I.; Spoerri, P.E. Feoktistov, I.; Biaggioni, I.; Shryock, J.C. and Belardenelli, L. 1999 Adenosine receptor activation induces vascular endothelial growth factor in human retinal endothelial cells. Circ. Res. 85:699-706.

Greenberg, M.E.; Thompson, M.A. and Sheng, M. 1992 Calcium regulation of immediate early gene transcription. J. Physiol. 86:99-108.

Guo, B.C. and Xu, Y. H. 2001 Bcl-2 over-expression and activation of PKC suppresses trail-induced apoptosis in Jurkat T cells. Cell Res. 11:101-106.

Gyulai, L.; Mitchell, S.; M'Laughlin, A.C.; Leigh, J.S. and Chance, B. 1987 Simultaneous ^{31}P- and ^{1}H-nuclear ,agnetic resonance studies of hypoxia and ischemia in the rat brain. J. Cereb. Blood Flow Metab. 7:543-551.

Haas, H.L. and Selback, O. 2000 Functions of neuronal adenosine receptors. Naunyn-Schmiedeberg Arch Pharmacol. 362:375-381.

Haddad, G.G. and Jiang, C. 1994 Mechanisms of neuronal survival during hypoxia: ATP-sensitive potassium channels. Biol. Neonate 65:160-165.

Hagerdal, M.; Harp, J. and Siesjö, B.K. 1975 The effect of induced hypothermia upon oxygen consumption in the rat brain. J. Neurochem. 24:311-316.

Halliwell, B. 1999 Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic. Res. 31:261-272.

Hara, H.; Onodera, H.; Kawagoe, J. and Kogure, K. 1991 Failure of basic fibroblast growth factor to prevent postischemic neuronal damage in the rat. Eur. J. Pharmacol. 209:195-198.

Harada, H.; Asano, O.; Hoshino, Y.; Yoshikawa, S.; Matsukura, M. and Kabasawa, Y. 2001 2-alkynyl-8-aryl-9-methyladenines as novel adenosine receptor antagonists: their synthesis and structure activity relationships toward hepatic glucose production induced via agonism of the A2b receptor. J. Med. Chem. 44:170-179.

Harris, E.; Witter, M.P.; Weinstein, G. and Stewart, M. 2001 Intrinsic connectivity of the rat subiculum: dendritic morphology and patterns of axonal arborization by pyramidal neurons. J. Comp. Neurol. 436:490-505.

Hatashita, S.; Hoff, J.T. and Salamat, S.M. 1990 An osmotic gradient in ischemic brain edema. Adv. Neurol. 52:85-92.

Headrick, J.P.; Gauthier, N.S.; Berr, S.S.; Morrison, R.R. and Matherne, G.P. 1998 Transgenic A1 adenosine receptor overexpression markedly improves myocardial energy state during ischemia reperfusion. J. Mol. Cell Cardiol. 30:1059-1064.

Heart and Stroke Foundation of Canada. 1999 The changing face of heart disease and stroke in Canada. Heart and Stroke Foundation of Canada, Ottawa, Ontario.

Heart and Stroke Foundation of Canada in collaboration with Health Canada, Laboratory Centre for Disease Control, Statistics Canada and the University of Saskatchewan. 1997 Heart Disease and Stroke in Canada. Heart and Stroke Foundation of Canada, Ottawa, Ontario.

Heart and Stroke Foundation of Canada. 1994 Cardiovascular disease in Canada. Heart and Stroke Foundation of Canada, Ottawa, Ontario.

Hendrikse, J.; Hartkamp, M.J.; Hillen, B.; Mali, W.P. and van der Grond, J. 2001 Collateral ability of the circle of Willis in patients with unilateral internal carotid artery occlusion. Stroke 32:2768-2773.

Héron, A.; Lekieffer, K.; LePeillet, E.; Lasbennes, F.; Seylaz, J.; Plotkine, M. and Boulu, R.G. 1994 Effects of an A1 adenosine receptor agonist on the neurochemical, behavioural and histological consequences of ischemia. Brain Res. 641:217-224.

Héron, A.; Lasbennes, F. and Seylaz, J. 1992 Effect of two different routes of administration of r-PIA on glutamate release during ischemia. Neurosci. Lett. 147:205-208.

Heurteaux, C.; Lauritzen, I.; Widmann, C. and Lazdunski, M. 1995 Essential role of adenosine: adenosine A1 receptors, and ATP-sensitive K^+ channels in cerebral ischemic preconditioning. Proc. Natl. Acad. Sci. USA 92:46666-4670.

Heurteaux, C.; Bertaina, V.; Widmann, C. and Lazdunski, M. 1993 K^+ channel openers prevent global ischemic-induced expression of c-*fos*, c-*jun*, heat shock protein, and amyloid β-protein precursor genes and neuronal death in rat hippocampus. Proc. Natl. Acad. Sci. USA 90:9431-9435.

Hevner, R.F. and Wong-Riley, M.T.T. 1992 Entorhinal cortex of human, monkey and rat: Metabolic map as revealed by cytochrome oxidase. J. Comp. Neurol. 326:451-469.

Hicks, T.P. and Conti, F. 1996 Amino acids as a source of considerable excitation in cerebral cortex. Can. J. Physiol. Pharmacol. 74:341-361.

Hoag, J.B.; Quian, M.A.; D'Angelo, N.M. and Kukreja, R.C. 1997 ATP-sensitive potassium channel mediates delayed ischemic protection by heat stress in rabbit heart. Am. J. Phys. 273:H2458-H2464.

Hock, N. 1998 Neuroprotective and thrombolytic agents: advances in stroke treatment. J. Neurosci. Nurs. 30:175-184.

Hockenbery, D.; Oltvai, Z.N.; Yin, X.M.; Milliman, C.L. and Korsmeyer, S.H. 1993 Bcl-2 functions in an antioxidant pathway to prevent apoptosis. Cell 75:241-251.

Hong, S.C.; Goto, Y.; Lanzino, G.; Soleaw, S.; Kassell, N.F. and Lee K.S. 1994 Neuroprotection with calpain inhibitor in a model of focal cerebral ischemia. Stroke 25:663-669.

Hösli, E. and Hösli, I. 1988 Autoradiographic studies on the uptake of adenosine and on binding of adenosine analogues in neurons and astrocytes of cultured rat cerebellum and spinal cord. Neurosci. 24:261-268.

Hsu, M. and Buzsaki, G. 1993 Vulnerability of mossy fibre targets hippocampus to forebrain ischemia. J. Neurosci. 13:3964-3979.

Hu, K.; Li, G.R. and Nattel, S. 1999 Adenosine-induced activation of ATP-sensitive channels in membrane patches is mediated by PKC. Am. J. Phys. 276:H488-H495.

Insausti, R.; Tuñón, T.; Sobreviela, T.; Insausti, A.M. and Gonzalo, L.M. 1995 The human entorhinal cortex: a cytoarchitectonic analysis. J. Comp. Neurol. 355:171-198.

Ivanics, T.; Miklos, Z.; Dezsi, L.; Ikrenyi, K.; Toth, A.; Roemen, T.H.; van der Vusse, G.J. and Ligeti, L. 2001 Concomitant accumulation of intracellular free calcium and arachidonic acid in the ischemia-reperfused rat heart. Mol. Cell Biochem. 226:119-128.

Iwasa, Y. and Hosey, M.M. 1984 Phosphorylation of cardiac sarcolemma proteins by the calcium-activated phospholipid-dependent kinases. J. Biol. Chem. 259:534-540.

Jacobson, K.A.; von Lubitz, D.K.J.E.; Daly, J.W. and Fredholm, B.B. 1996 Adenosine receptor ligands: differences with acute versus chronic treatment. Trends Pharmacol. Sci. 17:108-113.

Jacobson, K.A.; Nikodijevic, O.; Shi, D.; Gallo-Rodriguez, C.; Olah, G.L.; Stiles, G.L. and Daly, J.W. 1993 A role for central A3-adenosine receptors: Mediation of behavioural depressant effects. FEBS Lett. 336:57-60.

Jarvis, M.F.; Jackson, R.H. and Williams, M. 1989 Autoradiographic characterization of high affinity A2 receptors in the rat brain. Brain. Res. 484:111-118.

Jarvis, M. F. and Williams, M. 1989 Direct autoradiographic characterization of high affinity A2 receptors in the brain using the A2-selective agonist, [^{3}H]-CGS 21680. Eur. J. Pharmacol. 168:243-246.

Ji, X.-D.; von Lubitz, D.K.J.E.; Olah, M.E.; Stiles, G.L. and Jacobson, K.A. 1994 Species differences in ligand affinity at central A3 adenosine receptors. Drug Dev. Res. 33:51-54.

Jiang, N.; Kowaluk, E.A.; Lee, C.H.; Mazdiyasni, H. and Chopp, M. 1997 Adenosine kinase inhibition protects brain against transient focal ischemia in rats. Eur. J. Pharmacol. 320:131-137.

Jockers, R.; Linder, M.E.; Hohenegger, M.; Nanoff, C.; Bertin, B.; Strosberg, A.D.; Marrulo, S. and Freissmuth, M. 1994 Species difference in the G-protein selectivity of human and bovine A1 adenosine receptor. J. Biol. Chem. 269:32077-32084.

Johnston, M.V.; Trescher, W.H.; Ishida, A. and Nakajima, W. 2001 Neurobiology of hypoxic-ischemic injury in the developing brain. Ped. Res. 49:735-741.

Juurlink, B.H.J. 1999 Management of oxidative stress in the CNS: The many roles of glutathione. Neurotox. Res. 1:119-140.

Juurlink, B.H.J. and Sweeney, M.I. 1997 Mechanisms that result in damage during and following cerebral ischemia. Neurosci. Biobehav. Rev. 21(1):121-128.

Kalaria, R.N. and Harik, S.I. 1986 Adenosine receptors of cerebral microvessels and choroid plexus. J. Cereb. Blood Flow Metab. 6:463-470.

Kato, H.; Liu, Y.; Araki, T. and Kogure, K. 1991 Temporal profile of the effects of pretreatment with brief cerebral ischemia on the neuronal damage following secondary ischemic insult in the gerbil: cumulative damage and protective effects. Brain Res. 553:238-242.

Keller, J.N.; Kindy, M.S.; Holtsberg, F.W.; St. Claire. D.; Yen, H.C.; Germeyer, A.; Steiner, S.M.; Bruce-Keller, A.J.; Hutchings, J.B. and Mattson, M.P. 1998 Mitochondrial MnSOD prevents neuronal apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J. Neurosci. 18:687-697.

Kermer, P.; Klöcker, N. and Bähr, M. 1999 Neuronal death after brain injury: Models, mechanisms, and therapeutic strategies in vivo. Cell Tiss. Res. 298:383-395.

Kerr, J.F.R.; Wyllie, A.H. and Currie, A.R 1972 Apoptosis: A basic biological phenomenon with wide ranging implication in tissue kinetics. Br. J. Cancer 26:239-257.

Kessey, K.; Trommer, B.L.; Overstreet, B.S.; Ji, T. and Mogul, D.J. 1997 A role for adenosine A2 receptors in the inhibition of long-term potentiation in the CA1 region of the hippocampus. Brain Res. 2:184-190.

Kirino, T.; Tamura, A. and Sano, K. 1985 Delayed neuronal death in the rat hippocampus following transient forebrain ischemia. Acta Neuropathol. 64:139-147.

Kirino, T. 1982 Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res. 239:57-69.

Kirk, I.P. and Richardson, P.J. 1995 Inhibition of striatal GABA release by the adenosine A2a receptor is not mediated by increases in cyclic AMP. J. Neurochem. 64:2801-2809.

Kitagawa, K.; Matsumoto, M.; Tagaya, M.; Hata, R.; Ueda, H.; Niinobe, M.; Handa, N.; Fukunaga, R.; Kimura, K.; Mikoshiba, K. and Kamata, T. 1990 Ischemic tolerance phenomenon found in the brain. Brain Res. 528:21-24.

Klijn, C.J.M.; Kapelle, L.J.; Tulleken, C.A.F. and van Gijn, J. 1997 Symptomatic carotid artery occlusion: a reprisal of hemodynamic factors. Stroke 28:2084-2093.

Knutsen, L.J.; Lau, J.; Petersen, H.; Thomsen, C.; Weis, J.; Shalmi, M.; Judge, M.; Hansen, A.. And Sheardown, M. 1999 N-substituted adenosines as novel neuroprotective A1 agonists with diminished hypotensive effects. J. Med. Chem. 42:3463-3477.

Kobayashi, S.; Zimmerman, H. and Millhorn, D.E. 2000 Chronic hypoxia enhances adenosine release in rat PC12 cells by altering adenosine metabolism and membrane transport. J. Neurochem. 74:621-632.

Kogure, K. and Nakano, S. 1992 Dismetabolism in lipid brain ischemia. In: Neurochemical Correlates of Ischemia. Bazan, N.G.; Bracket, P. and Ginsberg, M. (eds) Plenum Press. New York. 103-116.

Korenkov, A.I.; Pahnke, J.; Frei, K.; Warzok, R.; Schoeder, H.W.; Frick, R.; Muljana, L.; Piek, J.; Yonekawa, Y. and Gaab, M.R. 2000 Treatment with nimodipine or mannitol reduces programmed cell death and infarct size following focal cerebral ischemia. Neurosurg. Rev. 23:145-150.

Kreutzberg, G.W.; Baron, K. and Schubert, P. 1976 Cytochemical localization of 5'nucleotidase in glial plasma membranes. Brain. Res. 158:247-257.

Latini, S.; Corsi, C.; Pedata, F. and Pepeu, G. 1995 The source of brain adenosine outflow during ischemia and electrical stimulation. Int. Neurochem. 27:239-244.

Lauritzen, I.; De Weille, J.R. and Lazdunski, M. 1997 The potassium channel opener (-)-cromakalin prevents glutamate-induced cell death in hippocampal neurons. J. Neurochem. 69:1570-1579.

Lee, K.S. and Lowenkopf, T. 1993 Endogenous adenosine delays the onset of hypoxic depolarization in the rat hippocampus *in vitro* an action at A1 receptors. Brain Res. 609:313-315.

Lee, K.S.; Reddington, M.; Schubert, P. and Kreutsberg, G. 1983 Regulation of the strength of modulation in the hippocampus by a differential distribution of the density of A1 receptors. Brain Res. 269:156-159.

Leist, M and Nicotera, P. 1999 Calcium and cell death. In: Cell Death and Diseases of the Nervous System. Koliatsos, V.E. and Ratan, R.R. (eds) Humana Press, New Jersey. 69-90.

Levy, L.M.; Warr, O. and Atwell, D. 1998 Stoichiometry of the glial glutamate transporter GLT-1 expressed inducibly in a Chinese hamster ovary cell line selected for low endogenous Na^+ - dependent glutamate uptake. J. Neurosci. 19:9620-9628.

Li, P.A.; Liu, G.J.; He, Q.P.; Floyd, R.A. and Siesjö, B.K. 1999 Production of hydroxyl free radical by brain tissues in hyperglycemic rats subjected to transient forebrain ischemia. Free Radic. Biol. Med. 27:1033-1040.

Li, X-.G.; Somogyi, P.; Ylinen, A. and Buzsaki, G. 1994 The hippocampal CA3 network: An *in vivo* intracellular labelling study. J. Comp. Neurol. 339:181-208.

Li, Y.; Chopp, M.; Jlang, N.; Yao, F. and Zaloga, C. 1995 Temporal profile of in situ DNA fragmentation after transient middle cerebral artery occlusion in the rat. T. Cereb. Blood Flow Metab. 15:389-397.

Liang, B.T. and Jacobson, K.A. 1998 A physiological role of the adenosine A3 receptor: sustained cardioprotection. Proc. Natl. Acad. Sci. USA 95:6995-6999.

Linden, J. 2001 Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. Annu. Rev. Pharmacol. Toxicol. 41:775-787.

Linden, J. 1995 Structure and function of A1 adenosine receptors. J. FASEB 12:2668-2676.

Linnick, M.D.; Zahos, P.; Gschwind, M.D. and Fereroff, H.J. 1995 Expression of Bcl-2 from a defective herpes simplex virus-1 vector limits neuronal death in focal cerebral ischemia. Stroke 26:1670-1674.

Lipton, P. 1999 Ischemic cell death in brain neurons. Physiol. Rev. 79:1531-1568.

Ljunggren, B.L.; Ratcheson, R. and Siesjö, B.K. 1974 Changes in energy state and acid base parameters of the rat during complete compression ischemia. Brain Res. 398:369-373.

Logan, M. and Sweeney, M.I. 1997 Adenosine A1 receptor activation preferentially protects cultured cerebellar neurons versus astrocytes against hypoxia-induced death. Molec. Chem. Neuropath. 31:119-133.

Lohse, M.J.; Klotz, K-N.; Lindenborn-Fotinos, J.; Reddington, M.; Schwabe, U. and Olsson, R.A. 1987 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) - a selective high affinity antagonist radioligand for A1 adenosine receptors. Nauny-Schmiedeberg Arch. Pharmacol. 336:204-210.

Londos, C. and Wolff, J. 1980 Two distinct adenosine sensitive sites on adenylyl cyclase. Proc. Natl. Acad. Sci. USA 74:5482-5486.

Lorenzen, A.; sebastião, A.M.; Sellink, A.; Vogt, H.; Schwabe, U.; Ribeiro, J.A. and Ijzerman, A.P. 1997 Biological activities of N^6-, C8-disubstituted adenosine derivatives as partial agonists at rat brain A1 receptors. Eur. J. Pharmacol. 334:299-307.

Lynch, D.R. and Guttman, R.P 2002 Excitotoxicity: Perspectives based on NMDA receptor subtypes. J. Pharmacol. Exp. Ther. 300:717-723.

MacDonald, M.C.; Mota-Philipe, H.; Parl, H.; Cuzzocrea, S.; Abdelrahman, M.; Harwood, S.; Plevin, R.; Chatterjee, P.K.; Yagoob, M.M. and Thiemermann, C. 2001 Calpain inhibitor ± reduces the activation of NF-κB on organ dysfunction in hemorrhagic shock. FASEB 15:171-186.

MacMillan, V.H. 1989 Cerebral energy metabolism in cyanide encephalopathy. J. Cereb. Blood Flow Metab. 9:156-162.

Mager, R.; Ferroni, S. and Schubert, P. 1990 Adenosine modulates a voltage dependent chloride conductance in cultured hippocampal neurons. Brain Res. 532:58-62.

Majima, H.J.; Oberley, T.D.; Furukawa, K.; Mattson, M.P.; Yen, H.C.; Szweda, L.I. and St Claire, D.K. 1998 Prevention of mitochondrial injury by manganese superoxide dismutase reveals a primary mechanism for alkaline-induced cell death. J. Biol. Chem. 273:8217-8224.

Malenka, R.C.; Kauer, J.A.; Perkel, D.J. and Nicoll, R.A. 1989 The impact of postischemic calcium on synaptic transmission: its role in long-term potentiation. Trends Neurosci. 12:444-450.

Marangos, P.J. 1990 Adenosinergic approaches to stroke therapeutics. Med. Hypoth. 32:45-49.

Marangos, P.J. and Boulanger, J.P. 1985 Basic and clinical aspects of adenosinergic neuromodulation. Neurosci. Biobehav. Rev. 9:421-430.

Mark, S.D.; Wang, W.; Fraumeri, J.F.; Li, J.Y.; Taylor, P.R.; Wang, G.Q.; Dausy, S.M.; Li, B.; and Blot, W.J. 1998 Do nutritional supplements lower the risk of stroke or hypertension. Epidemiology 9:9-15.

Martinow, J.-C.; Dubois-Dauphin, M.; Staple, J.K.; Rodriguez, I.; Frankowski, H.; Missoten, M.; Albertini, P.; Talabot, D.; Catsicas, S. and Petra, C. 1994 Overexpression of bcl-s in transgenic mice protects neurons from naturally occurring cell death in experimental ischemia. Neuron 13:1017-1030.

Matherne, G.P.; Linden, J.; Byford, A.M.; Gauthier, N.S. and Headrick, J.P. 1997 Transgenic A1 adenosine receptor overexpression increases myocardial resistance to ischemia. Proc. Natl. Acad. Sci. USA. 94:479-481.

Mathôt, R.A.A.; van Schaik, E.A.; Langemeijer, M.W.E.; Soudijn, W.; Breimer, D.D.; Ijzerman, A.P. and Danhof, M. 1994 Pharmacokinetic-pharmacodynamic relationship of the cardiovascular effects of adenosine A1 receptor agonist N^6-cyclopentyladenosine in the rat. J. Pharmacol. Exp. Ther. 268:616-624.

Mathôt, R.A.A.; Appel, S.; van Schaik, E.A.; Soudijn, W.; Ijzerman, A.P. and Danhof, M. 1993 High-performance liquid chromatography of the A1 agonist N^6-cyclopentyladenosine and the A1 antagonist 8-cyclopentyltheophylline and its application in a pharmacokinetic study in rats. J Chromatog. 620:113-120.

Matsuyama, T. 1997 Free-radical-mediated cerebral damage after hypoxia / ischemia and stroke. In: Clinical Pharmacology of Cerebral Ischemia. Ter Horst, G.T. and Korf, J. (eds) Humana Press, New Jersey. 153-184.

Mattson, M.P.; Culmsee, C.; Yu, Z. and Cmandola, S. 2000 Roles of nuclear factor κB in neuronal survival and plasticity. J. Neurochem. 74:443-456.

Mattson, M.P.; Goodman, Y.; Luo, H.; Fu, W. and Furukawa, K. 1997 Activation of NF-κB protects hippocampal neurons against oxidative stress-induced apoptosis: evidence for induction of MnSOD and suppression of peroxynitrite production and protein tyrosine nitration. J. Neurosci. Res. 49:681-697.

Mattson, M.P.; Cheng, B.; Baldwin, S.; Smith-Swintosky, V.L.; Keller, J.; Geddes, J.W.; Scheff, S.W. and Christakos, S. 1995 Brian injury and tumor necrosis factors induces expression of calbindin D-28k in astrocytes: a cytoprotective response. J. Neurochem. Res. 42:357-370.

Mattson, M.P.; Murrain, M.; Guthrie, P.B. and Kater, S.B. 1989 Fibroblast growth factor and glutamate: opposing roles in the generation of hippocampal neuroarchitecture. J. Neurosci. 9:3728-3740.

Meghji, P. 1993 Storage, release, uptake and activation of purines. Drug Dev. Rev. 28:214-219.

Melani, A.; Pantoni, L.; Corsi, C. and Bianchi, L. 1999 Striatal outflow of adenosine, EAA, γ-aminobutyric acid and taurine in awake freely moving rats after MCAO. Stroke 30:2448-2455.

Mendonca, A. and Ribiero, J.A. 1997 Influence of metabotropic glutamate receptor agonists on the inhibitory effects of adenosine A1 receptor activation in the rat hippocampus. Br. J. Pharmacol. 121:1541-1548.

Menzies, S.A.; Betz, A.L. and Hoff, J.T. 1993 Contributions of ions and albumin to the formation and resolution of ischemic brain edema. J. Neurosurg. 78:257-266.

Michowiz, S.D.; Melamed, E.; Pilarsky, E. and Rappaport, J.H. 1990 Effects of ischemia induced by MCAO on superoxide dismutase activity in rat brain. Stroke 21:1613-1617.

Miller, L.P.; Jelovich, L.A.; Yao, L.; DaRe, J.; Ugarkar, B. and Foster, A.C. 1996 Pre- and peristroke treatment with the adenosine kinase inhibitor, 5'-deoxyiodotubercidin, significantly reduces infarct volume after temporary occlusion of the middle cerebral artery in rats. Neurosci. Lett. 220:73-76.

Miyamoto, O. and Auer, R.N. 2000 Hypoxia, hyperoxia, ischemia and brain necrosis. Neurol. 54:362:371.

Miyasaka, N.; Nagoka, T.; Kuroiwa, T.; Akimo, H.; Haku, T.; Kubota, T. and Aso, T. 2000 Histopathologic correlates of temporal diffusion changes in a rat model of cerebral hypoxia/ischemia. Am. J. Neuroradiol. 21:60-66.

Moore, R.S; Mayo, Y.; Zhang, J. and Clarke, K. 1997 Economic burden of illness in Canada 1993. Ottawa Health Center and Laboratory Center for Disease Control. In: The changing face of heart disease and stroke in Canada 2000. Heart and Stroke Foundation, Ottawa Canada.

Moreau, J.-L. and Huber, G. 1999 Central adenosine A2a receptors: an overview. Brain Res. Rev. 31:65-82.

Mori, T.; Muramatsu, T.; McKee, A. and Asano, T. 2000 Possible role of the superoxide anion in the development of neuronal tolerance following ischemic preconditioning in rats. Neuropathol. Appl. Neurobiol. 26:31-40.

Mori, A.; Shindou, T.; Ichimura, M. and Miyataka, T. 1996 The role of adenosine A2a receptors in regulating GABAergic synaptic transmission in striatal medium spiny neurons. J. Neurosci. 2:605-611.

Mori, H. and Mishina, M. 1995 Structure and function of the NMDA receptor channel. Neuropharmacol. 34:1219-1237.

Morris, D.C.; Zhang, L.; Zhang, Z.G.; Berens, K.L.; Brown, P.M. and Chopp, M. 2001 Extension of the therapeutic window for the recombinant tissue plasminogen activator with argatroban in the rat model of embolic stroke. Stroke 32:2635-2640.

Naber, P.A.; Witter, M.P. and Lopes da Silva, F.H. 2000 Networks of the hippocampal memory system of the rat: The pivotal role of the subiculum. Ann. NY Acad. Sci. 911:392-403.

Nakamura, M.; Nakakimura, K.; Matsumoto, M. and Sakabe, T. 2002 Rapid tolerance to focal cerebral ischemia in rats is attenuated by adenosine A1 receptor antagonist. J. Cereb. Blood Flow Metab. 22:161-170.

Nakamura, T.; Nakamura, K.; Lasser-Ross, N.; Barbara, J.G.; Sandler, V.M. and Ross, W.N. 2000 Inositol 1,4,5-triphosphate IP_3 -mediated Ca^{2+} release evoked by metabotropic agonists and back propagating action potentials in hippocampal CA1 pyramidal neurons. J. Neurosci. 20:8365-8376.

Negulescu, P.A.; Shastri, N. and Cahalan, M.D. 1994 Intracellular calcium dependent gene expression in single T lymphocytes. Proc. Natl. Acad. Sci. USA 91:2873-2877.

Nemoto, E.M; Evans, R.W. and Kochanek, P.M. 1992 Free fatty acid liberation in the pathogenesis and therapy of ischemic brain damage. In: Neurochemical Correlates of Ischemia. Bazan, M.G.; Braquet, P. and Ginsberg, M. (eds). Plenum Press. New York. 183-218.

Nicotera, P.; Zhvotovsky, B. and Orrenius, S. 1994 Nuclear calcium transport and the role of calcium in apoptosis. Cell Cal. 16:279-288.

Nogushi, J. and Yamashita, H. 2000 Adenosine inhibits voltage-dependent Ca^{2+} currents in rat dissociated supraoptic neurons via A1 receptors. J. Physiol. 526:313-326.

Okada, M.; Nutt, D.J.; Murakami, T.; Zhu, G.; Kamata, A.; Kawata, Y. and Kaneko, S. 2001 Adenosine receptor subtype modulate two major functional pathways for hippocampal serotonin release. J. Neurosci. 21:628-640.

O'Kane, E.M. and Stone, T.W. 1998 Interaction between A1 and A2 receptor mediated responses in the rat hippocampus *in vitro*. Eur. J. Pharmacol. 362:17-25.

Olah, M.E. and Stiles, G.L. 1995 Adenosine receptor subtypes: characterization and the therapeutic regulation. Toxicology 35:581-606.

Ongini, E. 1997 SCH58261: A selective A2a adenosine receptor antagonist. Drug Dev. Res. 42:581-606.

Ouyang, Y.B.; Tan, Y.; Comb, M.; Liu, C.L.; Martone, M.E.; Siesjö, B.K. and Hu, B.R. 1999 Survival and death promoting events after transient cerebral ischemia phosphorylation of Akt, release of cytochrome C and activation of caspase like proteases. J. Cereb. Blood Flow Metab. 19:1126-1135.

Ozawa, S.; Kamiya, H.; Tsuzuki, K. 1998 Glutamate receptors in the mammalian central nervous system. Prog. Neurobiol. 54:581-618.

Palmer, T.M. and Stiles, G.L. 1997 Structure-function analysis of inhibitory adenosine receptor regulation. Neuropharmacol. 9:1141-1147.

Palmer, T.M.; Gettys, T.W. and Stiles, G.L. 1995 Differential interaction with and regulation of multiple G-proteins by the rat A3 adenosine receptor. J. Biol. Chem. 270:16895-16902.

Park, T.S.; Beetch, J.W.; Gonzales, E.R.; Shah, A.R. and Gidday, M. 1997 Modulation of leukocyte-endothelial adherence in normoxic and ischemia brain by adenosine A2 and A3 receptors. Neurosci. Lett. 55:47-53.

Park, T.S.; Gidday, J.M. and Gonzales, E. 1991 Local cerebral blood flow response to locally infused 2-chloroadenosine during hypotension in piglets. Brain Res. Dev. Brain. Res. 1:73-77.

Park, C.K.; Nehls, D.G.; Graham, D.I.; Teasdale, G.M. and M^{c}Culloch, J. 1988 The glutamate antagonist MK-801 reduces focal ischemia damage in the rat. Ann. Neurobiol. 24:543-551.

Parkinson, F.E.; Zhang, Y.; Shepel, P.N.; Greenway, S.C.; Peeling, J. and Geiger, J.D. 2000 Effects of nitrobenzythioinosine on neuronal injury, adenosine levels and adenosine receptor activity in rat forebrain ischemia. J. Neurochem. 75:795-802.

Parkinson, F.E.; Johansson, B.; Lindstom, K. and Fredholm, B.B. 1995 Adenosine A1 and A2a receptors and nitrobenzylthiothiosine-sensitive transporters in the gerbil brain: no changes following long-term treatment the adenosine transport inhibitor propentofylline. Neuropharmacol. 35:79-89.

Parkinson, F.E.; Rudolphi, K.A. and Fredholm, B.B. 1994 Propentofylline: a nucleoside transport inhibitor with neuroprotective effects in cerebral ischemia. Gen. Pharmacol. 25:10531058.

Parkinson, F.E.; Patterson, A.R.; Young, J.D. and Cass, C.E. 1993 Inhibitory effects of propentofylline on [^{3}H]-adenosine influx. A study of three nucleoside transport systems. Biochem. Pharmacol. 46:891-897.

Parkinson, F.E. and Fredholm, B.B. 1990 Autoradiographic evidence for G-protein coupled A2 receptors in the rat neurostriatum using [^{3}H]-CGS 21680 as a ligand. Arch. Pharmacol. 342:85-89.

Pasini, F.L.; Guideri, F.; Picano, E.; Parenti, G.; Petersen, C.; Varga, A. and Di Perri, T. 1999 Increase in plasma adenosine during brain ischemia in man: A study during transient ischemic attacks and stroke. Brain Res. Bull. 51:327-330.

Patel, M.; Day, B.J.; Crapo, J.D.; Fridovich, I. and McNamara, J.O. 1996 Requirement for superoxide in excitotoxic cell death. Neuron 16:345-355.

Pearigen, P.; Gwinn, R. and Simon, R.P. 1996 The effects of *in vivo* hypoxia on brain injury. Brain Res. 725:184-191.

Pedata, F.; Pazzagli, M.; Tillis, S. and Pepeu, G. 1990 Regional differences in the electrically stimulated release of endogenous and radioactive adenosine and purine derivatives from rat brain slices. Naunyn- Schmiedeberg Arch. Pharmacol. 342:447-453.

Peng, L.A.; Juurlink, B.H. and Hertz, L. 1991 Differences in transmitter release, morphology, and ischemia-induced cell injury in cerebellar granule cell cultures developing in the presence and in the absence of potassium concentration. Brain Res. Dev. Brain Res. 63:1-12.

Pérez-Pinzón, M.A.; Xu, G.-P.; Dietrich, W.D.; Rosenthal, M. and Sick, T.J. 1997 Rapid preconditioning protects rats against ischemia neuronal damage after 3 but not 7 days of reperfusion following global cerebral ischemia. J. Cereb. Blood Flow Metab. 17:175-182.

Peters, O.; Back, T.; Lindauer, U.; Busch, C.; Megow, D.; Dreier, J. and Dirnagl, U. 1998 Increased formation of reactive oxygen species after permanent and reversible middle cerebral artery occlusion in the rat. J. Cereb. Blood Flow Metab. 18:196-205.

Phillis, J.W.; O'Regan, M.H. and Walker, G.A. 1989 Effects of two nucleoside transport inhibitors dipyridamole and soluflazine on purine release from the rat cerebral cortex. Brain Res. 481:309-316.

Phillis, J.W.; Walter, G.A.; O'Regan. M.H. and Stair, R.E. 1987 Increases in cerebral cortical perfusate adenosine and inosine concentrations during hypoxia and ischemia. J. Cereb. Blood Flow Metab. 7:679-686.

Picklo, M.J.; Amarnath, V.; McIntyre, J.O.; Graham, D.G. and Montine T.J. 1999 4-hydroxy-2(E)-nonenol inhibits CNS mitochondrial respiration at multiple sites. J. Neurochem 72:1617-1624.

Plamondon, H.; Blondeau, N.; Heurteaux, C. and Lazdunski, M. 1999 Mutually protective actions of kainic acid epileptic preconditioning and sublethal global ischemia on hippocampal neuronal death: involvement of adenosine A1 receptors and $K_{(ATP)}$ channels. J. Cereb. Blood Flow Metab. 19:1296-1308.

Powers, W.J. 1991 Cerebral hemodynamics in ischemic cerebrovascular disease. Ann. Neurol. 29:231-240.

Powers, W.J.; Press, G.A.; Grubb, R.L.; Gado, M. and Raichle, M.E. 1987 The effect of hemodynamically significant carotid artery disease on the hemodynamic status of the cerebral circulation. Annals of Int Med. 106:27.

Propoli, P.; Betto, P.; Reggio, R. and Ricciarello, G. 1995 Adenosine A2a receptor stimulation enhances striatal extracellular glutamate release in rats. Eur. J. Pharmacol. 287:215-217.

Pulsinelli, W. 1995 The ischemic penumbra in stroke. Sci. Am. Sci. Med. 2:16-25.

Purves, D.; Augustine, G.L.; Fitzpatrick, D.; Katz, L.C.; Lamatia, A-.S. and McNama, J.O. 1997 Neuroscience. Sinaver Associates Inc., Sutherland, Massachusetts, USA.

Ralevic, V. and Burnstock, G. 1998 Receptors for purines and pyrimidines. Pharmacol. Rev. 50:413-492.

Randall, R.D. and Thayer, S.A. 1992 Glutamate-induced calcium transient triggers delayed calcium overload and neurotoxicity in rat hippocampal neurons. J. Neurosci. 12:1882-1895.

Ratan, R.R.; Murphy, T.H. and Baraban, J.M. 1994 Oxidative stress induces apoptosis in embryonic cortical neurons. J. Neurochem. 62:376-379.

Reshef, A.; Sperling, O. and Shani, E. 2000 The adenosine -induced mechanism for the acquisition of ischemic tolerance in primary rat neuronal cultures. Pharmacol. Ther. 87:151-159

Reshef, A.; Sperling, O. and Zoref-Shani, E. 1999 Opening of K_{ATP} channels is mandatory for acquisition of ischemic tolerance by adenosine. Neuroreport 11:463-465.

Reshef, A.; Sperling, O. and Shani, E. 1998 Opening of ATP-sensitive potassium channels by cromakalin confers tolerance against chemical ischemia in rat neuronal cultures. Neurosci. Lett. 250:111-114.

Reshef, A.; Sperling, O. and Shani, E. 1997 Activation and inhibition of protein kinase C protect neuronal cultures against ischemia-reperfusion insult. Neurosci. Lett. 238:37-40.

Reshef, A.; Sperling, O. and Shani, E. 1996 Preconditioning of primary rat neuronal cultures against ischemic injury: characterization of the "time window of protection". Brain Res. 741:252-257.

Rice, J.E.; Vannucci, R.C. and Brierly, J.B. 1981 The influence of immaturity on hypoxia-ischemia brain damage in the rat. Ann. Neurol. 9:131-141.

Rie, M.A. and Bernad, P.G. 1980 Prolonged hypoxia in man without circulatory compromise fails to demonstrate cerebral pathology. Neurology 30:443-444.

Riepe, M.W.; Esclaire, F.; Kasischke, K.; Schreiber, S. and Nakase, H. 1997 Increased hypoxic tolerance by chemical inhibition of oxidative phosphorylation: "chemical preconditioning." J. Cereb. Blood Flow Metab. 17:257-264.

Rigley, S.T.; Sweeney, M.I. and Tasker, R.A.R. 2000 Cyclopentyladenosine decreases apoptotic cell death in cerebellar granule neurons after oxygen-glucose deprivation. Soc. Neurosci. Abstract 26:2067.

Ringleb, P.A.; Schellinger, P.D.; Schranz, C. and Hacke, W. 2002 Thrombolytic therapy within 3 to 6 hours after onset of ischemic stroke: useful or harmful? Stroke 33:1437-1441.

Rivkees, S.A.; Price, S.L. and Zhou, F.C. 1995 Immunohistochemical detection of A1 adenosine receptors in the rat brain with emphasis on localization in the hippocampus formation cerebral cortex cerebellum and basal ganglia. Brain Res. 528:193-203.

Roberts-Lewis, J.M.; Savage, M.J.; Marcy, V.R.; Pinsker, L.R. and Siman, R. 1994 Immunolocalization of calpain I-mediated spectrin degradation to vulnerable neurons in ischemic gerbil brain. J. Neurosci. 14:1934-3944.

Rodnitzky, R.L. 1995 Clinical correlation stroke. In: Neuroscience in Medicine. Conn, P.M. (ed) Lippincott Company, Philadelphia, Pennsylvania. 167-170.

Rolls, E.T.; Treves, A.; Robertson, R.; Georges-Francois, P. and Panzeri, S. 1998 Information about spacial view in an ensemble of primate hippocampal neurons. J. Comp. Neurol. 399:671-676.

Rosenberg, G.A. 1999 Ischemic brain edema. Prog. Cardiovasc. Dis. 42:209-216.

Rothman, S.M. and Olney, J.W. 1986 Glutamate and the pathophysiology of hypoxic-ischemic brain damage. Ann Neurol. 19:105-111.

Rubino, A.; Ralevic, V. and Burnstock, G. 1995 Contribution of P1-(A2b subtype) and P2-purinoceptors to the control of vascular tone in the rat isolated mesenteric arterial bed. Br. J. Pharmacol. 115:648-652.

Rudolphi, K.A.; Schubert, P.; Parkinson, F.E. and Fredholm. B.B. 1992a Neuroprotective role of adenosine in cerebral ischemia. Trends Pharmacol. Sci. 13:439-445.

Rudolphi, K.A.; Schubert, P.; Parkinson, F.E. and Fredholm. B.B. 1992b Adenosine and brain ischemia. Cerebrovasc. Brain Metab. Rev. 4:346-369.

Salvatore, C.A.; Jacobson, M.A.; Taylor. H.E.; Linden, J. and Johnson, R.G. 1993 Molecular cloning and characterization of the human A3 adenosine receptor. Proc. Natl. Acad. Sci. USA 90:10365-10369.

Sattler, R. and Tymianski, M. 2001 Molecular mechanisms of glutamate receptor mediated excitotoxic neuronal cell death. Mol. Neurobiol. 24:107-129

Saura, C.A.; Mallol, J.; Camela, E.I.; Lluis, C. and Franco, R. 1998 Adenosine deaminase and A1 adenosine receptors internalize together following agonist-induced receptor desensitization. J. Biol. Chem. 273:17610-17617.

Schoepp, D.D. 2001 Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J. Pharmacol. Exp. Ther. 299:12-20.

Schoepp, D.D. and Conn, P.J. 1999 Metabotropic glutamate receptors in brain function and pathology. Trends Pharmacol. Sci. 14:13-20.

Schomer, D.F.; Marks, M.P.; Steinberg, G.K.; Johnston, I.M.; Boothroyd, D.M.; Ross, M.R.; Pelc, N.J. and Enzmann, D.R. 1994 The anatomy of the posterior communicating artery as a risk factor for ischemic cerebral infarction. N. Eng. J. Med. 330:1565-1570.

Schrader, J.; Schütz, W. and Bardenheuer, B. 1981 Role of S-adenosylhomocysteine hydrolase in adenosine metabolism in mammalian heart. J. Biochem. 196:65-70.

Schubert, P.; Rudolphi, K.A.; Fredholm, B.B. and Nakamura, Y. 1994 Modulation of nerve and glial functions by adenosine - role in development of ischemia damage. Int. J. Biochem. 26:1227-1236.

Schubert, P. and Mager, R. 1991 The critical input frequency for NMDA-mediated neuronal Ca^{2+} frequency depends on endogenous adenosine. Int. J. Purine Pyrimidine Res. 2:11-16.

Schwartzman, R.A. and Cidlowski, J.A. 1993 Apoptosis: The biochemistry and molecular biology of programmed cell death. Endocr. Rev. 14:133-151.

Sebastião, A.M. and Robeiro, J.A. 1996 Adenosine A2 receptor-mediated excitatory actions on the nervous system. Prog. Neurobiol. 48:167-189.

Segal, M.; Rogawski, M.A. and Barker, J.L. 1984 A transient potassium conductance regulates the excitability of cultured hippocampal and spinal neurons. Brain Res. 4:604-609.

Shaller, C.A; Jacques, S. and Sheldon, H. 1980 The pathophysiology of stroke: A review with molecular considerations. Surg. Neurol. 14:433-443.

Sheardown, M.J. and Knutsen, L.J.S. 1996 Unexpected neuroprotection observed with the adenosine A2a receptor agonist CGS21680. Drug Dev. Res. 39:108-114.

Sheardown, M.J.; Hansen, A.J.; Thomsen, C.; Judge, M.E. and Knutsen, L.J.S. 1995 Novel adenosine agonists: A strategy for stroke therapy. In: Stroke Clinical Trials: Issues in Design Conduct and Analysis. Grotta, J.; Miller, M.P. and Buchan, A.M. (eds). International Business Communications. Worcester, Mass. 187-214.

Shimizu, S.; Nagayama, T.; Jin, K.L.; Zhu, L.; Loeffert, J.E.; Watkins, S.C.; Graham, S.H. and Simon, R.P. 2001 bcl-2 antisense treatment prevents induction of tolerance to focal ischemia in the rat brain. J. Cereb. Blood Flow Metab. 21:233-243.

Shimizu, H.; Graham, S.H.; Chang, L.-H.; Mintorovich, J.; James, T.L.; Faden, A.I. and Weinstein, P.R. 1993 Relationship between extracellular neurotransmitter amino acids and energy metabolism during cerebral ischemia in rats monitored by microdialysis and *in vivo* magnetic resonance spectroscopy. Brain Res. 605:33-42.

Shuaib, A. and Breker-Klassen, M.M. 1997 Inhibitory mechanisms in cerebral ischemia. Neurosci. Biobehav. Rev. 21:219-226.

Siesjö, B.K. 1992 Physiology and treatment of focal cerebral ischemia. J. Neurosurg. 77:337-354.

Siesjö, B.K. and Bengtsson, F. 1989 Calcium fluxes, calcium antagonists and calcium related pathology in brain ischemia, hypoglycemia and spreading depression: A unifying hypothesis. J. Cereb. Blood Flow Metab. 9:127-140.

Siesjö, B.K. 1975 Brain Energy Metabolism. John Wiley and Sons, New York. 12-20.

Simon, R.P. and Shiraishi, K. 1990 NMDA antagonist decreases stroke size and regional glucose metabolism. Ann. Neurol. 27:606-611.

Simpson, R.E.; O'Regan, M.H.; Perkins, L.M. and Phillis J.W. 1992 Excitatory amino acid release from the ischemic rat cerebral cortex: effects of adenosine receptor agonists and antagonists. J. Neurochem. 58:1683-1690.

Sinclair, C.D.J.; LaRivière, C.G.; Young, J.D.; Cass, C.E.; Baldwin, S.A. and Parkinson, F.E. 2000 Purine uptake and release in rat glioma cells. J. Neurochem. 75:1528-1538.

Sollevi, A. 1986 Cardiovascular effects of adenosine in man: Possible clinical implications. Prog. Neurobiol. 27:319-345.

Sorimashi, H.; Saido, T.C. and Suzuki, K. 1994 New era of calpain research: Discovery of tissue specific calpains. FEBS Lett. 343:1-5.

Stagliano, N.E.; Pérez-Pinzón, M.A. Moskowitz, M.A. and Huang, P.L. 1999 Focal ischemic preconditioning induces rapid tolerance to middle cerebral artery occlusion in mice. J. Cereb. Blood Flow Metab. 19:757-761.

Stambaugh, K.; Jacobson, K.A.; Jiang, J.L. and Liang, B.T. 1997 A novel cardioprotective function of adenosine A1 and A3 receptors during prolonged simulated ischemia. Am. J. Physiol. Heart Circ. 273:H501-H505.

Strohmeier, G.R.; Reppert, S.M.; Lencer, W.I. and Madara, J.L. 1995 The A2b adenosine receptor mediates c-AMP responses to adenosine receptor agonists in human intestinal epithelia. J. Biol. Chem. 270:2387-2394.

Spira, M.E.; Oren, R.; Dormann, A.; Ilouz, N. and Lev, S. 2001 Calcium, protease activation and cytoskeleton remodelling underlie growth cone formation and neuronal regulation. Cell Mol. Neurobiol. 21:591-604.

Statistics Canada. 2000 Heart and Stroke Disease: Quick Facts. http://www.hsf.ab/hrt_stats.html.

Sussmann, M.S.; Buchman, T.G. and Bulkey, G.B. 1989 Free radical-mediated reperfusion injury: The fundamental mechanism and its potential for clinical application. In: Free Radicals, Diseased States and Anti-Radical Interventions. Rice-Evans, C. (ed) Richelieu, London. 13-56.

Sutherland, G.; Bose, R.; Louw, D. and Pinsky, C. 1991 Global elevation of brain superoxide dismutase activity following forebrain ischemia. Neurosci. Lett. 128:169-172.

Suzuki, W.A.. And Amaral, D.G. 1994a Topographic organization of the reciprocal connections between the monkey entorhinal cortex and the perirhinal and parahippocampal cortices. J. Neurosci. 14:1856-1877.

Suzuki, W.A.. And Amaral, D.G. 1994b Perirhinal and parahippocampal cortices of the macaque monkey: Cortical afferens. J. Comp. Neurol. 350:497-533.

Swanson, L.W. 1981 A direct projection from Ammon's horn to the prefrontal cortex in the rat. Brain Res. 217:150-154.

Swanson, L.W. and Cowan, W.M. 1976 Autoradiographic studies of the development and connections of the septal area in the rat. Science 189:303-304.

Swanson, T.H.; Drazba, J.A. and Rivkees, S.A. 1995 Adenosine A1 receptors are located predominantly on axons in the rat hippocampus. J. Comp. Neurol. 4:517-531.

Sweeney, M.I. 1997 Neuroprotective effects of adenosine in cerebral ischemia: window of opportunity. Neurosci. Behav. Rev. 21:207-217.

Sweeney, M.I. and Dolphin, A.C. 1995 Adenosine A1 agonists and the Ca^{2+} channel agonist Bay K 8644 produce a synergistic stimulation of the GTPase activity in rat frontal cortical membranes. J. Neurochem. 64:2034-2042.

Szatkowski, M. and Atwell, D. 1994 Triggering and execution of neuronal death in brain ischemia: two phases of glutamate release by different mechanisms. Trends Neurosci. 17:359-365.

Taber's Cyclopedic Medical Dictionary (19^{th} ed.) 2001 Venes, D. (ed) F.A. Davis Company. Philadelphia, Pennsylvania.

Tamura, A.; Kawai, K. and Takagi, K. 1997 Animal models used in cerebral ischemia and stroke research. In: Clinical Pharmacology of Cerebral Ischemia. Ter Horst, G.J. and Korf, J. (eds) Humana Press, New Jersey. 265-294.

Tan, S.; Zhou, F.; Nielson, V.G.; Wang, Z.; Gladson, C.L. and Parks, D.A. 1999 Increased injury following intermittent fetal hypoxia-reoxygenation is associated with increased free radical production in fetal rabbit brain. J. Neuropathol. Exp. Neurol. 58:972-981.

Tan, S.; Wood, M. and Maher, P. 1998 Oxidative stress induces a form of programmed cell death with characteristics of both apoptosis and necrosis in neuronal cells. J. Neurochem. 71:95-105.

Tanaka, H.; Calderon, A.; Jover, T.; Grooms, S,Y.; Yokota, H.; Zukin, S. and Bennett, M. 2002 Ischemic preconditioning acts up-stream of GluR2 down-regulation to afford neuroprotection in the hippocampal CA1. Proc. Natl. Acad. Sci. 99:2362-2367.

Thompson, S.M.; Capogna, M. and Scanziani, M. 1993 Presynaptic inhibition in the hippocampus. Trends Neurosci. 16:222-227.

Toms, N.J. and Roberts, P.J. 1999 Group I mGlu receptors elevate $[Ca^{2+}]$I in rat cultured cortical type 2 astrocytes: $[Ca^{2+}]$i synergy with adenosine A1 receptors. Neuropharmacol. 38:1511-1517.

Toyoda, T.; Kassel, N.F. and Lee, K.S. 1997 Induction of ischemic tolerance and antioxidant activity by brief focal ischemia. Neuroreport 8:847-851.

Trigger, D.J. 1990 Calcium antagonists, history and perspective. Stroke 21:49-58.

Uchiyama, Y. 1995 Apoptosis: the history and trends of its studies. Arch Histol. Cytol. 58:127-137.

van Calker, D.; Muller, M. and Hamprecht, B. 1978 Adenosine inhibits the accumulation of cyclic AMP in cultured brain cells. Nature 276:839-841.

van Everdingen, K.J.; Visser, G.H.; Klijn, C.K. Kappelle, L.J. and van der Grond, J. 2001 Role of collateral flow on cerebral hemodynamics in patients with unilateral internal carotid artery occlusion. Ann. Neurol. 44:167-176.

Vianna, M.R.M.; Alonso, M.; Viola, H.; Quevedo, J.; de Paris, F.; Furman, M.; Levi de Stein, M.; Medina, J. and Izquierdo, I. 2000 Role of hippocampal signalling pathways in long-term memory formation of a nonassociative learning task in the rat. Learn. Memor. 7:333-340.

von Lubitz, D.K.E.J.; Lin, R.C.S.; Bischofberger, N.; Beenhakker, M.; Boyd, M.; Lipartowska, R. and Jacobson, K.A. 1999 Protection against damage by adenosine amine cogener, a potent and selective adenosine A1 receptor agonist. Eur. J. Pharmacol. 369:313-317.

von Lubitz, D.K.E.J.; Lin, R.C.S. and Jacobson, K.A. 1995a Cerebral ischemia in gerbils: effects of acute and chronic treatment with adenosine A2a receptor agonist and antagonist. Eur. J. Pharmacol. 287:295-302.

von Lubitz, D.K.E.J.; Carter, M.F.; Deutsch, S.I.; Lin, R.C.-S.; Mastropaolo, J.; Meshulam, Y. and Jacobson, K.A. 1995b The effects of adenosine A3 receptor stimulation on seizures in mice. Eur. J. Pharmacol. 275:23-29.

von Lubitz, D.K.E.J.; Lin, R.C.S.; Melman, N.; Xiao-duo, J.; Carter, M.F. and Jacobson, K.A. 1994a Chronic administration of selective adenosine A1 receptor agonist or antagonist in cerebral ischemia. Eur. J. Pharmacol. 256:161-167.

von Lubitz, D.K.E.J.; Lin, R.C.; Popik, P.; Carter, M.F. and Jacobson, K.A. 1994b Adenosine A3 receptor stimulation and cerebral ischemia. Eur. J. Pharmacol. 263:59-67.

von Lubitz, D.K.E.J.; Dambrosia, J.M. and Redmond, D.J. 1989 Protection effect of cyclohexyladenosine in treatment of cerebral ischemia in gerbils. Neurosci. 30:451-462.

von Lubitz, D.K.J.E.; Dambrosia, A.; Kempski, O. and Redmond, D.J. 1988 Cyclohexyladenosine protects against neuronal death following ischemia in the CA1 region of the hippocampus. Stroke 19:1133-1139.

Wann, K.T. 1993 Neuronal sodium and potassium channels: structure and function. Br. J. Anesth. 71:2-14.

Webb, R.L.; Barclay, B.W and Crayhill, S.C. 1991 Cardiovascular effects of adenosine A2 agonists in the conscious spontaneously hypertensive rat: A comparative study of three structually distinct ligands. J. Pharm. Exp. Ther. 259:1203-1212.

White, P.J.; Rose-Myer, R.B and Hope, J.W. 1996 Functional characterization of adenosine receptors in the NTS mediating hypertensive responses in rat. Br. J. Pharmacol. 2:305-308.

Whittingham, T.S. 1990 Aspects of brain energy metabolism and cerebral ischemia. In: Cerebral Ischemia and Resuscitation. Schurr, A. and Rigor, B.M. (eds) CRC Press. Boca Raton, Florida. 101-121.

Williams, M. 1993 Purigenic drugs: Opportunities in the 1990's. Drug Dev. Res. 28:438-441.

Wong, G.H.; Elwell, J.H.; Oberley, L.W. and Goeddell, D.V. 1989 Mangenese superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. Cell 58:923-931.

Xu, H.; Abiki, M. and Nagoya, J. 2002 Neuroprotective effects of hyperthermic preconditioning on infarcted volume after middle cerebral artery occlusion in rats: role of adenosine receptors. Crit. Care Med. 30:1126-1130.

Zalewska, T. 1996 Calpain as proposed target for neuroprotective treatment of brain ischemia. Neuropathol. 34:121-127.

Zhao, Z.; Francis, C.E. and Ravid, K. 1997 An A3-subtype adenosine receptor is highly expressed in rat vascular smooth cells: its role in attenuating adenosine induced increase in c-AMP. Microvasc. Res. 3:243-252.

Zhongzhen, N.; Mei, Y.; Ford, M.; Rybak, L.; Marcuzzi, A.; Ren, H.; Stiles, G. and Ramkumar, V. 1998 Oxidative stress increases A1 adenosine receptor expression by activating nuclear factor κB. Mol. Pharmacol. 53:663-669.

Zhou, J.; Meno, J.; Hsu, S.S.-F. and Winn, H.R. 1994 Effects of theophylline and cyclohexyladenosine on brain injury following normo- and hyperglycemic ischmeia: a histopathologic study in the rat. J. Cereb. Blood Flow Metab. 14:166-173.

Zhou, Q.Y.; Olah, M.E.; Johnson, R.A. and Li, C. 1992 Molecular cloning and characterization of an adenosine receptor: The A3 adenosine receptor. Proc. Natl. Acad. Sci. USA 89:7433-7436.

Zhu, C.Z. and Auer, R.N. 1995 Graded hypotension and MCA occlusion duration: effect in transient focal ischemia. J. Cereb. Blood Flow Metab. 15:980-988.

Zhu, P.J. and Krnjevic, K. 1998 Persistent block of synaptic function by prolonged hypoxia. J.Cereb. Blood flow Metab. 16:566-577.

Zimmermann, H.; Braun, N.; Kegal. And Heine, P. 1998 New insights into the molecular structure and function of ectonucleotidases in the nervous system. Neurochem. 32:421-425.

Zivin, J. and Miller, L.P. 1999 Stroke: Present and future therapy. In: Stroke Therapy. Basic Preclinical and Clinical Directions. Miller L.P. (ed) Wiley-Liss Inc. New York. 401-421.