LIPID METABOLISM IN ISCHEMIC NEURONAL DEATH

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ABSTRACT

Alterations in lipid metabolism including the release of arachidonic acid (**AA**) are key events which eventually lead to neuronal death following cerebral ischemia/ reperfusion. **AA** is released by the activation of phospholipases and the alteration of phosphatidylcholine synthesis. Released **AA** is either re-incorporated into membranes or metabolized by cyclooxygenases (**COX**) /lipoxygenases to form prostaglandins, leukotrienes, and reactive oxygen species (**ROS**). ROS formed by **AA** metabolism generate lipid peroxides and the cytotoxic products 4-hydroxynonenal and acrolein, which covalently bind to cellular proteins and alter their function. **AA** can also stimulate sphingomyelinase to produce ceramide, a potent pro-apoptotic agent. Ceramide and oxygen radicals have been implicated as causative agents in mitochondrial dysfunction and release of cytochrome c, which initiates the cellular death cascade by activation of caspase-3.

These pathways may be clinically important since accumulation of ceramide and induction of COX-2 have been demonstrated in human brain following cerebral ischemia. Our studies have shown that attenuating **AA** release and limiting **AA** conversion to oxidative metabolites significantly protected the vulnerable hippocampal CA_1 neurons of the cornu Ammonis (CA) after transient ischemia.

INTRODUCTION

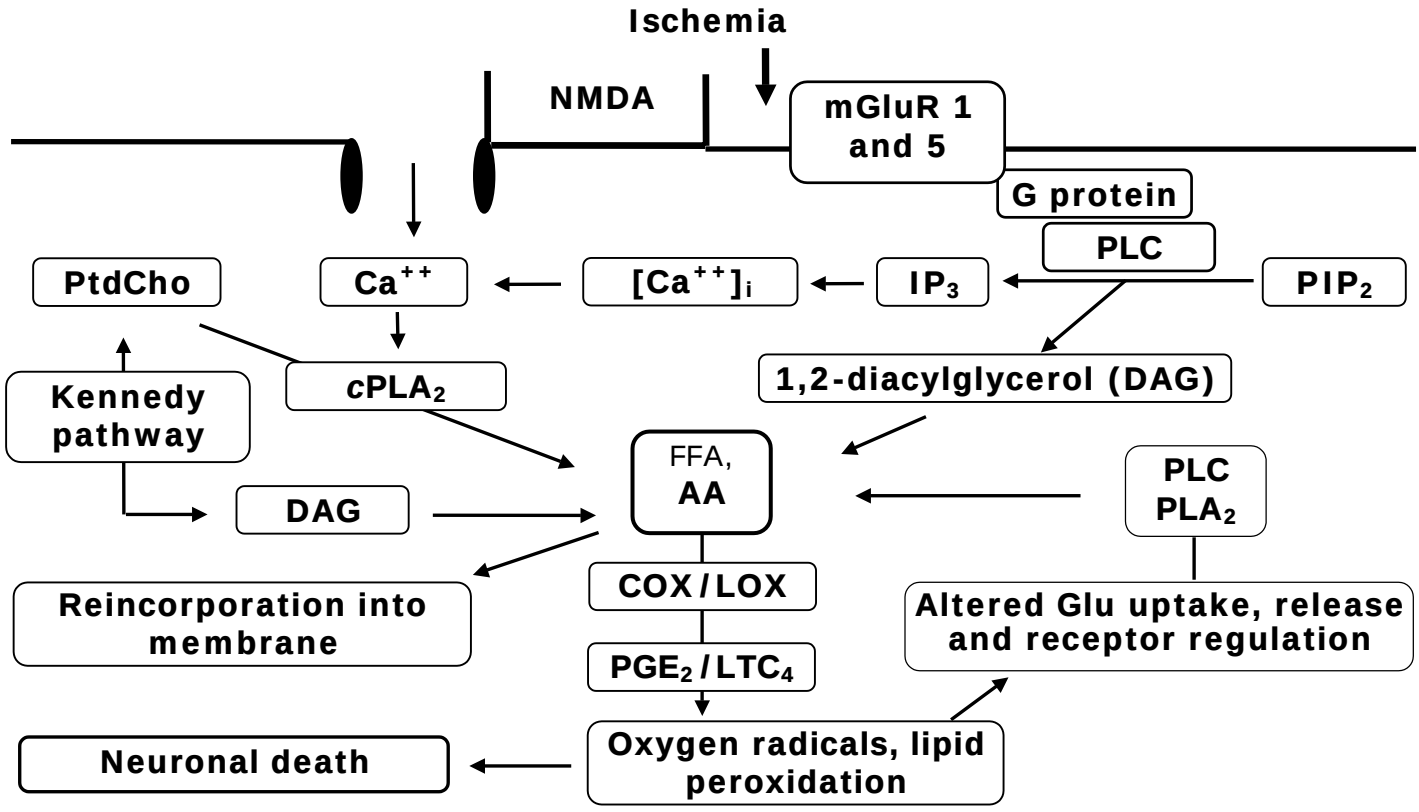
Cerebral ischemia is caused by reduced blood supply to the brain and can be focal (regional) or global (forebrain). Cerebral ischemia/reperfusion initiates a complex series of metabolic events leading to neuronal death involving necrotic and apoptotic (programmed cell death) processes [1]. Within hours after severe forebrain ischemia, some neurons die in the striatum, hippocampus and lateral thalamus. However, in the hippocampus, CA_1 pyramidal neurons undergo selective delayed death several days after the injury. Because the process of tissue destruction in both forms of brain ischemia is not completed for several days, a therapeutic window may be present. Understanding the sequence of molecular events leading to

neuronal death is critical for the development of therapeutic strategies. The exact mechanisms which predispose the hippocampus to ischemic injury are largely unknown, but appear to include anatomical, physiological and neurochemical factors [2,3]. One such critical factor is the hydrolysis of membrane phospholipids, resulting in the accumulation of arachidonic acid (**AA**).

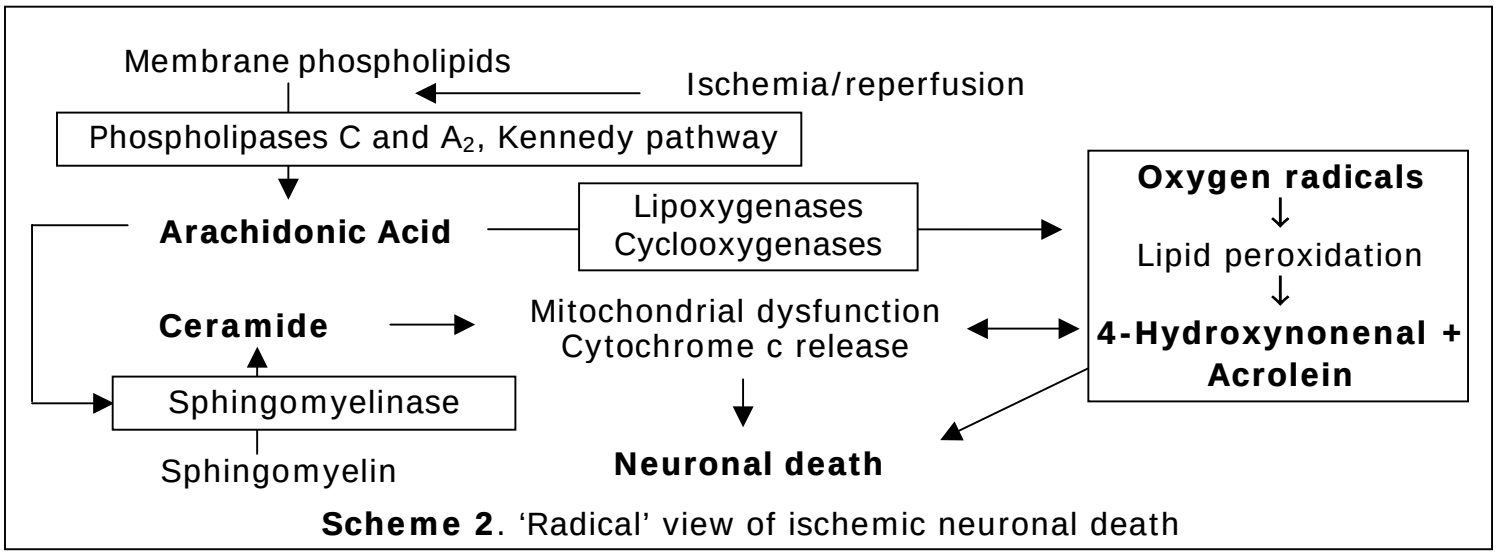
Poly-unsaturated fatty acids including **AA** are integral components of membrane phospholipids, which are important in maintaining structural and functional characteristics of cell membranes. Hydrolysis of phospholipids as a cellular response to various external stimuli, such as cerebral ischemia, results in alteration of the membrane lipid bilayer and release of free fatty acids (**FFA**), including **AA**. **AA** is one of the most important fatty acids in biological systems, including the brain. **AA** metabolism is highly regulated as part of the normal physiological function of the nervous system, whereas pathological **AA** liberation and metabolism contributes to neuronal death (Schemes **1** and **2**) [3,4].

Several pathways contribute to the hydrolysis of membrane phospholipids and the release of **AA**. Glutamate released during ischemia stimulates neuronal receptors, resulting in increases in intracellular Ca^{++} (**$[Ca^{++}]_i$**) and activation of phospholipases C (**PLC**) and A_2 (**PLA_2**) [5]. PLC hydrolyzes phosphatidylinositol lipids to release 1,2-diacylglycerol (**DAG**), which is further hydrolyzed to FFA including **AA**. PLA_2 hydrolyzes **AA** at the *sn*-2 position of phosphatidylcholine (**PtdCho**).

PtdCho is synthesized from DAG and cytidine-5'-diphosphocholine (**CDP-choline**) (Kennedy pathway, Scheme **1**). This reaction results in the formation of cytidine 5'-monophosphate, which is normally converted to its triphosphate. Energy failure and the loss of ATP during ischemia cause accumulation of cytidine 5'-monophosphate, stimulating the release of DAG



Scheme 1. Lipid metabolism in ischemic neuronal death.



Scheme 2. 'Radical' view of ischemic neuronal death

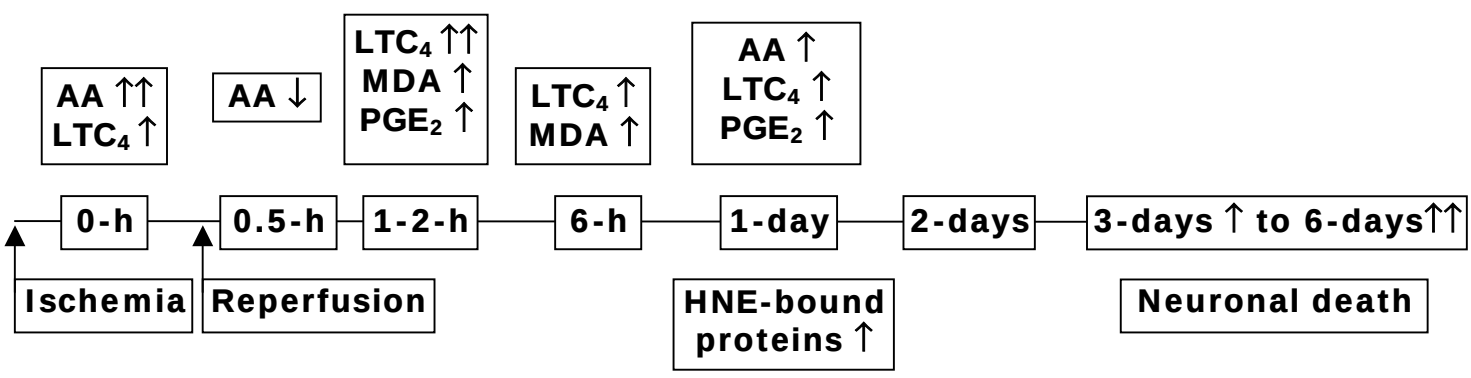
from PtdCho (Scheme **1**) [6].

AA is a common substrate for cyclooxygenases (**COX**) and lipoxygenases (**LOX**). Oxidative metabolism of **AA** to biologically-active prostaglandins, leukotrienes and thromboxanes produces oxygen radicals that cause lipid peroxidation and cytotoxic aldehydes [7], including malondialdehyde (**MDA**), 4-hydroxynonenal (**HNE**), and acrolein. Oxygen radicals and prostaglandin E_2 (**PGE_2**) may feed back to phospholipase activation by altering glutamate uptake and release (Scheme **1**) [8].

The released **AA** may also stimulate sphingomyelinase to generate ceramide [9]. Ceramide has been implicated as a potent inducer of apoptosis (Scheme **2**) [10-13] by inhibiting the mitochondrial electron transport [14] and releasing cytochrome c [15]. Oxygen radical production and lipid peroxidation have been implicated as both initiators and consequences of mitochondrial dysfunction (Scheme **2**) [16-19].

In the gerbil ischemia model, the time course of events related to **AA** release and neuronal death are presented in Scheme **3.** Forebrain ischemia/reperfusion in gerbil is a model for human cerebral ischemia resulting from transient cardiac arrest. In this model, delayed neuronal death occurs in the CA_1 region, beginning ~4-days after cerebral ischemia. Transient forebrain ischemia in gerbil provides a practicable model for understanding mechanisms and evaluating neuroprotective agents.

HIPPOCAMPAL CA_1 NEURONS ARE SUSCEPTIBLE TO ISCHEMIA-INDUCED NEURONAL DEATH. Neurons of the hippocampal CA_1 are more vulnerable to forebrain ischemia compared to CA_3 and other regions and undergo delayed death [20,21] with features of both apoptosis [1,22,23] and necrosis [24]. It has been



Scheme 3. Time course of events after gerbil transient forebrain ischemia.

suggested that apoptosis is induced in neurons following a necrotic injury as an adaptation to remove catastrophically injured cells through a non-inflammatory process. However, due to variable energy collapse in injured neurons, only a subset of the classical energy-dependent apoptotic process can be carried to completion, usually coincident with necrotic features [25].

Several important factors may pre-dispose CA_1 neurons to ischemic injury. CA_1 neurons are less proficient than CA_3 neurons at sequestering and exporting excess Ca^{++} [26] and have a higher density of NMDA and inositol-1,4,5-trisphosphate (**IP_3**) receptors [27,28].

Ischemia induces a number of genes, however the general decline in RNA to protein translation may affect neuronal survival the most [29]. The bcl-2 family of genes are up-regulated following ischemia and are involved in either pro-apoptotic (bax, bcl-x_S, bad) or anti-apoptotic (bcl-2 and bcl-x_L) signaling. The neuroprotective effects of Bcl-2 expression have been attributed to its ability to regulate mitochondrial dysfunction [17,30]. Mitochondrial dysfunction results in 1) release of cytochrome c and apoptosis inducing factor 2) activation of caspases after transient ischemia [17,23,31-34]. Anti-oxidant functions have also been proposed, since cells over-expressing Bcl-2 had elevated levels of glutathione (which scavenges toxic aldehydes) and superoxide dismutase (**SOD**) [35,36] and were resistant to oxidative stress induced apoptosis [7,37]. The hippocampal CA_1 neurons destined to die cease making Bcl-2 protein even though bcl-2 *m*RNA expression is induced following ischemia, whereas the *m*RNA is translated in the CA_3 region [38]. Thus hippocampal CA_1 neurons may be particularly vulnerable to ischemic injury resulting from free radical-induced lipid peroxidation.

REACTIVE OXYGEN SPECIES (ROS) CAUSE LIPID PEROXIDATION AND NEURONAL DAMAGE [39]. The prominent free radicals generated during transient ischemia are ROS and nitric oxide (**NO•**). Oxygen radical generation during ischemia/reperfusion may occur from mitochondria and oxidative metabolism of **AA** [4,40]. Both ROS (O_2^-• and OH•) and NO• (formed by nitric oxide synthase (**NOS**)) are involved in glutamate neurotoxicity and contribute to neuronal death. O_2^-• and NO• can act independently, or also concertedly to form highly reactive peroxynitrite ($ONOO^-$). Recent studies using transgenic models (altered expression of NOS and SOD) demonstrated the role of NO• and O_2^-• in neuronal death after cerebral ischemia [41-43]. BN 80933, which exerts the dual effects of NOS inhibition and ROS scavenging, provided significant neuroprotection even when administered after the CNS injury [44].

Formation of ROS, including O_2^-•, H_2O_2, and OH•, and the ensuing peroxidation of membrane lipids is a well-recognized mechanism of tissue damage in ischemia/ reperfusion [45,46]. Lipid peroxidation may be particularly detrimental to neurons by damaging ion transport proteins and other regulatory systems in membranes. The peroxidation of poly-unsaturated fatty acids results in the formation of aldehydic by-products, such as MDA, plus cytotoxic HNE and acrolein [47-51]. HNE and acrolein form covalent cross-links with proteins to lysine, cysteine and histidine residues [7,48,50,52]. HNE modifies ion homeo-stasis [53], decreases Na^+/K^+-ATPase activity [54], impairs glutamate transport [55,56], disrupts neuronal calcium homeo-stasis, causes mitochondrial dysfunction

[57], caspase activation [58] and oxidative stress-induced neuronal apoptosis [7]. Acrolein depletes the cellular antioxidant glutathione and contributes to mitochondrial dysfunction and neuronal death [47,59].

LIPID PEROXIDATION INCREASED AFTER TRANSIENT CEREBRAL ISCHEMIA. MDA levels were assessed as thiobarbituric acid reactive species as a marker of lipid peroxidation [60]. Gerbils subjected to 10-min transient cerebral ischemia showed significant elevation of MDA levels in the hippocampus (55 ± 6 and 59 ± 6 nmol/g tissue at 2-h and 6-h reperfusion, respectively) compared to shams (22 ± 4 nmol/g tissue) [61]. The increase in MDA at 2-h corresponds with the observed increase in leukotriene C_4 (**LTC_4**) [61,62], suggesting increased lipid peroxidation due to metabolism of **AA** by COX/LOX, which are closely associated with oxygen radical generation [4,40,63]. Between 2 to 6-h, LTC_4 levels declined whereas MDA levels remained elevated during this period. Other pathways, including cyclooxygenases, contribute to the lipid peroxidation and these may account for the sustained elevation of MDA.

We adopted HNE immunochemical methods [7] to localize cellular distribution of the HNE modified proteins in the hippocampus. Our immunochemical studies showed that the CA_1 neurons stained with HNE-antibody at 1-day after ischemia in the gerbil hippocampus. No HNE reactive cells were observed in the sham-operated gerbils (Fig. **1**). Recent studies also reported immunohistochemical detection of HNE after experimental brain injury in rat [64].

AA RELEASE AND METABOLISM CONTRIBUTES TO THE FORMATION OF ROS AND NEURONAL DEATH. Cerebral ischemia causes large increases in FFA including **AA** by alteration of PtdCho synthesis (Kennedy pathway) from DAG, and the action of phospholipases [65,66]. On reperfusion, with restoration of ATP and oxygen, the **AA** released during ischemia is either reincorporated into phospholipids or is oxidized by COX/LOX. Release of **AA** from cardiolipin, an inner mitochondrial membrane lipid that is essential for electron transport [67], has been demonstrated following cerebral ischemia [68]. The amount of **AA** in cardiolipin is relatively small compared to the total phospholipid pool, and loss of **AA** from cardiolipin likely contributes little to the total free **AA**. However, **AA** and other poly-unsaturated fatty acids are essential components to

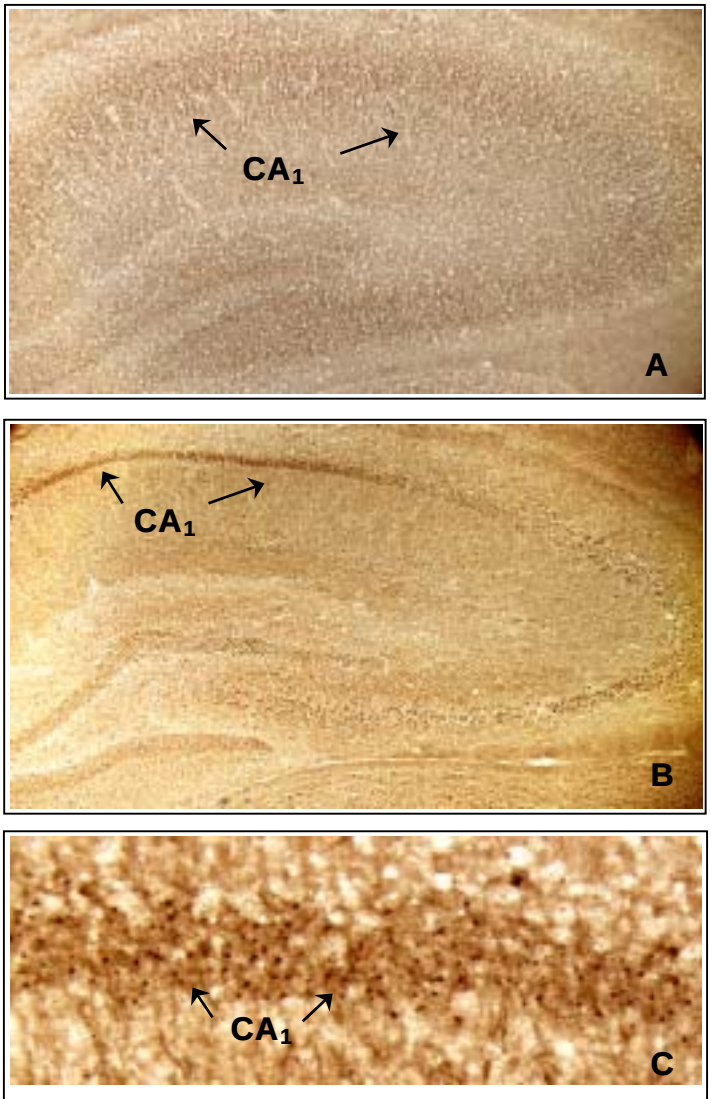


Figure 1. HNE immunochemistry. A. Sham, 100x; B. Ischemic, 1-day, 100x; C. Ischemic, 1-day, 400x

maintain the functionality of cardiolipin. Therefore, the degradation of cardiolipin and its effect on mitochondrial energy status may be more important than the **AA** released from cardiolipin.

The activation of PLC and PLA_2 in ischemia is initiated by stimulation of group I metabotropic glutamate receptors (**mGluR** 1 and 5), and increases in $[Ca^{++}]_i$ through NMDA-receptors [69] (Scheme **1**). The oxidative metabolism of **AA** by COX/LOX is considered to be a major source of ROS in ischemia/reperfusion [63,70]. **AA**, its metabolites and oxygen radicals promote the release and inhibit the uptake of excitatory amino acids [71], which may further stimulate phospholipases and the release of **AA** (Scheme **1**). **AA** may also stimulate sphingomyelinase to generate pro-apoptotic ceramide [9]. **AA**-induced ceramide generation could represent a novel pathway by which serial production of lipid mediators regulates neuronal fate after ischemia/reperfusion.

DOES mGluR 1 OR 5 CONTRIBUTE TO NEURONAL INJURY? A major mechanism of neuronal damage during ischemia in those brain areas destined to die is the massive release of endogenous glutamate/ aspartate [69] and over-stimulation of excitatory amino acid receptors. Excitotoxic neuronal injury appears to be mediated through stimulation of ionotropic (NMDA-, AMPA-, kainate) receptors, and G-protein coupled mGluR [72-74]. Of the three subtypes of mGluR (Table **1**) [75], group I mGluR (mGluR 1 and 5) are coupled to PLC through G-proteins [76]. Antagonists of group I mGluR proved to be neuroprotective [77,78]. (S)-α-Methyl-4-carboxyphenylglycine (**MCPG**), a mGluR 1 and 5 antagonist, completely blocked the glutamate-stimulated phosphatidylinositol hydrolysis [79] and showed neuroprotection in the CA_1 region [78]. Other studies suggest that PLC-coupled mGluR 5 might be involved in ischemic brain injury [73]. The role of group I mGluR in CNS injury has not been clearly defined since a number of studies provided conflicting results [73,80-83].

MCPG, MPEP, AIDA, CDP-CHOLINE AND $AACOCF_3$ ATTENUATED AA RELEASE [84,85]. Our results indicated that a significant amount of **AA** was released immediately after 10-min ischemia (0-min reperfusion) which returned to sham levels by 30-min reperfusion. A later release of **AA** occurred after 1-day [61]. Treatment with MCPG, 2-methyl-6-(phenylethynyl) pyridine (**MPEP**), 1-aminoindan-1,5-dicarboxylic acid (**AIDA**), CDP-choline, or the cytosolic PLA_2 (***c*PLA$_2$**) inhibitor, arachidonyl trifluoromethylketone (**$AACOCF_3$**) prior to ischemia did not significantly alter the **AA** levels following 10-min ischemia with no reperfusion. Our studies then focused on the effects of these agents on **AA** release after 1-day. Treatment with MCPG or AIDA (25 mg/kg i.p.), MPEP (10 mg/kg i.p.), CDP-choline (500-mg/kg i.p.), or $AACOCF_3$ (2 mg/kg i.p.) immediately and 3-h after ischemia significantly reduced **AA** levels

Table 1: Classification of cloned metabotropic glutamate receptors (mGluR)

	Group I	Group II	Group III	PLD coupled
Subtypes	mGluR1 (a,b,c,d) mGluR5 (a,b)	mGluR2, mGluR3	mGlurR4 (a,b), mGluR6, mGluR7 (a,b), mGluR8	undetermined
Transduction mechanism	stimulate Phospholipase C	inhibition of adenylyl cyclase	inhibition of adenylyl cyclase	stimulate Phospholipase D

after 1-day reperfusion (Fig. **2**).

Agent	mGluR 1	mGluR 5
MCPG	non-specific antagonist	
AIDA	strong antagonist	weak antagonist
MPEP	weak antagonist	strong antagonist

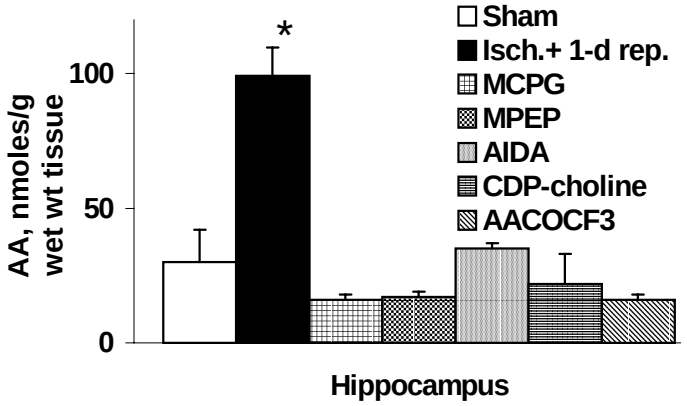


Figure 2. Effect of MCPG, MPEP, AIDA, CDP-choline and $AACOCF_3$ on **AA** levels in FFA after 10-min ischemia/1-day reperfusion. * $p < 0.05$ compared to shams.

The fact that all these agents attenuated **AA** levels at 1-day suggests the involvement of both PLC and PLA_2. The levels of **AA** at any given time are determined by a combination of dynamic processes of release and metabolism (Scheme **1**). Accumulation of **AA** may occur only when release from phospholipids exceeds subsequent metabolism. PLC and PLA_2 are major pathways of **AA** release in ischemia/reperfusion, and their activities may be inter-linked. The COX-2 product PGE_2 has been shown to stimulate the release of glutamate from astrocytes [8], which in turn activates PLC and PLA_2. Thus, changes in activity of either PLC or PLA_2 may affect other pathways through this feedback mechanism.

MCPG, MPEP, CDP-CHOLINE AND $AACOCF_3$ ARE NEUROPROTECTIVE [84,85]. Hippocampal CA_1 neurons are vulnerable to ischemia/reperfusion injury [20]. Fig. **3** shows no obvious neuronal death at 2-days after 10-min transient ischemia. Significant CA_1 neuronal death was apparent after 3-days but the maximum was observed at 6-days reperfusion (Fig. **3**) [21]. Our results indicate that MCPG, MPEP [85], CDP-choline [84] and $AACOCF_3$ provided neuroprotection in the hippocampal CA_1 region (Fig. **4**). These results are in agreement with other studies that MCPG provided neuroprotection in the CA_1 region after hypoxic injury [78]. MCPG (mGluR 1 and 5 antagonist, Fig. **4**) and MPEP (mGluR 5 antagonist, Fig. **4**) [86] showed neuroprotection whereas no significant effect was obtained with AIDA (mGluR 1 antagonist, data not shown). These findings are consistent with other studies suggesting that mGluR 5 might be more efficiently linked than mGluR 1 to G-protein coupled PLC [82] and that mGluR 5 is expressed more than mGluR 1 in CA_1 pyramidal neurons [87,88]. In our studies, AIDA when administered systemically (i.p.) did not offer significant neuroprotection [85]. However, in other studies, intracerebroventricular (**i.c.v.**) administration of AIDA showed neuroprotection in *in vivo* models [76,89]. The

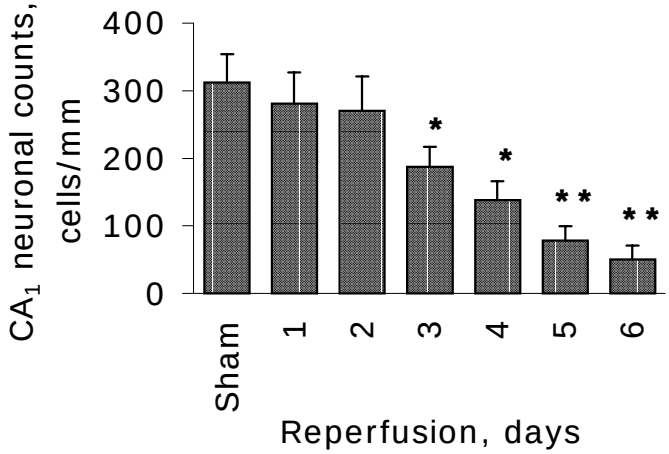


Figure 3. Hippocampal CA_1 neuronal counts over 6-days reperfusion after 10-min ischemia. * $p < 0.05$ and ** $p < 0.01$ compared to sham.

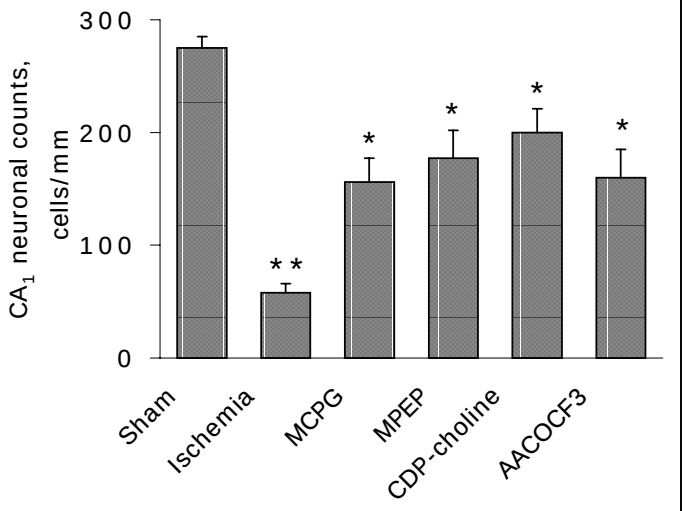


Figure 4. Hippocampal CA_1 neuronal counts at 6-days after transient forebrain ischemia in gerbil. ** $p < 0.01$ compared to sham; *$p < 0.05$ compared to untreated ischemic.

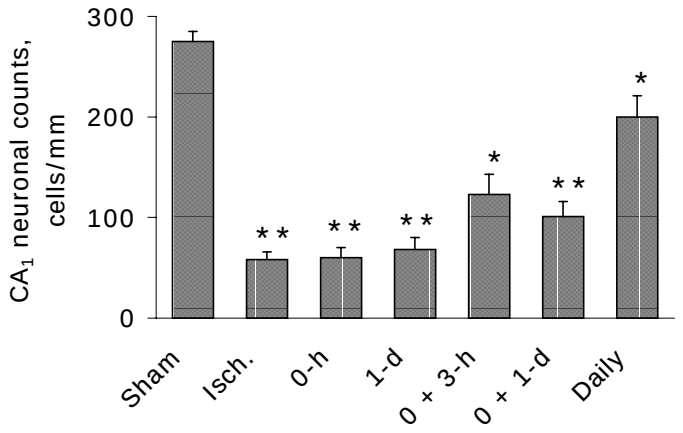


Figure 5. Effect of CDP-choline dose on hippocampal CA_1 neuroprotection.
** $p < 0.01$ compared to sham;
*$p < 0.05$ compared to untreated ischemic.

discrepancy between these results may be attributable to the degree of ischemic injury (5-min *vs*. 10-min) and route of administration of the agent (i.c.v. *vs* i.p.).

DAILY ADMINISTRATION OF CDP-CHOLINE PROVIDED SIGNIFICANT NEUROPROTECTION [90]. CDP-choline (500 mg/kg i.p.) administered as one dose immediately after ischemia or after 1-day reperfusion did not provide significant neuroprotection. When two doses were given at 0 and 1-day or at 0 and 3-h reperfusion, CDP-choline provided ~20% and ~30% neuroprotection, respectively. CDP-choline daily for 5-days protected ~65% of the CA_1 hippocampal neurons [90] (Fig. **5**).

PLC IS INVOLVED IN SIGNAL TRANSDUCTION. PLC activation catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (**PIP_2**) to generate DAG and IP_3 (Scheme **1**). DAG is further converted to FFA including **AA**. IP_3 mobilizes $[Ca^{++}]_i$ [69] which activates Ca^{++}-dependent PLA_2 and NOS. PIP_2 hydrolysis and the generation of DAG have been demonstrated in cerebral ischemia [61,91]. DAG and the increase in $[Ca^{++}]_i$ activate neuronal protein kinase C enhancing phosphorylation of neuronal proteins and contributing to neuronal death after ischemia [92-94].

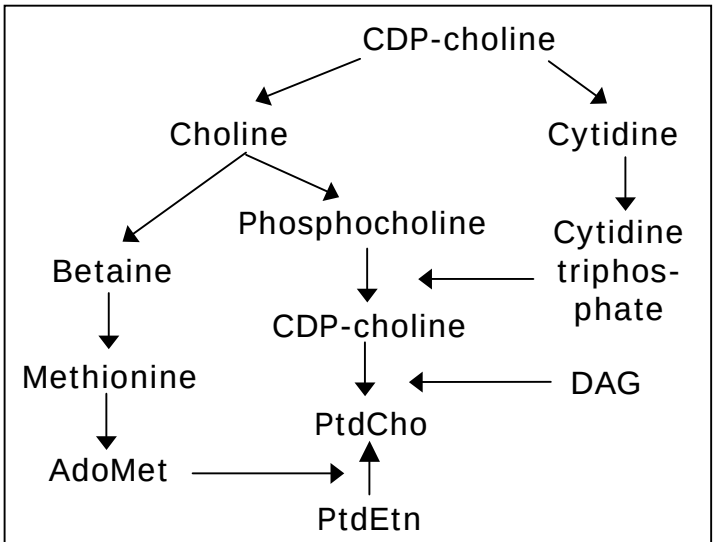
PLA_2 ACTIVATION RELEASES AA. PLA_2 catalyzes the hydrolysis of **AA** at the *sn*-2 position of phospholipids such as PtdCho and phosphatidylethanolamine (**PtdEtn**). Multiple forms are present in the brain [95] and have been classified as secretory PLA_2 and *c*PLA_2 (Ca^{++}-dependent and independent). In cerebral ischemia, Ca^{++}-dependent *c*PLA_2 is a major source for **AA** release [96,97]. In unstimulated cells, *c*PLA_2 is present in the cytosol, but an increase in $[Ca^{++}]_i$ induces translocation to cellular membranes where its substrate is located [98]. Activation of PLA_2 is harmful to neurons in several ways: 1) membrane phospholipid degradation, 2) increased calcium influx, 3) formation of lyso-PtdCho that can be metabolized to platelet activating factor [99,100] and 4) **AA** release and metabolism by COX/LOX.

cPLA_2 deficient mice developed smaller infarcts, demonstrating a role for *c*PLA_2 in the pathophysiology of transient ischemia [101]. Since the *c*PLA_2 gene was inactivated embryonically in these studies, the reduc-

tion in infarct volume represents the overall contribution of *c*PLA$_2$ both during the ischemia and the reperfusion period. Activation of phospholipases during reperfusion following cerebral ischemia has been inferred based on accumulation of **AA** [65,66] or its metabolites. There exists relatively little direct data on activity of phospholipases following transient cerebral ischemia. In a recent study, increased *c*PLA$_2$ immunoreactivity was selectively expressed in areas undergoing neuronal death, especially in the CA$_1$ region [102].

CDP-CHOLINE STIMULATES PtdCho SYNTHESIS. CDP-choline is an essential rate-limiting intermediate in the PtdCho biosynthesis (Kennedy pathway) [6,103]. In cerebral ischemia, ATP depletion results in the accumulation of cytidine 5'-monophosphate and conversion of PtdCho to DAG and **AA**. Exogenous CDP-choline stimulates the PtdCho synthesis and attenuates the release of **AA** by restoring the phosphotransferase reaction [104,105]. Choline liberated from CDP-choline can be converted to S-adenosyl-L-methionine (**AdoMet**) via metabolism to methionine [84]. AdoMet can serve as the methyl donor in the biosynthesis of PtdCho from PtdEtn in cell membranes. Thus CDP-choline may increase PtdCho synthesis via two pathways (Scheme **4**): 1) biosynthesis of AdoMet and 2) conversion of DAG to PtdCho. Our results also show that CDP-choline attenuated **AA** release (Fig. **2**), LTC$_4$ levels (Fig. **6**), and neuronal death in transient ischemia (Fig. **4**) [84]. Since CDP-choline does not have any known effects on 5-LOX, the reduction in LTC$_4$ levels may be directly related to attenuating **AA** release.

CDP-CHOLINE MAY ALSO STIMULATE SPHINGOMYELIN SYNTHESIS AND ATTENUATE CERAMIDE LEVELS. PtdCho, predominantly derived from *de novo* synthesis, serves as the phosphocholine donor to ceramide to form sphingomyelin [84,106,107] (Scheme **5**).



Scheme 4. CDP-choline metabolism and PtdCho biosynthesis

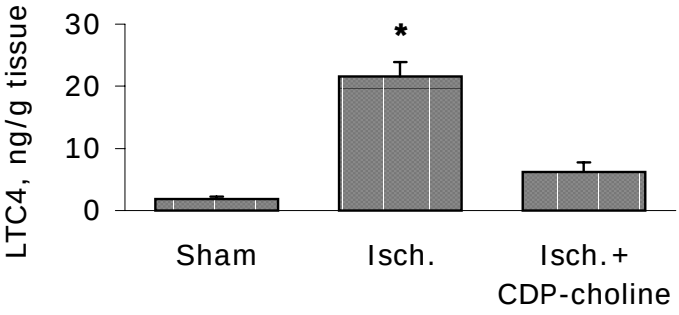
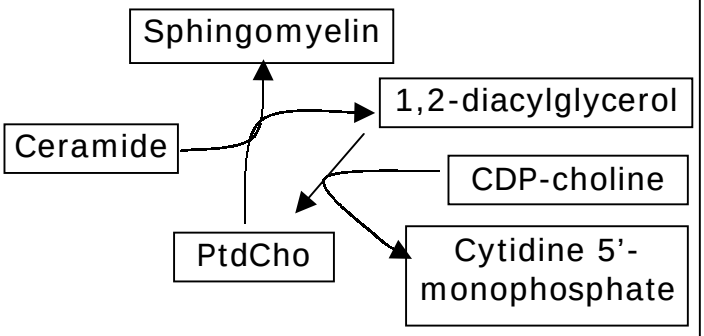


Figure 6. Effect of CDP-choline on hippocampal LTC$_4$ levels 1-day after transient ischemia. * $p < 0.05$ compared to shams.

$$\text{CDP-choline} + \text{DAG} \rightarrow \text{PtdCho}$$
$$\text{PtdCho} + \text{ceramide} \rightarrow \text{Sphingomyelin} + \text{DAG}.$$

CDP-choline itself may also serve as the phosphocholine donor to ceramide [108].



Scheme 5. CDP-choline: PtdCho and sphingomyelin synthesis.

There is no study to date examining the effect of enhanced PtdCho and sphingomyelin synthesis on neuronal survival after transient forebrain ischemia. It is conceivable that a distinct novel pathway of neuroprotection by CDP-choline involves stimulation of sphingomyelin synthesis, thus attenuating neuronal death induced by ceramide. Furthermore, CDP-choline inhibited the mitochondrial PLA_2 activation [109] and might have prevented the mitochondrial phospholipid degradation.

5-LOX IN CEREBRAL ISCHEMIA. 5-LOX converts **AA**, released from membrane phospholipids upon external stimulation, to biologically active LTC_4. Immunohistochemistry of gerbil brains showed intense reactivity for 5-LOX in hippocampal CA_1 neurons [110] with no immunoreactivity in glial cells. Ischemia/reperfusion resulted in translocation of 5-LOX from cytosol to membrane with an increase in LTC_4 levels [110]. A previous study [111] which did not show neuroprotection with AA861 used 100 mg/kg. Our studies [61,62] showed that complete inhibition of LTC_4 synthesis was achieved with the 5-LOX inhibitor, 2-(12-hydroxydodeca –5, 10 -diynyl)- 3,5,6-trimethyl-1,4-benzoquinone (**AA861**) at 1000 mg/kg (i.g.), which also provided partial but significant neuroprotection. This indicated that the other arm of **AA** metabolism, COX-2, plays a critical role in transient cerebral ischemia.

COX-2 IS INDUCED AFTER TRANSIENT ISCHEMIA [112]**.** COX is present in brain tissue as two isozymes: constitutive (COX-1) and inducible (COX-2). Cerebral ischemia up-regulates COX-2 mRNA [113], increases neuronal COX-2 immunoreactivity and elevates PGE_2 levels [112]. COX-2 expression may be induced by **AA** liberated from cell membranes via activation of glutamate receptors [114].

Our studies demonstrated that blockade of the COX pathway with indomethacin reduced PGE_2, 6-Keto-$PGF_{1\alpha}$ and TXB_2 levels (Table **2**). Indomethacin has also shown beneficial effects through attenuation of neuronal damage [115,116]. Indomethacin and other non-selective COX inhibitors also block COX-1, resulting in gastrointestinal and renal toxicity, thus limiting their therapeutic effectiveness [117]. The COX-2 specific inhibitor {1-[(4-methylsulfonyl) phenyl] -3- trifluoromethyl -5- [(4-fluoro) phenyl] pyrazole (**SC58125**) showed neuroprotection to CA_1 neurons after transient ischemia [118], which may be due to attenuation of ROS and lipid peroxidation (Scheme **1**). Since COX-2 is also induced in human brain following cerebral ischemia [119,120], this pathway may be clinically relevant.

AA AND ITS METABOLITES CAUSE BLOOD-BRAIN BARRIER (BBB) DYSFUNCTION AND EDEMA. Cerebral edema can be either cytotoxic (due to ionic imbalance) or vasogenic (resulting from BBB dysfunction). Glutamate may increase the BBB permeability via NO•-dependent mechanisms [121], producing vasogenic edema through extravasation of serum proteins [122,123] after ischemia/

Table 2. Hippocampal cyclooxygenase products (pg/mg protein) after10-min transient ischemia (n=4 gerbils per group). * $p < 0.05$ compared to shams

	Prostaglandin E_2		6-Keto-$PGF_{1\alpha}$		Thromboxane B_2	
	2-h R	1-day R	2-h R	1-day R	2-h R	1-d R
Sham	112 ± 10	71 ± 10	176 ± 15	221 ± 40	24 ± 59	5 ± 2
Ischemia	256 ± 35*	228 ± 13*	263 ± 48*	487 ± 59*	46 ± 59*	21 ± 2*
Indomethacin	78 ± 20	129 ± 28	80 ± 8	344 ± 85	15 ± 3	12 ± 2

reperfusion. It has been proposed that astrocyte swelling in edema may exacerbate neuronal injury through 1) glutamate release, causing additional excitotoxic damage [124] and 2) alteration of extracellular Ca^{++} and neuronal excitability due to reduction of the extracellular space [125]. Cerebral edema is a phenomenon of clinical importance in CNS injury. However, recent studies have indicated that plasma proteins do not of themselves cause significant neurodegeneration and suggested that BBB dysfunction is an event independent of excitotoxic neuronal death [126].

Of the poly-unsaturated fatty acids, **AA** is one of the most potent inducers of BBB dysfunction [127] and brain edema [128-131]. Loss of BBB integrity occurs at 6-h [61] and CDP-choline significantly attenuated BBB dysfunction after transient ischemia (Fig. **7**) [84]. This could be due to stabilizing the membrane by decreasing phospholipid hydrolysis (attenuation of **AA** levels) and increasing the PtdCho synthesis.

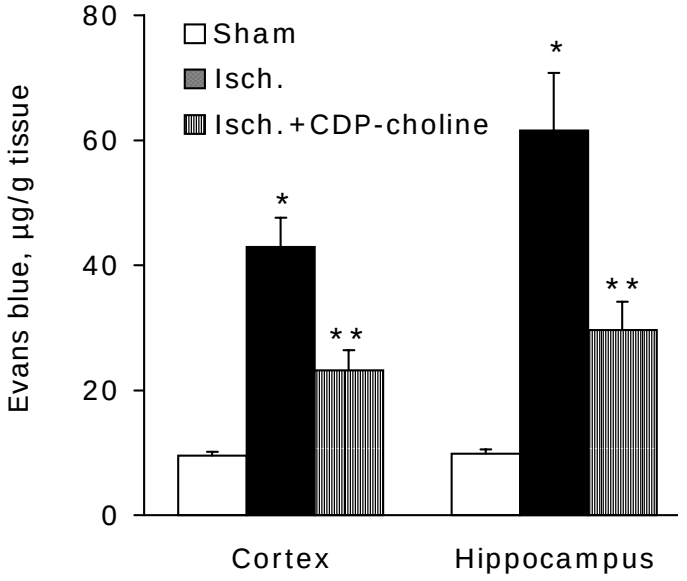


Figure 7. CDP-choline effect on BBB dysfunction at 6-h after transient ischemia. * $p < 0.01$ compared to shams. ** $p < 0.05$ compared to untreated-ischemic.

The time-course of edema after transient ischemia is shown in Fig. **8A**. Significant edema was observed in the hippocampus by 1-day, but reached maximum at 3-days. In our studies, MCPG, AIDA [85], and CDP-choline [84] decreased the hippocampal edema at 3-days after transient ischemia (Fig. **8B**), which may be directly related to decreased **AA** release. AIDA was less effective in attenuating **AA** release (Fig. **2**) and also was less potent in decreasing edema (Fig. **8B**).

AA metabolites LTC_4/PGE_2, oxygen radicals and lipid peroxides have also been

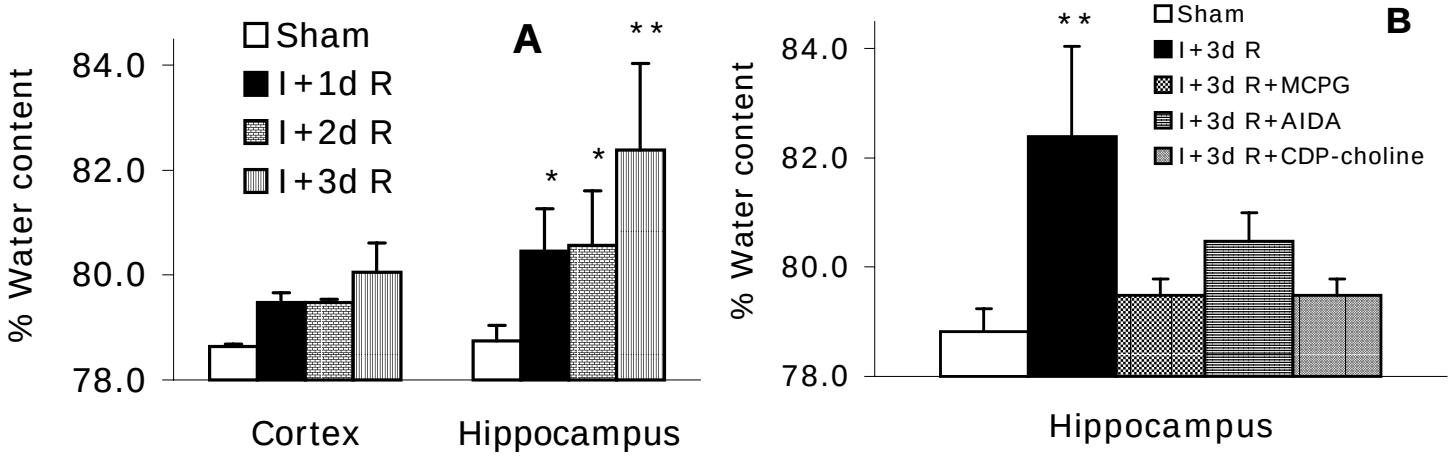


Figure 8. A) Time course of edema and **B)** the effect of MCPG, AIDA and CDP-choline (25, 25 and 500 mg/kg i.p., respectively) on edema in gerbil hippocampus after ischemia and 3-day reperfusion. Sham animals were treated with corresponding agents and showed no effect on edema. ** $p < 0.01$ and * $p < 0.05$ compared to shams.

implicated in BBB dysfunction and edema [132-135]. γ-Glutamyl transpeptidase, which converts LTC_4 to LTD_4, has been shown to decrease after ischemia [132] and this may contribute to accumulation of LTC_4 and an increase in edema [61].

Targeted disruption of the 5-LOX gene resulted in markedly attenuated **AA** induced inflammation [136]. Beneficial effects of the 5-LOX inhibitor, AA861 on cerebral edema in transient ischemia of gerbils and rats has been shown [62,63,137,138]. In our studies, inhibition of 5-LOX with AA861 almost completely inhibited the LTC_4 synthesis but did not attenuate the edema to the same extent. This suggests a role for LTC_4 as well as PGE_2 and other factors in the development of edema.

ISCHEMIC INJURY TO THE BRAIN IS MULTI-DIMENSIONAL. Due to the multiple pathways involved in ischemic injury (i.e., excitatory amino acids and their receptors, calcium channels, phospho-lipases, **AA** release and its oxidative metabolism, ceramide formation, free radicals, lipid peroxidation leading to HNE and acrolein formation), no single agent is likely to provide complete neuroprotection following transient ischemia. Combinations of agents with different mechanisms of action will probably be necessary for full recovery [139]. CDP-choline together with other neuroprotective agents was effective in ischemia models [140,141]. Approaches to limit the ischemic damage to brain should take into account both arms of **AA** metabolism since the COX/LOX pathways are linked by a common substrate [61].

NEUROPROTECTIVE AGENTS MAY CAUSE HYPOTHERMIA. Pharmacological intervention should be monitored for possible hypothermic effects. Mild brain hypothermia may result in significant increase in CA_1 neuronal survival [142, 143]. A reduction in brain temperature during ischemia and reperfusion markedly attenuated glutamate [144] and FFA release [145], OH• production [146], and CA_1 neuronal death [143,147]. Thus it is essential that neuroprotective agents be closely examined to rule out hypothermic effects [142,147].

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