Revised & Approved

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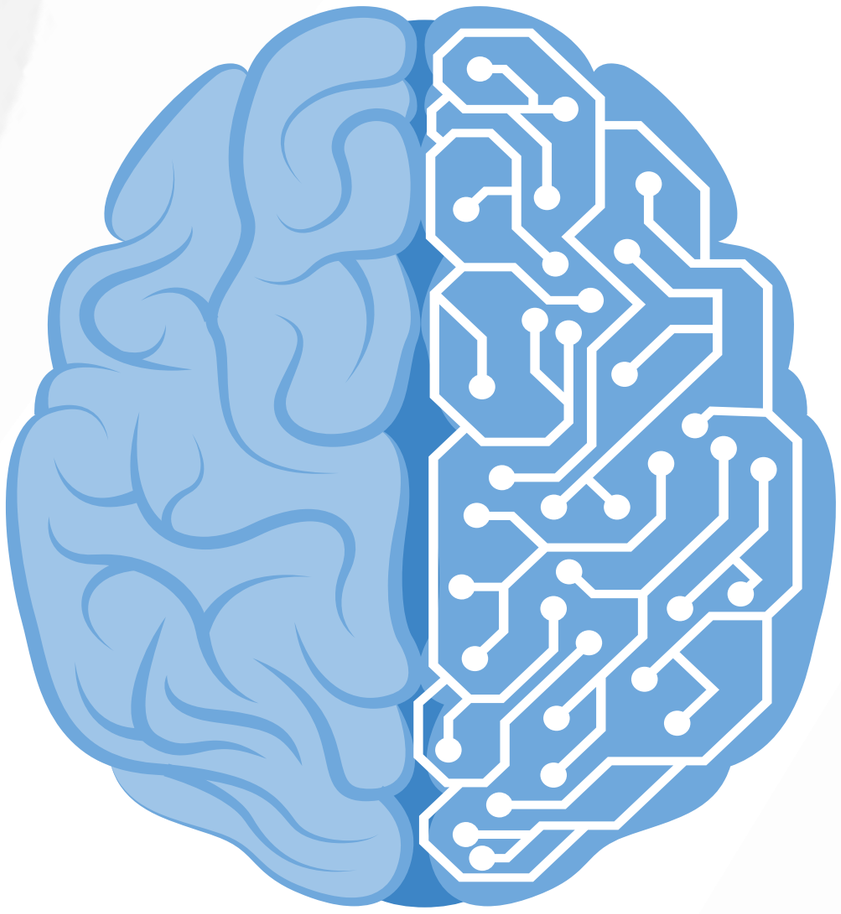
BIOCHEMISTRY TEAM
439

MED439
KING SAUD UNIVERSITY



Pathogenesis of Cerebral Infarction at Cellular & Molecular Levels

Editing File

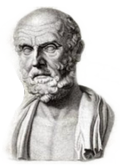
Color Index

- Main Text
- Important
- Extra
- Dr.'s Notes
- Girls slides
- Boys slides

Objectives

Click on the objective to go to the related slide

- Identify the possible cell death mechanisms implicated in the pathogenesis of ischemic brain injury.
- Acquire the knowledge of the important role played by oxidative stress and free radicals in the pathogenesis of cerebral infarction.
- Understand the various factors involved in ischemia-induced metabolic stress.
- Identify the Neurochemical changes involved in cerebral ischemia.



Level 3 (with each lecture you will level up and it will get harder to find the scientist)
Hello my name is Hippocrates, Find me in this lecture!
Then click me for more info about what I discovered.

Click the number between the [ ] to see the related notes.

Objective: 1

Cerebral ischemia (Stroke) [1]

Ischemic [2]
Global incidence: 68%

Thrombotic [3]

Embolic [4]

Risk Factors

- Age older than 40 years
- Heart disease
- High blood pressure
- Smoking
- Diabetes
- High blood cholesterol levels
- Illegal drug use
- Recent childbirth
- Previous history of transient ischemic attack
- Inactive lifestyle and lack of exercise
- Obesity
- Current or past history of blood clots
- Family history of cardiac disease and/or stroke

Hemorrhagic [5]
Global incidence: 32%

Intracerebral

Subarachnoid

Risk Factors

- High blood pressure
- Smoking
- Illegal drug use (especially cocaine and "crystal meth")
- Use of warfarin or other blood thinning medicines [6]

Risk factors of stroke

There are a number of risk factors for stroke:

- Some increase the risk of one type of stroke (hemorrhagic or ischemic).
- Some increase the risk of both types
- Occasionally, strokes occur in people who have no risk factors. [7]

Cell death mechanisms in cerebral ischemia [8]

- **Necrosis**: is commonly observed **early** after **severe** ischemic insults [9]
- **Apoptosis**: occurs with more **mild** insults and with **longer** survival periods
- The mechanism of cell death involves calcium-induced **calpain-mediated proteolysis** of brain tissue. [10]
- **Substrates for calpain include**: Cytoskeletal proteins, Membrane proteins and Regulatory and signaling proteins. [11]

Click the number between the [ ] to see the related notes.

Objective: 2

Biochemical Responses to Ischemic Brain Injury

1 Oxidative stress

2 Metabolic Stress

3 Neurochemical response

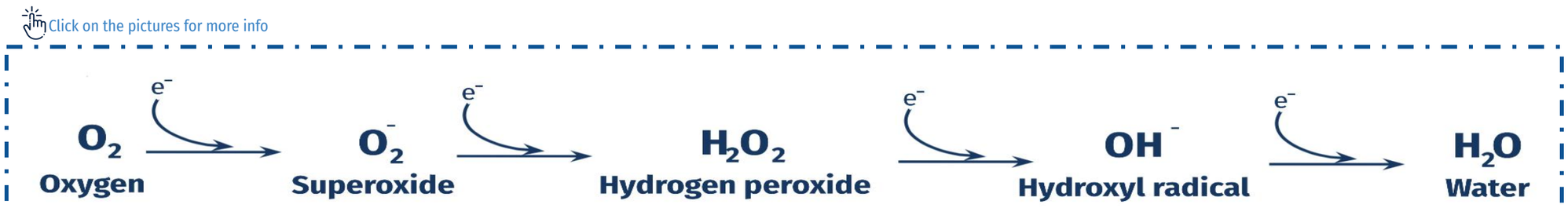
1. Oxidative Stress [12]

- A condition in which cells are subjected to excessive levels of **Reactive oxidizing species** (ROS) or **Reactive nitrative species** (RNS) & they are unable to counterbalance their deleterious effects with antioxidants.
- It has been implicated in the ageing process & in many diseases (e.g., atherosclerosis, cancer, neurodegenerative diseases, stroke)

The Role of Reactive Oxygen Species (ROS) & Reactive Nitrative Species (RNS) in Normal Brain Physiology

- They are mainly generated by microglia & astrocytes
- They modulate synaptic transmission & non-synaptic communication between neurons & glia
- During periods of increased neuronal activity, ROS & RNS diffuse to the myelin sheath of oligodendrocytes activating **Protein kinase C (PKC)** ⟶ posttranslational modification of myelin basic protein (MBP) by phosphorylation [13]
- They regulate neuronal signaling in both central & peripheral nervous systems
- They are required for essential processes as learning & memory formation

Click on the pictures for more info



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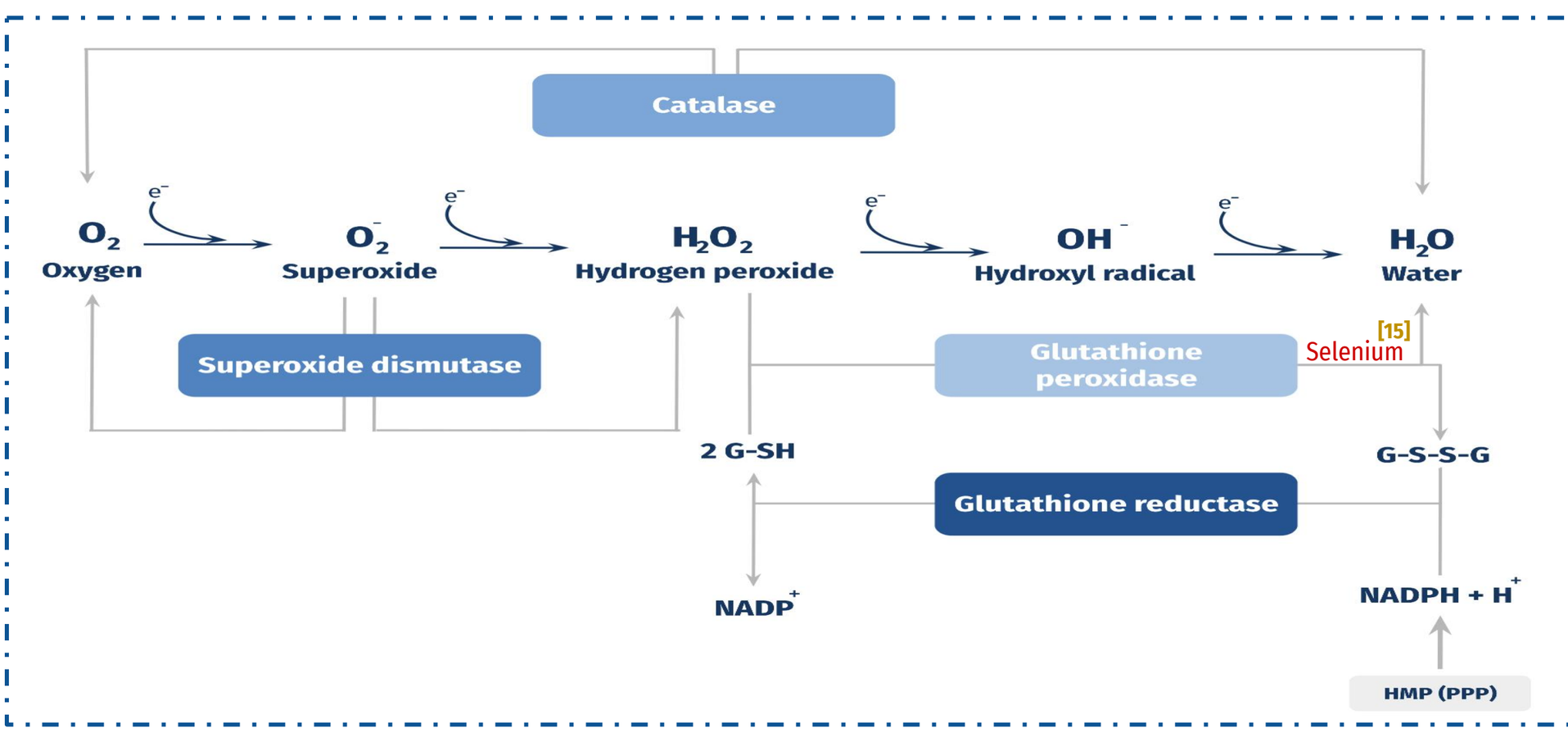

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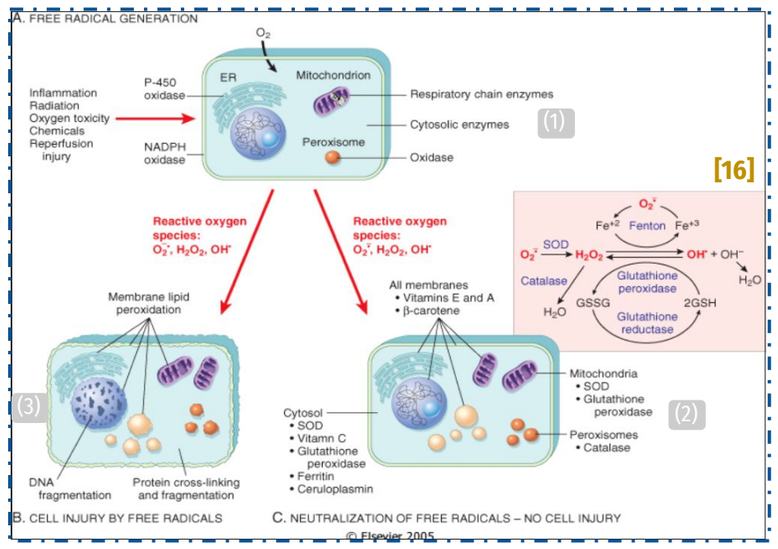
Objective: 2

1. Oxidative Stress

- (1) ROS produced 90% in mitochondria and the remaining 10% by cytosolic enzymes: oxidase, P-450 oxidase, NADPH oxidase.
- (2) Normally we have anti-oxidant enzymes to take care of them→ no cell injury
- (3) If ROS increased, they will enter the cells and degrade all proteins causing proteins DNA fragmentation which lead to cell death.

Some notes about the figure:
- When ROS are generated, they can cause membrane lipid peroxidation which means that the membrane gets degraded. If this happens to lysosomes (which contain proteases) their contents leak into the cell → cell death.
- The Fenton reaction: Fe interacts with H2O2 → hydroxyl radical (very reactive). Other ions like Cu can also lead to the generation of ROS.
- The brain has a lot of iron stores that are also involved in the formation of ROS.
  -special thanks to team 438



The Brain And Oxidative Stress

The brain is highly susceptible to ROS-induced damage because of:

- High oxygen consumption
- Low levels of protective antioxidants [17]
- High concentrations of peroxidizable lipids [18]
- High levels of iron (acts as pro-oxidants under pathological conditions) [19]
- The occurrence of reactions involving dopamine & Glutamate oxidase in the brain [20]

Molecular & Vascular effects of ROS in ischemic stroke

Molecular effects

- DNA damage
- Lipid peroxidation of unsaturated fatty acids
- Protein denaturation
- Inactivation of enzymes
- Cell signaling effects (e.g., release of Ca2+ from intracellular stores)
- Cytoskeletal damage
- Chemotaxis

Vascular effects

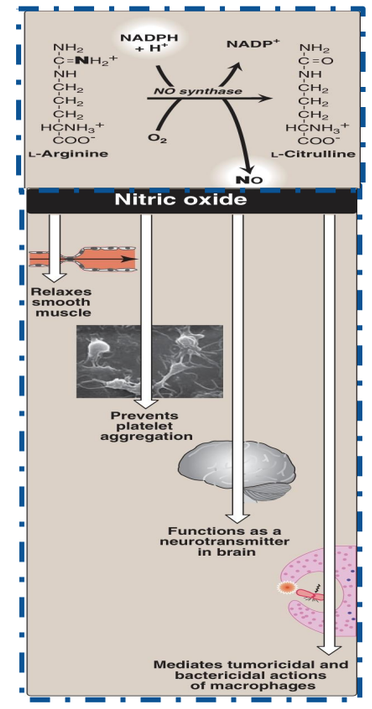
- Altered vascular tone and cerebral blood flow
- Increased platelet aggregability
- Increased endothelial cell permeability

The role of NO in the pathophysiology of cerebral ischemia [21]

- Ischemia ⟶ abnormal NO production
- This may be both beneficial and detrimental, depending upon when and where NO is released

- NO produced by endothelial NOS (**eNOS**) improving vascular dilation and perfusion (i.e. **beneficial**).
- In contrast, NO production by neuronal NOS (**nNOS**) or by the inducible form of NOS (**iNOS**) has **detrimental** (harmful) effects.
- Increased iNOS activity generally occurs in a delayed fashion after brain ischemia and trauma and is associated with inflammatory processes

Click on the pictures for more info



2. Metabolic Stress
(Biochemical changes in The brain during ischemia)

Ischemia

↓

interruption or severe reduction of blood flow, O2 & nutrients in cerebral arteries

↓

energy depletion (depletion of ATP & creatine phosphate)

↓

- Inhibition of ATP-dependent ion pumps
- Membranes depolarization
- Perturbance of transmembrane ion gradients

↓

- **Ca2+ Influx** (translocation from extracellular to intracellular spaces) ⟶ activation of cellular proteases (Calpains) & lipases ⟶ breakdown of cerebral tissue [22]
- **Na+ influx**
- **K+ efflux**
- K+ induced **release of excitatory** amino acids

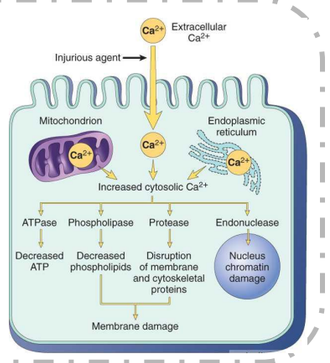
↑ Lactic acid in neurons ⟶ acidosis ⟶ promotes the pro- oxidant effect ⟶ ↑ the rate of conversion of $O2^{-\bullet}$ to H2O2 or to hydroxyperoxyl radical

Click the number between the [ ] to see the related notes.

Objective: 4

Sources & consequences of increased cytosolic Calcium in cell injury

Ca comes in from extracellular stores & intracellular stores (mitochondria+endoplasmic reticulum) and affects different enzymes causing the following changes



3. Neurochemical response

Following cerebral ischemia, extracellular levels of various neurotransmitters are increased e.g.,

- Glutamate
- Glycine
- GABA
- Dopamine

The Blood tests in patients with brain ischemia or hemorrhage

Complete blood count	Prothrombin time	Thrombin time and/or ecarin clotting time	Blood lipids	Enzymes
including hemoglobin, hematocrit, white blood cell count, and platelet count	international normalized ratio (INR), and activated partial thromboplastin time	if patient is known or suspected to be taking a direct thrombin inhibitor or a direct factor Xa inhibitor	including total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, and triglycerides.	Cardiac enzymes and troponin

Biochemical basis of pharmacological intervention

Examples of Potential Biochemical Intervention in Cerebral Ischemia:

- Inhibitors of glutamate release
- Ca2+ channel blockers
- Nitric oxide synthase inhibitors & free radical inhibition
- Calpain inhibitors

Doctors' Notes

Click the note to go back to the related slide

[1] stroke means when blood and nutrient supply to brain cells is blocked leading to the death of cells. It is happened due to either decreased in blood flow to the brain due to rupture of cerebral vessels"hemorrhagic stroke" or due to blockage of blood flow by a clot "ischemic stroke".

[2] ischemic stroke is caused by a clot, This clot either localized in the brain (thrombotic) , or it came from other side of the body such as the heart (emboli). Majority of strokes are ischemic strokes.

[3] Thrombus originated in the vessels of the brain

[4] Embolism is a detached part of a thrombus that might travel to the brain

[5] hemorrhagic stroke is two types according to the site of the ruptured vessel, **intracerebral** when the ruptured vessel inside the brain tissue, **subarachnoid** when the ruptured vessel on the surface of the brain which will increase the intracranial pressure causing severe headache.

[6] Aspirin is used by some individuals in low doses to prevent CVA but there's a debate whether its effective or not, in high doses it can cause hemorrhagic stroke.

[7] Rarely stroke can happen without any risk factors.

[8] Normal cell death is two type: **Apoptosis**:- programmed cell death, and **Necrosis:-** cell death induced by stress.

[9] Severity and duration of the stroke determine which type of cell death will more occur. core is necrotic cell death, surrounded by apoptotic cell death.

[10] Proteolysis means brain protein degradation by enzymes such as Ca-induced- Calpain-mediated proteolysis. This enzyme requires Ca to be activated.

[11] Cytoskeletal proteins control the shape and structure of the cell, regulatory and signaling proteins control the movement of substances from and to the cell.

[12] Oxidative stress means when there is imbalance between oxidants and antioxidants inside the cell.

[13] They increase PKC activity by increasing the presence of Ca that is required for PKC activities. once this enzyme being activated, it will phosphorylate MBP which is a protein that is normally present in myelin sheath". This step is important for MBP interactions with other cytoskeleton proteins such as actin.

[14] This figure shows antioxidant machinery which contains enzymes that take care of ROS and completely reduced them.

[15] Selenium is a mineral that is required for activation of glutathione peroxidase.

[16] Iron in **normal** amounts helps in anti-oxidation, but it can lead to production of hydroxyl free radicals by process called **Fenton** mainly will damage the DNA.

[17] The brain has less levels of antioxidants because it's not as permeable as other organs (BBB).

[18] Free radicals have alone pair of electron. This electron is highly active to interact with another ion, unsaturated lipids" double bond lipids" are sharing one pair of electron and they will accept any free electron. so when these lipids accept this free electron "free radical" these lipids will be damaged so they are highly vulnerable to be damaged by free radicals. And peroxidizable lipids are unsaturated**.**

[19] Like in hemochromatosis, iron in high levels causes pro-oxidant effect instead of antioxidant. Pro-oxidant means more production of free radicals.

[20] glutamate and dopamine oxidase increases ROS levels, dopamine plays a role in parkinson's pathogenesis while glutamate plays a role in the pathogenesis of alzheimer's. Any type of oxidase enzymes will produce free radicals "because they utilize O2".

[21] NO is normally present in the body as a neurotransmitter. when there is an excess amount of ROS, this NO will bind to ROS and produce Reactivated Nitrogen Species "RNS".

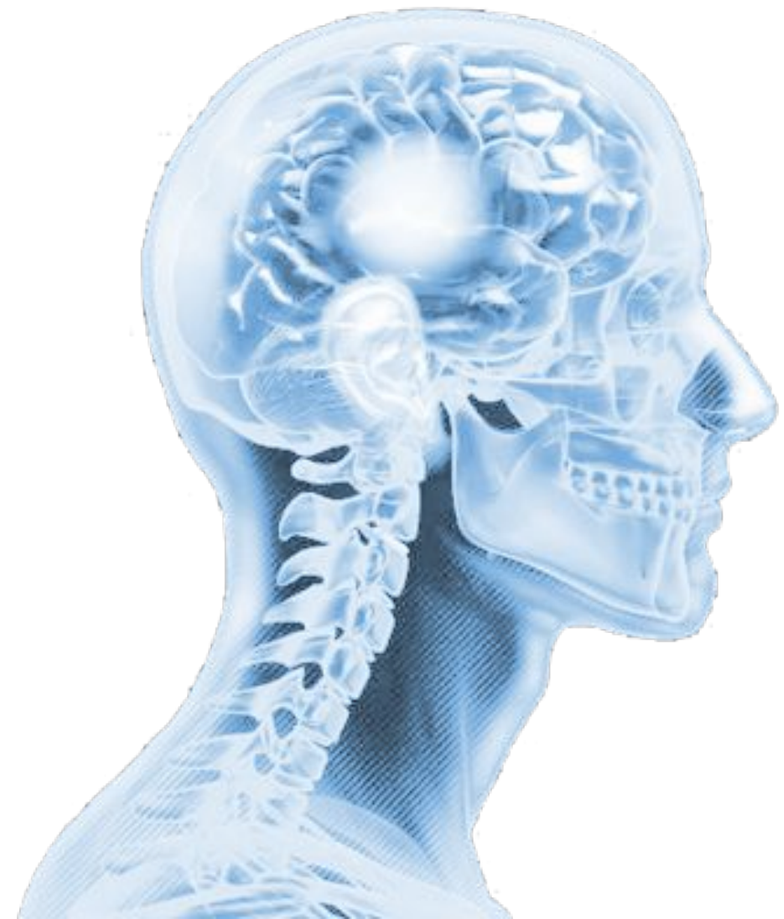
[22] When there's less ATP ion channels and pumps won't work properly, so Na/K atpase will be reversed as well as Ca, Ca influx causes release of calpins (proteolytic).

Take Home Messages

Severe cerebral ischemic insults lead to a **complex cascade of biochemical and molecular events**, including:

1- Cell death
2- Oxidative stress
3- Metabolic stress and neurochemical changes

Click on the picture to read more about strokes



Summary

From the doctors' slides

Energy Failure/ Depolarization/ Oxidative Stress
(Consequences of brain ischemia)

Neurotransmitter release and receptor activation

Ca^{2+} ↑

Lipolysis (DAG↑ → PKC↑)

Protein phosphorylation

Proteolysis

FFAs ↑

Membrane damage

Dysfunction of receptors and ion channels

Break down of cytoskeleton

Inhibition of axonal transport, blebbing

Ischemic cascade

Lack of oxygen supply to ischemic neurons

↓

ATP depletion

↓

Malfunctioning of membrane ion system

↓

Depolarisation of neurons

↓

Influx of calcium

↓

Release of neurotransmitters, activation of proteases

↓

Further depolarisation of cells

↓

Further calcium influx

MCQs

1-The enzyme that converts hydrogen peroxide to oxygen is:
A-Catalase
B-Superoxide dismutase
C-Glutathione peroxidase
D-NADPH oxidase

2-Which one of the following is a molecular effect of ROS in ischemic stroke:
A-Increased platelet aggregability
B-Increased endothelial cell permeability
C-Altered vascular tone
D-Protein denaturation

3-Which of the following is not one of the biochemical responses to ischemic brain injury:
A-Oxidative stress
B-Metabolic stress
C-Altered vascular tone
D-Neurochemical response

4-Which one of the following induces calpain:
A-Na+
B-Ca++
C-K+
D-O2

5-A result on energy depletion due to ischemia is:
A-Alkalosis
B-Acidosis
C-Na+ efflux
D-K+ influx

6-The cell death mechanism that occurs with more mild insults and with longer survival periods is:
A-Phagocytosis
B-Toxicosis
C-Apoptosis
D-Necrosis

Answers key

1- A 2- D 3- C 4- B 5- B 6-C

SAQs

1- Enumerate the risk factors of hemorrhagic stroke:

- High blood pressure
- Smoking
- Illegal drug use
- Use of warfarin or other blood thinning medicines

2- Mention the beneficial & the harmful types of NO & their effects:

- Beneficial: NO produced by endothelial NOS (eNOS)→improving vascular dilation and perfusion.
- Harmful: NO production by neuronal NOS (nNOS) or by the inducible form of NOS (iNOS)
- Increased iNOS activity is associated with inflammatory processes.

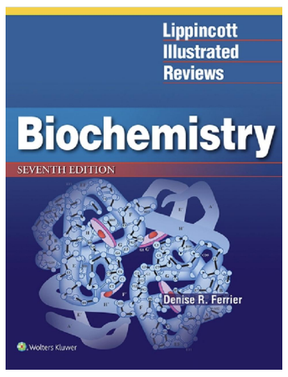
3- What are the effects of increased cytosolic Ca levels ?

activates:

- ATPase→ decreased ATP.
- Phospholipase→ decreased phospholipids. (Membrane damage)
- Protease→ disruption of membrane and cytoskeletal proteins (Membrane damage)
- Endonuclease→ nucleus chromatin damage.

Resources

Click on the book to download the resource



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Special thanks to Fahad AlAjmi for designing our team's logo.