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CEREBRAL ISCHEMIA AND REPERFUSION INJURY: A BRIEF REVIEW

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Cerebral injury, whether due to cardiac arrest, stroke, air embolism, head trauma, asphyxiation or cerebral edema, is a major medical challenge. The understanding of the basic physiology and biochemistry of brain damage helps lay a foundation for informed diagnosis and treatment, as well as the development of future intervention and treatment strategies. It is hoped that the review that follows will provide a useful collection of facts and concepts that can be integrated into a working understanding of brain injury induced by a compromised oxygen supply.

This review is the result of an extensive literature search and compilation of information on ischemia and reperfusion injury. This information was used, in part, for the preparation and construction of a research pre-proposal on the same subject matter.

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TABLE OF CONTENTS

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INTRODUCTION

Conscious and autonomic brain function is maintained by an exquisite balance between circulation, oxygenation, ionic equilibrium, and metabolism. However, this delicate interaction enhances the vulnerability of the cells comprising the functional structure of the brain and imposes moderately rigid constraints on the neuronal environment. Clinically, this presents an enormous challenge when confronted with the task of treating and minimizing the sequelae of an insult to the brain. Advances in resuscitation and life support techniques for cardio-pulmonary arrest and cerebral ischemia allows the survival of a large number of individuals who otherwise would confront a fatal outcome. Unfortunately, the consequence of increased survival is an associated increase in permanent neurological damage. Thus, the treatment strategies of the future are dependent on a solid understanding of the molecular and cellular mechanisms that interrupt normal tissue homeostasis and result in long-term or permanent brain tissue damage.

While there is an abundance of literature describing the effects of ischemia and reperfusion on the brain, much confusion and controversy still exists. Furthermore, a number of popular hypotheses are based on indirect measurements or data that yield multiple interpretation. This review is being written with the intent of pulling together some of the current literature in order to generate a more concise view and to provide some insights into the mechanisms of CNS damage.

BACKGROUND

As previously cited, there are various insults to the brain that can lead to significant tissue damage by disrupting the fragile homeostatic balance within this organ. One prominent avenue of destruction is the result of moderate to severe focal or global cerebral ischemia and the cascade of events that follow the ischemic episode (as a consequence of resuscitation). The catastrophic series of events that follow resuscitation is referred to as reperfusion injury.

The extent of cerebral (neuronal and glial) damage as the result of ischemia can vary depending on the location of the insult, the degree of ischemia (i.e., whether flow is partially or completely compromised), the duration of the occlusion, and the magnitude of tissue involvement. On the molecular and cellular level, ischemia-induced changes can be, for convenience, segregated into stages.

Ischemia Stage I: With the onset of an ischemic episode, blood flow to the affected areas can be severely depressed or stopped entirely, leading to an abrupt fall in the partial pressure of tissue oxygen (pO_2). The decrease in pO_2 in the hippocampus has been shown to fall from a pre-ischemic value of 30 mmHg O_2 to approximately 12 mmHg O_2 within 10 s, 6 mmHg O_2 in 30 s and essentially zero pO_2 at 1 min post occlusion (50). The most dramatic effect seen in the first 10 seconds paralleling the fall in tissue pO2 is the loss of consciousness, followed by a rapid disappearance of electrical activity (both EEG and unit activity) (17,18,35,46,47,53). The cellular level events leading to unconsciousness and electrical failure are not clearly understood. The disappearance of electrical activity has been ascribed to oxygen dependence of transmitter synthesis, pre- and/or postsynaptic depolarization, or reduction in membrane

resistance (17,18). However, 1) rate-dependent enzymes for transmitter synthesis have a high tolerance for diminished pO_2 and do not appear to change during hypoxia (47), 2) neither cellular depolarization nor decreased resistance (membrane "short circuit") are observed in the first 10 s of ischemia (50,53), and 3) ATP depletion is not a factor in this early phase of hypoxia (discussed below).

Changes occurring within the next 30 s of complete ischemia reveal a slow decline in tissue pH and a slow rise in the $[K^+]_o/[K^+]_i$ ratio (see Fig. 1). At 30-60 s, pH falls more abruptly (pH 7.2 -> 6.9) and $[K^+]_o/[K^+]_i$ continues its slow shift. The fall in extracellular pH is believed to result from an increase in intracellular $[HCO_3^-]$ consumption due to the anaerobic conversion of glucose to lactic acid. The decreased availability of $[HCO_3^-]_i$ provides less buffering of metabolically produced CO_2, thus increasing the outward CO_2 gradient. Extracellular pH decreases because CO_2 is trapped by the circulatory stasis (17,18). The mechanism by which K^+ shifts to the extracellular environment is not known. Although the increase in $[K^+]_o$ has been attributed to failure of the Na^+/K^+ ATPase due to a fall in ATP levels (47), the availability of ATP is reported to be within normal values within this 60-second time period (27). Even though **aerobic** ATP synthesis is interrupted in the face of continued ATP demand (utilization), sufficient levels are most likely preserved due to a small contribution from anaerobic glycolysis and a more significant input from phosphocreatine (PCr) metabolism (PCr + ADP + H^+ -> creatine + ATP) (17,46,47). This mechanism can support ATP levels at approximately 90-95% of control until the PCr stores are depleted (46,47).

Several reports (17,18,47,48) indicate that during this early stage of injury, $[Na^+]_o$ and $[Cl^-]_o$ do not change. However, one study indicates that because the interstitial space dehydrates by approximately 20% during this phase, these ions must be entering the cell, thus creating a situation where an extracellular concentration change is not detected (17,18).

Ischemia State II: At 60-90 s, larger shifts in $[K^+]$, $[Ca^{++}]$, $[Na^+]$, $[Cl^-]$, and $[HCO_3^-]$ occur and are probably dependent on the depletion of ATP (17,18,35,47,48,50). The large ionic changes induced by ATP disappearance herald the onset of the next wave of molecular/cellular insults to besiege the neuronal and glial tissues (Fig. 1). Presynaptic depolarization is believed to accompany the inward directed Na^+ and Ca^{++} fluxes and in turn trigger the release of excitatory amino acid transmitters (glutamate in particular) (3,6,8,10,25,26,46,47,53,54,56). However, this transmitter release may not be calcium dependent. One study (3) indicates that quantal pre-synaptic transmitter release is rapidly blocked in anoxia. Glutamate release may instead be coupled to the reverse transport of Na^+ and glutamate on the glutamate symporter (9,20,26,47). With the normal Na^+ gradient disrupted, this situation is possible. It is also important to note that the actual source of glutamate is unknown, i.e., it has not been determined whether the large efflux of transmitter is coming from either neuronal, glial or macrophage-like (microglial) cells or from the combination of these cells (9,20,25,28,39,40).

Since glutamate can activate multiple receptors, it stimulates both non-selective monovalent (Na^+, K^+, and H^+) ion conductance channels (i.e., the kainate and AMPA receptor-induced channels) and a mixed conductance channel (viz. the NMDA receptor/ionophore), which is permeable to both monovalent cations and calcium

(10,47,48,56). The former mentioned channels (kainate or AMPA) depolarize the membrane and probably contribute to a large DC potential shift seen in Stage II of ischemia. In addition, depolarization of the voltage-sensitive NMDA channels allow Ca^{++} entry into the postsynaptic neurons. Postsynaptic calcium entry through the NMDA channels and entry through voltage sensitive calcium channels (VOCC's) (i.e., the L- and T-type calcium channels) produce a substantial rise in intracellular calcium. Since H^{+} can displace Ca^{++} from various binding sites, ischemia-induced acidosis may further enhance the calcium concentration. Endoplasmic reticulum, calcisomes, and mitochondria (8,25,50) also serve as sources for calcium and actually may be quite substantial, considering that NMDA ·eceptors desensitize on the order of seconds and inactivate dramatically at low pH (pH 6.6 - 6.3) (54,57). This puts the contribution of NMDA channel-derived calcium in question. Note however, that in outlying areas where ischemia is mild to moderate (the penumbra) and pH and ATP levels are not totally depressed, the "NMDA mechanism" is probably more significant.

Ischemia State III: After 90-120 s the combination of elevated extracellular glutamate, high intracellular calcium, and significant tissue acidosis, sets an ominous and portentive proscenium, boding imminent disaster. In addition to activating receptor-gated ion channels (e.g., kainate, AMPA, and NMDA), glutamate binds with another type of receptor (quisqualate), which is coupled to a "G" protein that regulates phospholipase C (47,63). Phospholipase C cleaves membrane-bound phosphatidylinositol 4,5 phosphate into phosphatidylinositol 1,4,5 triphosphate (IP_3) and 1,2 diacyglycerol (DAG) (47,48). IP_3 triggers calcium release from vesicular stores (endoplasmic reticulum), further enhancing $[Ca^{++}]_i$. DAG activates protein kinase C, which

phosphorylates a large variety of phosphoproteins. DAG metabolism produces arachidonic acid, the significance of which will be discussed below. Another insult to the cell resulting from elevated glutamate is increased competition with cystine for a transport site which in turn suppresses cystine uptake. Cystine is reduced to form cysteine, a necessary substrate for reduced glutathione (GSH) synthesis. As will be discussed later, GSH is an important and critical antioxidant that scavenges reactive oxygen molecules (H_20_2 and organic peroxides) (7,15,16,55,63).

Since calcium functions as a "second messenger" and calcium removal is compromised by ischemia-induced energy failure (ATP depletion), an avalanche of calcium-activated events occur in tandem with the glutamate effects, producing more potentially cytotoxic reactions. Elevated $[Ca^{++}]_i$ stimulates phospholipase activity, which in turn generates free fatty acids and altered membrane phospholipids (25). These breakdown products can insert into the membrane bilayer and exert a detergent-like action on this structure (47). One polyunsaturated fatty acid of particular interest is arachidonic acid. The metabolism of this compound is of interest because in the presence of cyclooxygenase it can be metabolized to thromboxane A_2 and a series of prostaglandins, or in the presence of lipoxygenase, it can be converted to various eicosatetraenoic acids and leukotrienes (15,25,48,55). All these substances are biologically active and have important post-ischemic implications. Since both cyclooxygenase and lipoxygenase require O_2 to metabolize arachidonic acid, further discussion will be postponed until reperfusion is considered, however, it is paramount to note that arachidonic acid accumulates during this period of ischemia and in essence creates a cellular "time-bomb". Other calcium-dependent interactions involve calmodulin

(CaM) modulated proteins. There are several CaM-activated kinases found in the brain, which include myosin light chain kinase and the CaM kinases I, II and III. Functionally, (1) myosin light chain kinase may regulate molecular transport; (2) CaM I phosphorylates the synaptic vesicle associated protein, synapsin; (3) CaM kinase II, which is found in both the cytoplasm and membrane, also interacts with synapsin as well as phosphorylating proteins (tubulin, tyrosine hydroxylase, and tryptophan hydroxylase) in the postsynaptic density; and (4) CaM III phosphorylates a ribosomal elongation factor, eEF-2. Phosphorylation of eEF-2 inhibits protein synthesis. On the other hand, calcium stimulates the expression of various oncogenes such as c-fos and c-jun (32,36). Since ischemia has been shown to both induce or repress mRNA expression, Ca^{++} accumulation may have profound effects on protein synthesis. Yet another CaM-dependent enzyme, nitric oxide synthetase, catalyzes the conversion of L-arginine to L-citrulline and the generation of nitric oxide gas (NO) (1,10,15,48). Like cyclooxygenase and lipoxygenase, NO synthetase requires O_2, therefore, it is probable that arginine accumulates during ischemia and the role of NO is more significant during reperfusion.

The duration of the ischemic period becomes quite critical at this point. Whether resuscitation is instituted within minutes or hours will impact upon the severity of a focal lesion or result in a life or death outcome for a global ischemic event such as cardiac arrest. Assuming timely intervention, a new and possibly treacherous series of insults await the resuscitated and reoxygenated tissue.

Reperfusion: Many noxious and insalubrious events occur within this segment of time, making it probably the most dangerous period to befall the nervous tissue and in

which permanent brain damage would be predestined. Assuming the blood supply to the ischemic brain can be restored, various changes can be observed in the reperfused tissue. In one recent study, re-establishment of brain blood flow following 8 min of global ischemia demonstrated a reproducible hyperemic response peaking at approximately 3 min and reaching flows of greater than 170% of pre-ischemic values (50). Concomitant increases occurred in pO_2 with regional values exceeding 120% of pre-ischemic values. Blood flow and pO_2 levels can persist above pre-ischemic values for hours and become unstable and oscillate. These oscillations occur at a 4- to 6-minute periodicity and represent aberrant local vasomotor control and tissue metabolism (50).

Both unit and EEG activity can reappear in regional areas within 7-10 min, but discharge patterns are sporadic and irregular (50,53). Within 40-60 min neuronal activity settles into a more normal firing pattern (50,53). This normal electrical pattern does not persist throughout the damaged area, but shows profound regional variation (50,53).

Reperfusion impacts on ion movement relatively rapidly. Within 10 min, intracellular calcium levels decline at least one order of magnitude and approach normal amounts (60-100 nM) in 20 min. Cells appear to remain at this normal level for 1 or 2 h and then slowly rise again to reach intracellular concentrations of approximately 300-400 nM (50). This slightly elevated level may reflect the activation of various Ca^{++}-mediated events and may be sufficient to continue to wreak havoc upon cell function. Steady-state distribution for $[Na^+]_o/[Na^+]_i$ and $[K^+]_o/[K^+]_i$ occurs within 5 min (17,18,50) and probably reflects the fact that the Na^+-K^+ ATPase is unaffected by long periods of a a and that the pathways for ATP production are somewhat resilient.

Recovery of intracellular pH occurs in a biphasic manner with a rapid change occurring over the first 5 min, where pH attains a value of 6.8 from a low of 6.1. This is followed by a slower recovery phase over a one-hour time period, at which point the cell reestablishes a normal pH of 7.3.

As the cells involved in the ischemic episode strive to recover, other forces of disruption begin to appear. Accompanying recirculation and reoxygenation is the cascade of events that occur with the interaction of oxygen and the products of hypoxic metabolism. One such example is the series of oxygen-related reactions that generate free radicals (i.e., molecules that contain one or more unpaired electrons and which in many cases are highly reactive). It is proposed that during hypoxia ATP is metabolized to AMP, which is degraded to hypoxanthine (15,37,55). The hypoxanthine accumulates in the ischemic cells. Although nervous tissue does not contain much xanthine oxidase (XO) (the enzyme that converts hypoxanthine to xanthine with O_2 as an electron acceptor) it does contain significant amounts of xanthine dehydrogenase (XD) (an enzyme coupled to NAD^+ and cytochrome C as electron acceptors). During ischemic injury, Ca^{++}-activated protease can convert XD to XO. Upon reperfusion and O_2 availability, XO catalyzes the conversion of hypoxanthine to xanthine and xanthine to uric acid. In the process diatomic oxygen (O_2) undergoes a one-electron reduction, thus producing an oxygen free radical $\cdot O_2^-$. The $\cdot O_2^-$ can undergo further conversion to H_2O_2 generating two relatively cytotoxic compounds. Whether this reaction is significant in brain has been questioned. Mink et al. (31) failed to detect conversion of XD to XO after 30 min of ischemia or 60 min following reperfusion. However, XO was still considered a contributor to ischemic brain damage, since significant quantities of XO

was present in normal and injured brain, and allopurinol (a XO inhibitor) improved mortality and morbidity when administered before cerebral ischemia (22,29,31,55,61).

In the presence of tissue destruction and low pH, elevated quantities of ferric iron can accumulate in the damaged areas and in turn catalyze the reaction of $\cdot O_2^-$ + H_2O_2 to yield a hydroxyl free radical ($\cdot OH$). Both the $\cdot O_2^-$ and the $\cdot OH$ can generate lipid and lipid peroxy free radicals that can become self-propagating and regenerating, therefore causing the membrane lipids to peroxidize. This can produce severe membrane damage and cellular swelling. Compounding the effects of neuronal- and glial-generated free radicals is the infiltration of the ischemically compromised tissues with various leukocytes, macrophages, and microglia, which also release reactive oxygen compounds, eicosanoids, and proteolytic enzymes. The degree of damage caused by the reactive oxygen products is dependent, in part, on the naturally occurring substrates for inactivating these oxidizing materials (such as catalase, superoxide dismutase, or glutathione) (55). As previously mentioned, ischemia may interfere with GSH synthesis, thus creating a potential situation for decreased scavenging of H_2O_2 and $\cdot OH$ (15). This non-protein thiol compound serves as a proton donor for protein thiols, thus keeping them in a reduced state (19,52). The reduced GSH is itself oxidized to form a disulfide (GSSH). The GSSG can be regenerated in the presence of glutathione reductase (GR) and NADPH (19,52). In addition to the problem of decreased GSH synthesis, if the oxidizing environment overwhelms the regenerative capacity of the GR system or if the GR system is disabled or compromised, GSH remains oxidized and can no longer serve to buffer the effects of the reactive oxygen (19,52). Increased GSSH can produce mixed disulfides with proteins to cause, for example, enzyme failure (16).

Another aspect of reperfusion injury is the effect of ischemia and hypoxia on the endothelium itself. As emphasized by other investigators (14,41,48), endothelium is not a passive tissue. Normally it presents an anticoagulant surface to blood; however, post-ischemically it becomes highly reactive, presenting a pro-coagulant surface as well as a target for a barrage of vasodilating and vasoconstrictive compounds. The endothelial cells can produce platelet activating factor (PAF), endothelium-leukocyte adhesion molecules, a series of arachidonic acid (AA) metabolites, endothelium-derived relaxing factor (EDRF) (this is actually NO), and the endothelins (14,41,48).

Production of AA occurs in blood platelets as well as in endothelium. This substance is generated from the different actions of the membrane-bound phospholipases A_2 or C on various phosphoglycerides (Figs. 3 and 4). Through the peroxidation of AA by the dioxygenases, lipoxygenase, or cycloxygenase, a group of reactive substrates are produced (48,55). The lipoxygenases introduce oxygen into one of the pentadiene moieties of AA, resulting in a hydroperoxyecosatetraenoic acid (HPETE), with properties dependent on where the peroxy group is introduced. 5-HPETE generates the leukotrienes. The leukotrienes as a group have chemoattractant properties, constrict vessels, and increase vascular permeability. Cycloxygenase produces two groups of substances with antagonistic effects, namely the prostaglandins and thromboxanes. One prostaglandin, prostacyclin, functions as a vasodilator and anti-aggregant, whereas thromboxane A_2 has potent vasoconstrictive and platelet aggregation properties. Normally there is a balance between these two materials, however, inactivation of prostacyclin synthetase by post-ischemic-generated free radicals has been proposed to result in an excess of thromboxane (48).

As previously mentioned above, phospholipase A_2 can generate AA. However, it also can catalyze the formation of the precursor of PAF (see Fig. 3). Further catalysis by acetyltransferase yields PAF (48). The platelet activating factor causes platelet and leukocyte accumulation and aggregation, plus it stimulates platelet secretion, increases vascular permeability, and induces vasoconstriction. Calcium is essential for platelet adhesion and activation, thus there is further involvement of calcium in a potentially toxic process.

The endothelins are a group of bi-cyclic, 21 amino acid peptides that are derived from the activity of various endopeptidases on a 203 amino acid peptide, proendothelin. A 39 amino acid intermediate, big endothelin, is generated from proendothelin by further proteolytic action. Endothelin is finally produced by enzymatic conversion of big endothelin (38). In the CNS, the endothelins are present in neurons, glia, vascular smooth muscle, and endothelium of cerebral vessels (12,30,64). These compounds are powerful vasoconstrictors with a long duration of action (approximately 20 times longer than norepinephrine or prostaglandin F_2). The vasoconstrictor action is the result of local secretion, because systemic administration of endothelin has been shown to have no effect on cerebral resistance vessels. The failure to evoke a response with systemically injected endothelin is due to the blood-brain barrier created by the endothelium of the luminal surface blocking access to smooth muscle (30,64). Hypoxia, vasospasm, and subarachnoid hemorrhage have been shown to induce *de novo* synthesis and release of endothelin, however, it is questionable whether the absolute amount released in response to these stimuli is sufficient to enhance vasoconstriction (30).

As described earlier another substance liberated during reperfusion is nitric oxide (1,10,15,33,51,62). Hypoxia-induced accumulation of L-arginine and elevated levels of Ca^{++}, NADPH, oxygen, and NO synthase provide a prime situation for enhanced NO production. Since NO is a potent activator of vasodilation, part of the erratic blood flow seen during reperfusion may be attributed to this compound. Coupled to the reperfusion-induced elevation of the endothelins, PAF, and the products of arachidonic acid metabolism (leukotrienes, prostaglandins and thromboxanes), sheer chaos can be expected.

The recovery and residual damage produced by an ischemic event is dependent on (1) the type of injury, that is, whether the insult is global (e.g., cardiac arrest or asphyxia) or focal (e.g., cerebral infarction or gas emboli); (2) the infarct volume, if focal; and (3) the time elapsed from onset to resuscitation and reperfusion. The collapse of the cerebral milieu includes large ionic shifts; a decrease in both intra and extracellular pH; depressed ATP synthesis; efflux of excitatory amino acids, leading to enhanced intracellular calcium levels and activated phospholipase activity; alterations in calcium-modulated functions including enzyme activity, protein synthesis, and mRNA expression; generation of reactive oxygen intermediates; and stimulation of a variety of vaso-active substances. The understanding of these events influence current treatment and will direct the treatment protocols of the future. Thus, the development and use of various NMDA antagonists, calcium channel blockers, free radical scavengers, anti-inflammatory compounds or elevated levels of oxygen (hyperbaric oxygen?) may provide rational approaches for resuscitation and improving the outcome following the ischemic episode.

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GLOSSARY

AA - Arachidonic acid

ADP - Adenosine diphosphate

AMP - Adenosine monophosphate

AMPA - ∝-amino-3-hydroxy-5-methylisoxazole-4-propionic acid

ATP - Adenosine triphosphate

ATPase - Adenosine triphosphatase

CaM - Calmodulin

CNS - Central nervous system

DAG - 1,2 diacylglycerol

DC - Direct current

EDRF - Endothelium-derived relaxing factor

eEF-2 - Ribosomal elongation factor

EEG - Electroencephalogram

GR - Glutathione reductase

GSH - Glutathione

GSSG - Oxidized glutathione

H^+ - Hydrogen ion

H_2O_2 - Hydrogen peroxide

HPETE - Hydroperoxyecosatetraenoic acid

IP_3 - Phosphatidylinositol 1,4,5 triphosphate

K^+ - Potassium ion

mmHg - Millimeters mercury

mRNA - Messenger ribonucleic acid

Na^+ - Sodium ion

NADPH - Nicotinamide adenine dinucleotide, reduced form

NMDA - N-methy-D-aspartic acid

NO - Nitric oxide gas

O_2 - Diatomic oxygen

$\cdot O_2$ - Oxygen free radical

$\cdot OH$ - Hydroxyl free radical

PAF - Platelet activating factor

PCr - Phosophocreatinine

pO_2 - partial pressure of oxygen

VOCC - Voltage-sensitive calcium channel

XD - Xanthine dehydrogenase

XO - Xanthine oxidase

$[Ca^{++}]$ - Calcium ion concentration

$[Cl^-]$ - Chloride ion concentration

$[HCO_3^-]_i$ - Bicarbonate concentration, inside

$[HCO_3^-]_o$ - Bicarbonate ion concentration, outside

$[K^+]_i$ - Potassium ion concentration, inside

$[K^+]_o$ - Potassium ion concentration, outside

$[Na^+]_o$ - Sodium ion concentration, outside

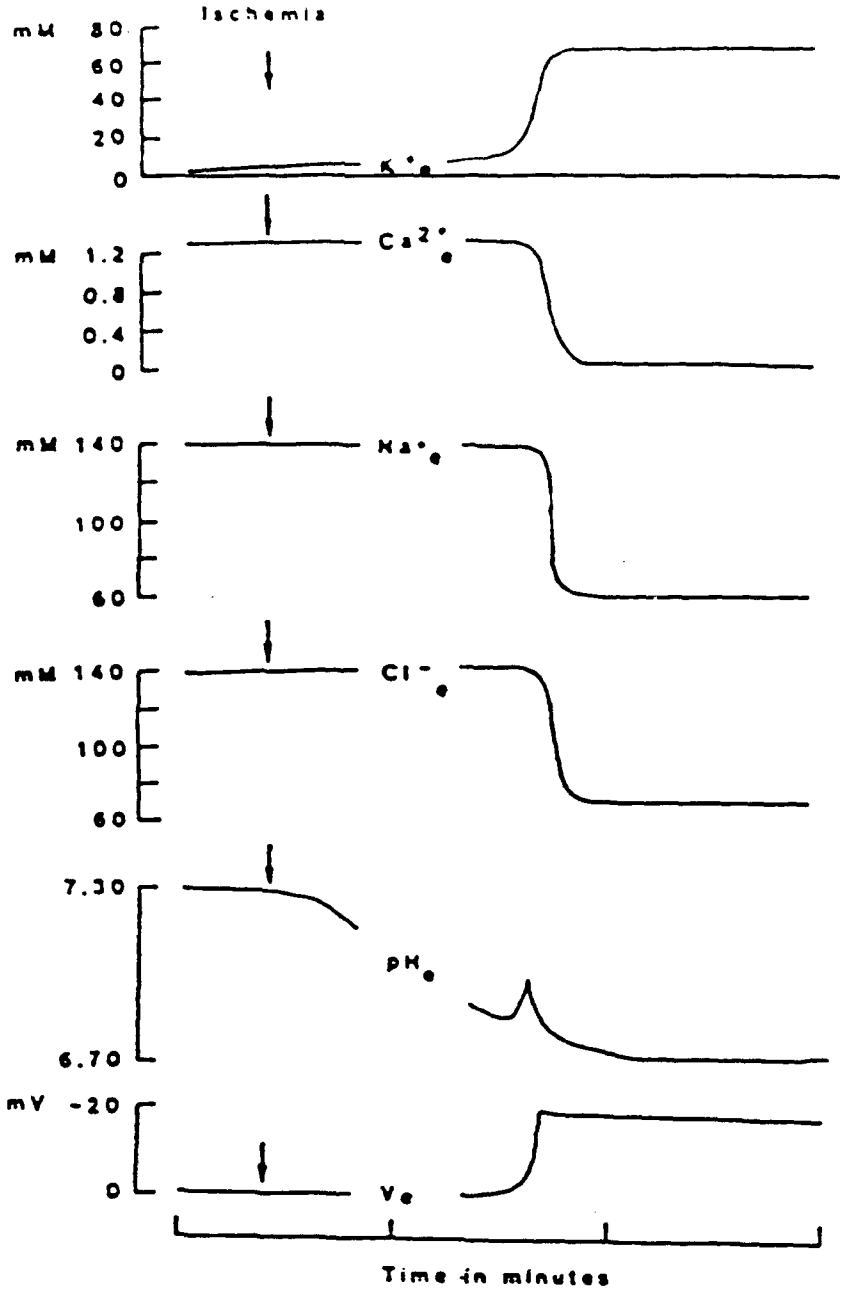

Figure 1. Line drawing depicting changes in extracellular cerebral cortical K^+, Ca^{2+}, Na^+ and Cl^- concentrations, pH and extracellular electrical potential. Extracellular K^+ shows a slow increase in concentration and pH falls during the first 90 seconds of ischemia. Major ionic shifts occur after 90 seconds. Just before the depolarizing shift there is a brief influx of H^+ and efflux of HCO^{3-} due to shifting electrochemical gradients. This produces the spike seen in the pH curve. (This diagram taken from refs. 17 and XI).

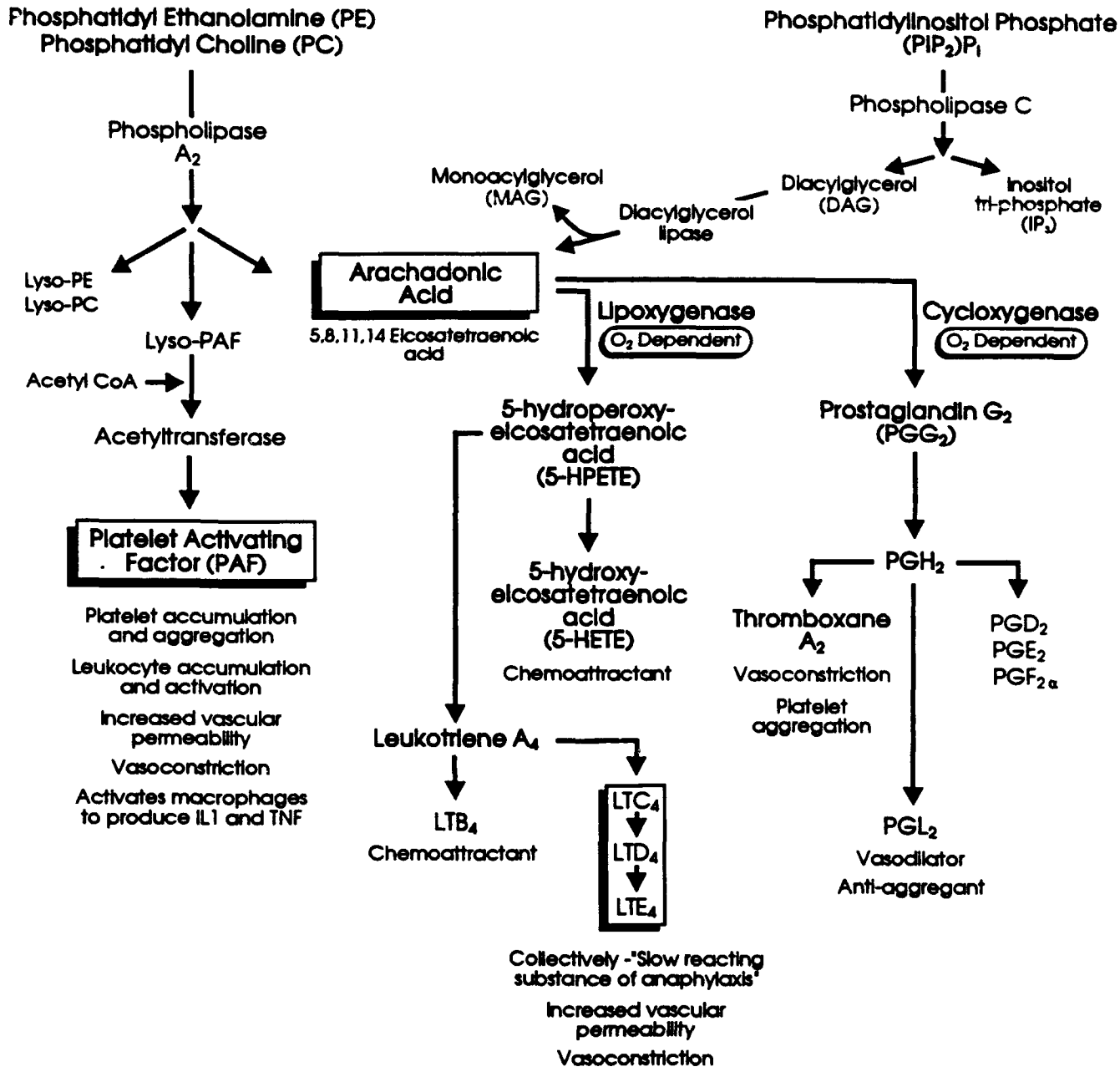

Figure 2. Schematic drawing of the pathway for phosphoglyceride and arachidonic acid metabolism.

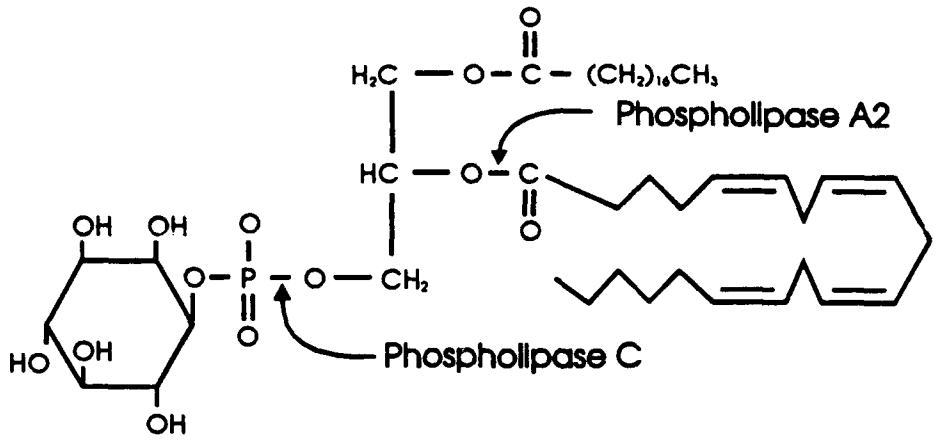

Figure 3. Graphic representation of a phosphoglyceride and location of lipase action

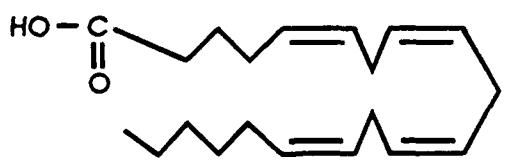

Figure 4. Graphic representation of arachidonic acid