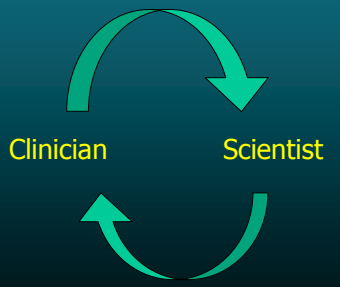
Universitätsspital Basel

Stroke
Focal ischemic brain lesion
Molecular Aspects of Neurological Diseases

Philippe Lyrer
29.05.2007

Aim

- Close the gap !



Overview

- What is a stroke
- Animal models
- The ischemic cascade
- Translation to clinical trials
- Diffuse subcortical ischemic lesions
- Failure of clinical trials
- Outlook

Stroke - Definition

There are two main types of stroke

- **Ischemic stroke** 84%
  - is caused by blockage in an artery that supplies blood to the brain, resulting in a deficiency in blood flow (ischemia)
- **Hemorrhagic stroke** 16%
  - is caused by the bleeding of ruptured blood vessels (hemorrhage) in the brain 10% intracerebral, 6% subarachnoidal.

Stroke – "biologic" Definition

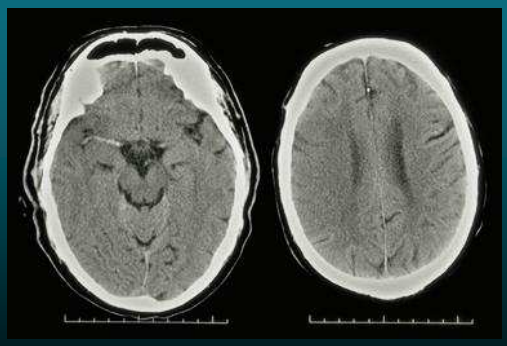
During ischemic stroke, diminished blood flow initiates a series of events (called **ischemic cascade**) that may result in additional, delayed damage to brain cells

Early medical intervention can halt this process and reduce the risk for irreversible complications.

Background Information

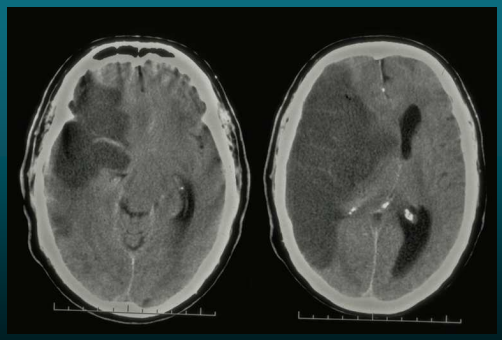
- 3rd most common cause of death
  - after heart attack and cancer
- Higher mortality with increasing age:
  - ≥ 60 years 2nd most common cause of death
- Men and women equally frequent
- Incidence: 150 - 200 / 100 000/year
  - Basel: 170 / 100'000/year (02-03)
  - 45-84 years old: 400 / 100 000/year

Ischemic brain lesion: cranial computer tomography early imaging (2 hours)



Dept. of Neuroradiology, University Hospital Basel, Switzerland

Ischemic brain lesion: delayed cranial computer tomography (48 hours)



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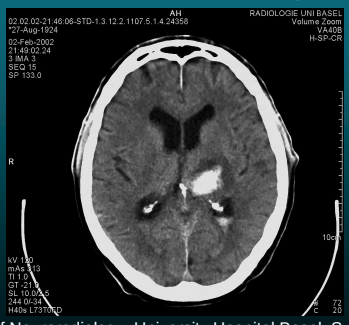
Hemorrhagic brain lesion: Cranial Computer tomography

Exclude intracranial Hemorrhage !



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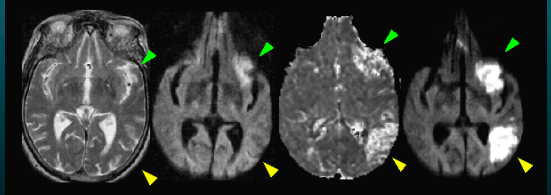
Penumbra: DWI-PWI Mismatch

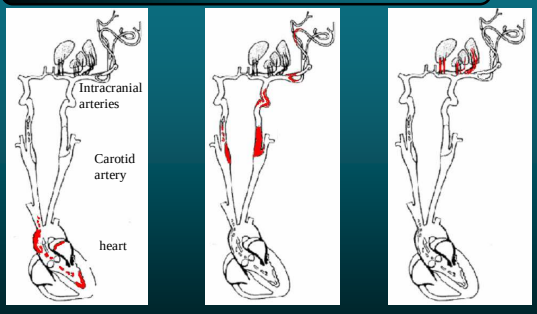
Diffusion / perfusion mismatch may be a marker for territory at risk.



T2 FSE | Initial DWI | Initial MTT | Follow-up DWI (5 days later)

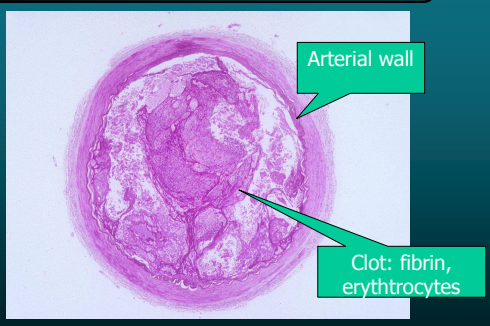
AGS/ MGH

Mechanism of cerebral ischemia



cardiac Emboly 25-35% | cerebral macroangiopathy 15-20 % | cerebral microangiopathy 30%

Embolic artrial occlusion



- Embolic clot occlusion of the middle cerebral artery
  (courtesy of A.Probst, Neuropathology Basel)

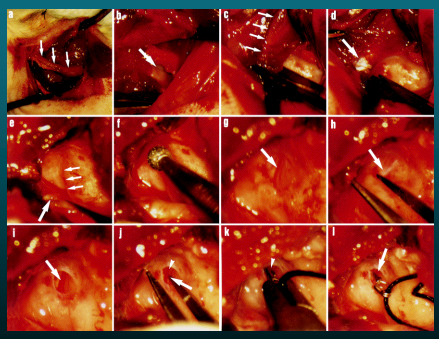
Animal models

- Rat: 4-Vessel occlusion (global)
  MCAO (focal)
- Mouse: MCAO (focal)
- Gerbil CCAO (focal, global)
- Rabbit: clot model (t-PA model)
- Cat: cardiac arrest models
- Dog: cardiac arrest cardiac arrest
- Non-human primates: behavioral models

- Note: Transient vs. Permanent vessel occlusion

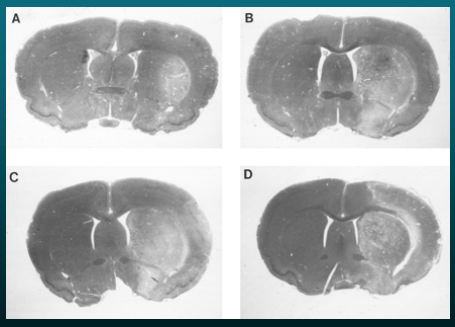
Lit.: Hoyte L. et al.: Exp. Neurology 2004

MCAO: Three-vessel occlusion using a micro-clip for the proximal left middle cerebral artery



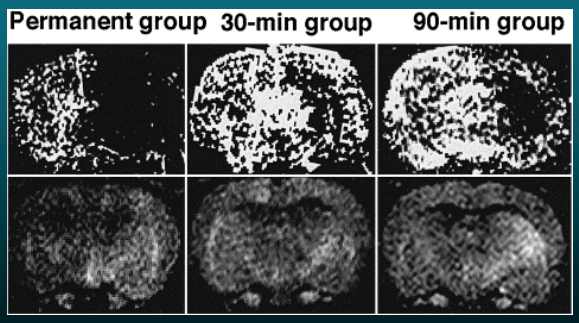
Lit.: Hiroji Yanamoto, Izumi Nagata, Nobuo Hashimoto and Haruhiko Kikuchi

MCA occlusion – permanent (autoradiography)



Lit.: Belayev L, et al. Stroke. 1996

MCA occlusion - permanent and temporary

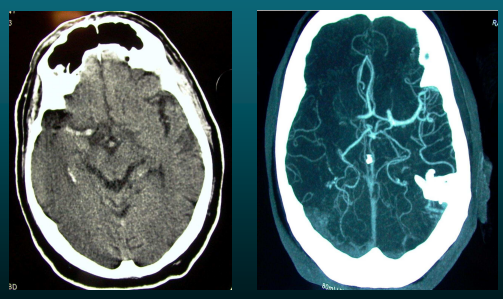


Yasuki Onoa, Shigehiro Morikawab, Toshiro Inubushib, Hiroaki Shimizua, *, and Takashi Yoshimotoa

CT Schädel

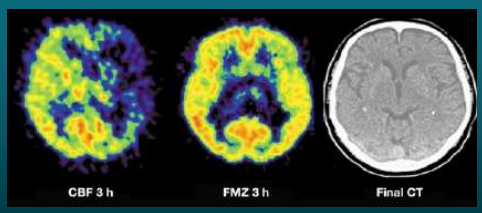
Nativ

Spiral-CT mit KM



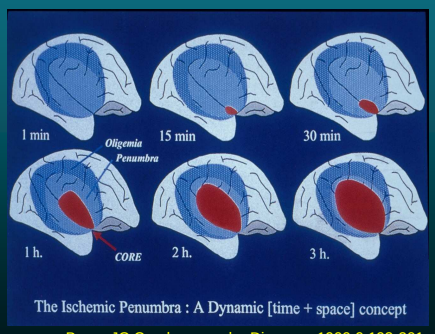
(Aufnahme: Neuroradiologie Universitätskliniken Basel, Prof. F.W. Hodler)

PET from patient with acute MCA-occsion



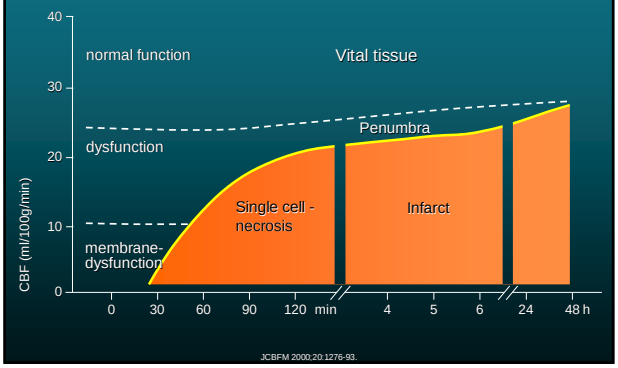
- Regional hypoperfusion at 3 hours after beginning of symptoms
- By reperfusion, no lesion on CT few days later

The penumbra concept



Baron JC Cerebrovascular Diseases 1999;9:193-201.

„Time is Brain"



Perfusion Basics

- CBF - Cerebral Blood Flow (ml/100ml/min)
  - Gray matter 45-70 ml/100ml/min
  - White Matter 20-24 ml/100ml/min
- CBV - Cerebral Blood Volume (ml/100ml)
  - Gray matter 4-7 %
  - white matter 2-3%
- MTT - Mean Transit Time (seconds)
  - Mean time to traverse vasculature (NOT pixel!)
  - CBV:CBF ratio

What happens in stroke ?

Blood supply drops suddenly threshold values:

Cerebral blodd flow values:

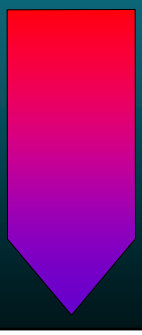
→80-60 ml/min/100g:
normal state

→37-60 ml/min/100g:
protein syn., selective gene exp.

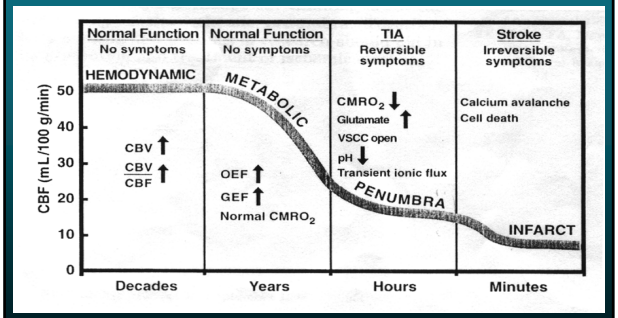
→20 –36 ml/min/100g:
lactic acidosis, cytotoxic edema

→10-20 ml/min/100g:
energy deficit, glutamate tox.

→0-10 ml/min/100g:
anoxic depolarisation, infarction

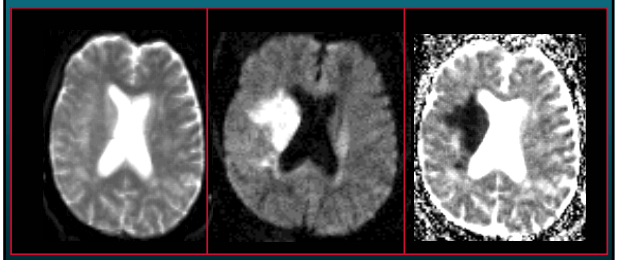


Cerebral blood flow



(from Hakim; Neurology:51(supp3):S44-6)

Magnetic resonance imaging



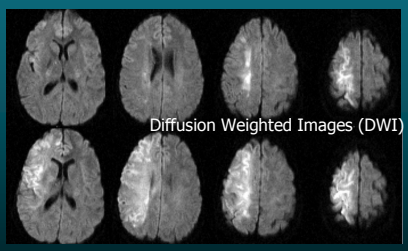
T2 DWI ADC

52 y/o WF with migraines, awoke with right H/A, L hemiparesis

Diagnostic Information in Acute Stroke: Diffusion Weighted Imaging

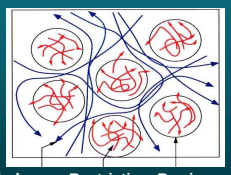
At 5 hours...

After 5 days...

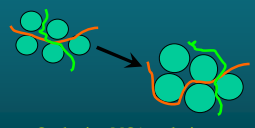


- Diagnostic Info: Assess initial infarct by DWI
- Prognostic Info: Predict infarct growth by PWI

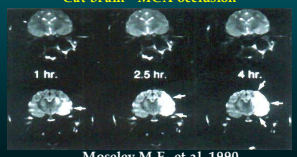
Cytotoxic Edema: Diffusion Weighted Imaging



Hindrance Restriction Barriers



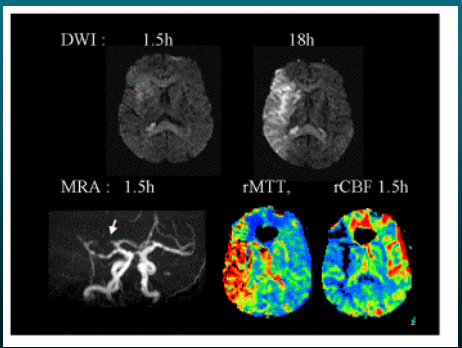
Cat brain - MCA occlusion



Moseley M.E, et al. 1990

ATP-depletion
Na^+-K^+ pump
Cytotoxic edema
Increased extracellular tortuosity
Hindrance of water movement
Diffusion weighted images appear bright

Penumbra: DWI-PWI Mismatch



Patient with acute occlusion of A. cerebri media

What happens in stroke ?

Consequences are disturbence :

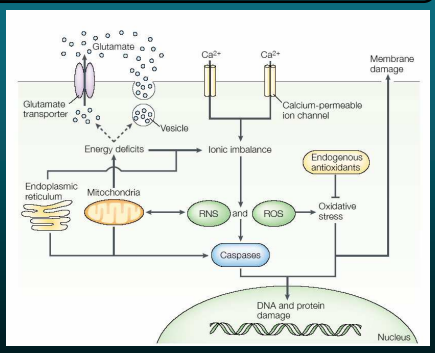
- neurons, glial cell, astrocytes – simultaneously
- vessel lesion
- interstitial space
- secondary damages: edema, space occupation, recanalisation injury, bleeding

Major pathways implicated in ischaemic cell death

- **excitotoxicity**
- **ionic imbalance**
- **oxidative and nitrosative stresses**
- **apoptotic-like mechanisms**
- **Disturbance of the neurovascular unit:**
  - **Proteolysis**
  - **inflammation**

Lo H., et al. nature rev. 2003

Major pathways implicated in ischemic cell death

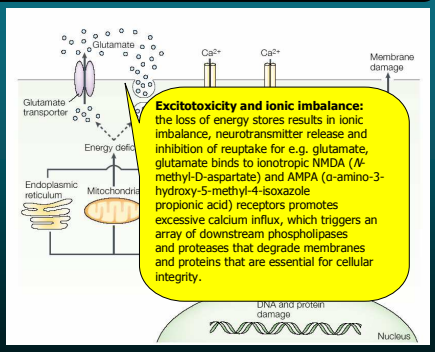


RNS reactive nitrogen species

ROS reactive oxygen species

Lo, E.H., T. Dalkara, and M.A. Moskowitz, Nat Rev Neurosci, 2003. **4**(5): p. 399-415.

Major pathways implicated in ischemic cell death



Excitotoxicity and ionic imbalance: the loss of energy stores results in ionic imbalance, neurotransmitter release and inhibition of reuptake for e.g. glutamate, glutamate binds to ionotropic NMDA (*N*-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors promotes excessive calcium influx, which triggers an array of downstream phospholipases and proteases that degrade membranes and proteins that are essential for cellular integrity.

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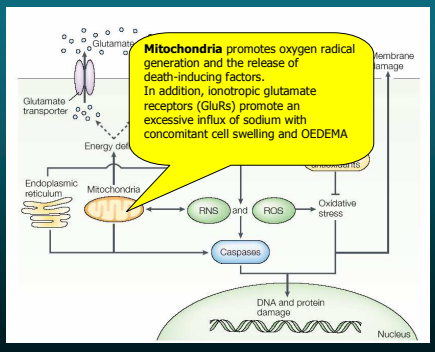
Major pathways implicated in ischemic cell death



Mitochondria promotes oxygen radical generation and the release of death-inducing factors.
In addition, ionotropic glutamate receptors (GluRs) promote an excessive influx of sodium with concomitant cell swelling and OEDEMA

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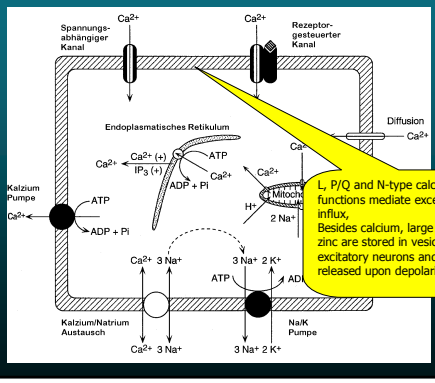
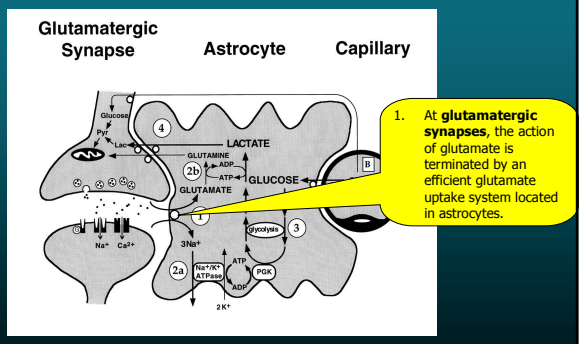
Ionic imbalance -Calcium metabolism



L, P/Q and N-type calcium channel functions mediate excessive calcium influx,
Besides calcium, large amounts of zinc are stored in vesicles of excitatory neurons and are co-released upon depolarization

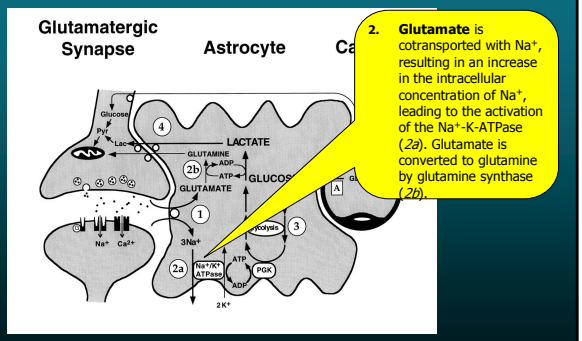
Glutamate toxicity - glucose



1. At **glutamatergic synapses**, the action of glutamate is terminated by an efficient glutamate uptake system located in astrocytes.

Magistretti P., Pellerin L.;News Physiol Sci. 1999 Oct;14:177-182.
Pyr, pyruvate; Lac, lactate; Gln, glutamine; G, G protein; PGK, phosphoglycerate kinase

Glutamate toxicity - glucose



2. **Glutamate** is cotransported with Na^+, resulting in an increase in the intracellular concentration of Na^+, leading to the activation of the Na^+-K-ATPase (*2a*). Glutamate is converted to glutamine by glutamine synthase (*2b*).

Magistretti P., Pellerin L.;News Physiol Sci. 1999 Oct;14:177-182.
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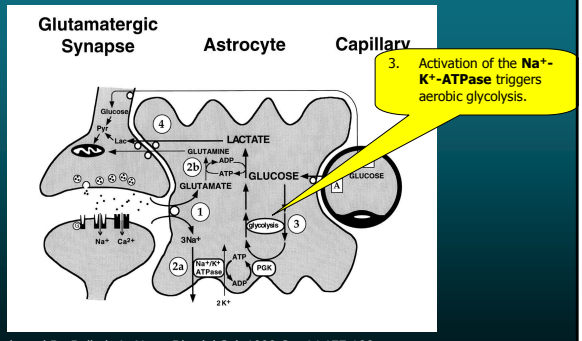
Glutamate toxicity - glucose



3. Activation of the **Na^+-K^+-ATPase** triggers aerobic glycolysis.

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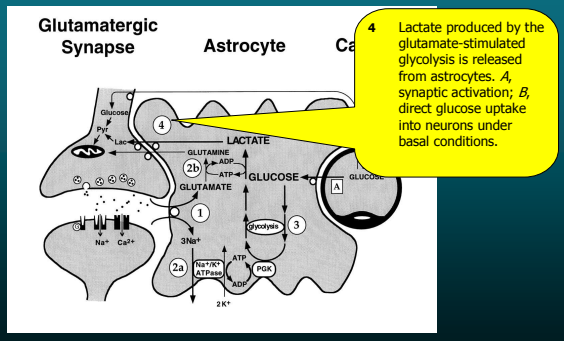
Glutamate toxicity - glucose



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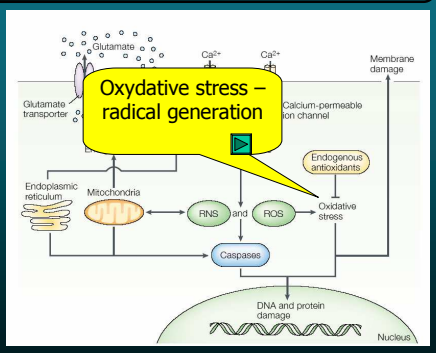
Pyr, pyruvate; Lac, lactate; Gln, glutamine; G, G protein; PGK, phosphoglycerate kinase

Summary of glutamate-calcium release

- K^+ efflux
- Excesive glutamate release and over excitation of glutamate receptors
- Ca^{2+} release from organelles
- Ca^{2+} influx and sequestration in the cell
- Intracellular accumulation of Ca^{2+} and Zn^{2+}

  ➢ ends in activation of calmodulin dependent intracellular enzymes (phospholipases, endonucleases and protein kianses)

Major pathways implicated in ischemic cell death



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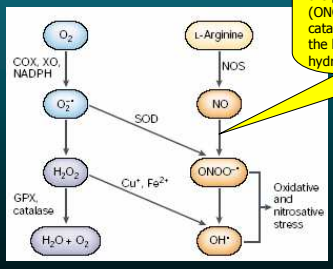
Generation of oxygen and nitrogen radicals



Combination of superoxide (O2-•) and nitric oxide (NO) generates the potent radical peroxynitrite anion (ONOO-•). Metal (Cu+ and Fe2+) catalysed pathways can also produce the hydroxyl radical (OH•) from hydrogen peroxide (H2O2).

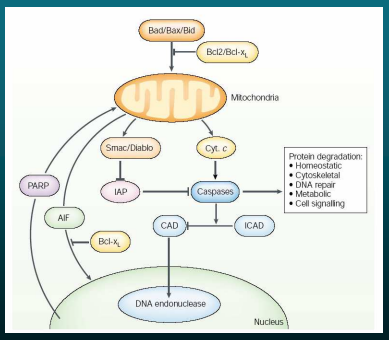
COX - cyclooxygenase
GPX - glutathione peroxidase
NOS - nitric oxide synthase
SOD - superoxide dismutase
XO - xanthine oxidase

apoptotic-like mechanisms Cell death pathways that are relevant to an apoptotic-like mechanism in cerebral ischaemia

IAP inhibitors of apoptosis

PARP poly-(ADP ribose) polymerase

AIF Apoptosis inducing factor



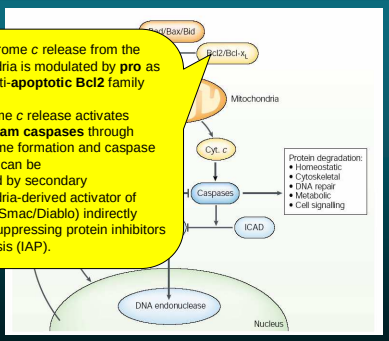
Smac Secondary mitochondria derived activator of caspase

CAD caspase-activated deoxyribo nuclease

Lo, E.H., T. Dalkara, and M.A. Moskowitz, Nat Rev Neurosci, 2003. **4**(5): p. 399-415.

apoptotic-like mechanisms Cell death pathways that are relevant to an apoptotic-like mechanism in cerebral ischaemia

1. Cytochrome *c* release from the mitochondria is modulated by **pro** as well as anti-**apoptotic Bcl2** family members.
Cytochrome *c* release activates **downstream caspases** through apoptosome formation and caspase activation can be modulated by secondary mitochondria-derived activator of caspase (Smac/Diablo) indirectly through suppressing protein inhibitors of apoptosis (IAP).



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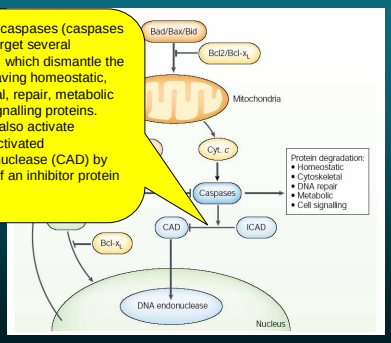
apoptotic-like mechanisms Cell death pathways that are relevant to an apoptotic-like mechanism in cerebral ischaemia

2. Effector caspases (caspases 3 and 7) target several substrates, which dismantle the cell by cleaving homeostatic, cytoskeletal, repair, metabolic and cell signalling proteins. Caspases also activate caspase-activated deoxyribonuclease (CAD) by cleavage of an inhibitor protein (ICAD)..



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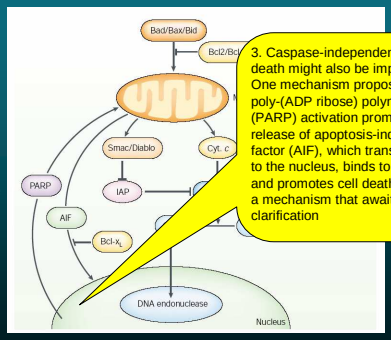
apoptotic-like mechanisms Cell death pathways that are relevant to an apoptotic-like mechanism in cerebral ischaemia

IAP inhibitors of apoptosis

PARP poly-(ADP ribose) polymerase

AIF Apoptosis inducing factor

3. Caspase-independent cell death might also be important. One mechanism proposes that poly-(ADP ribose) polymerase (PARP) activation promotes the release of apoptosis-inducing factor (AIF), which translocates to the nucleus, binds to DNA and promotes cell death through a mechanism that awaits clarification



activated deoxyribonuclease

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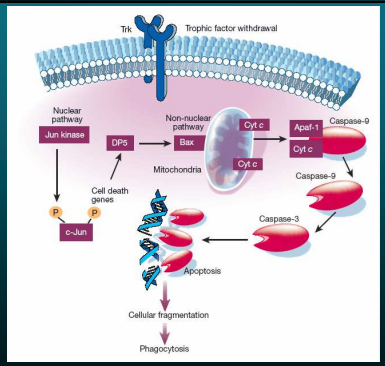
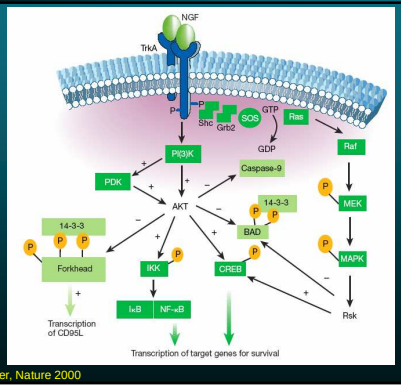
Apoptosis



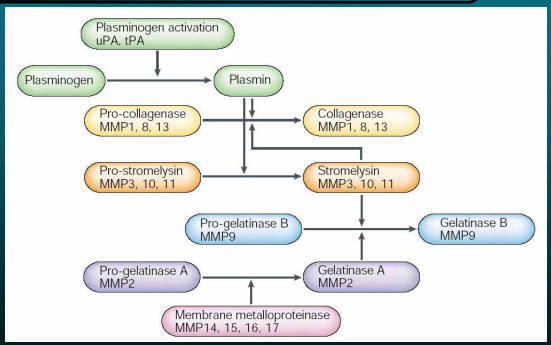
Yuang & Yankner, Nature 2000

Apoptosis



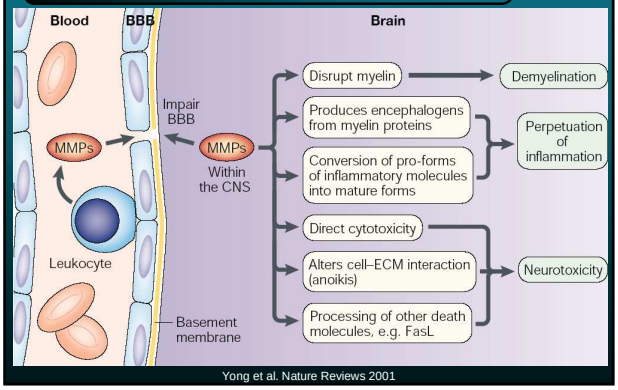
Yuang & Yankner, Nature 2000

protease cascade involving members of the matrixmetalloproteinase



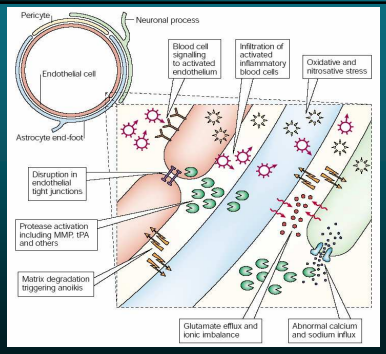
Lo, E.H., T. Dalkara, and M.A. Moskowitz, Nat Rev Neurosci, 2003. **4**(5): p. 399-415.

Metalloproteinases



Yong et al. Nature Reviews 2001

Schematic view of the neurovascular unit or module, and some of its components



Lo, E.H., T. Dalkara, and M.A. Moskowitz, Nat Rev Neurosci, 2003. **4**(5): p. 399-415.

Cause: Perfusion deficit, CBF <20ml/100g/min, complete, partial, time-dependent

Stop of metabolism, depletion of ATP, accumulation of lactate

Breakdown of the ATP-dependent Na-K-pump, loss of electric gradient, missing re-uptake and loss of glutamate, excitotoxicity

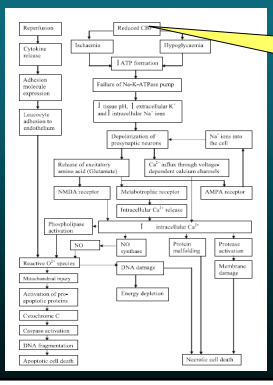
Increase of intracellular Ca^{2+}-due to inflow of Ca^{2+} from extracellular space and release of Ca^{2+} from endoplasmatic reticulum and mitochondria

Activation of proteases, early gene expression, phospholipidase A2 triggers production of arachidonic acid → cell mediators: leucotriens, adhesion molecules, platelet aggregation factor

Generation of free radicals, arachidonic acid -> prostaglandin, oxidation of lipids, release of iron

Cellular edema, irreversible cellular destruction, apoptosis via programmed metabolism due to proteases and endonucleases

Overview of the ischemic cascade



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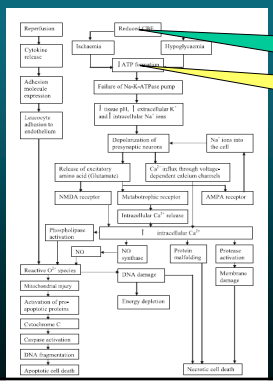

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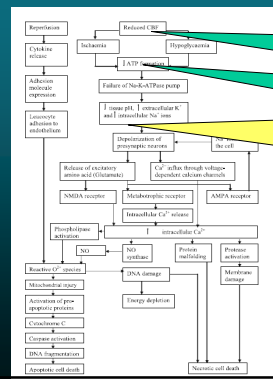

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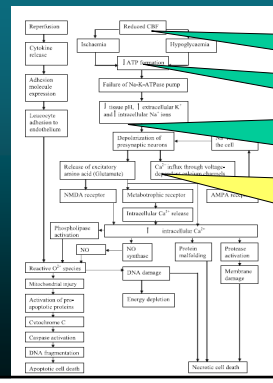

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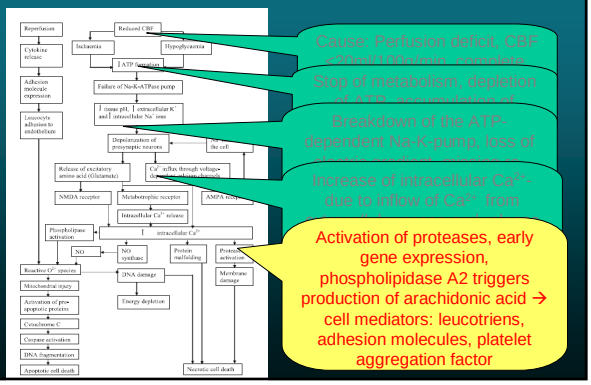

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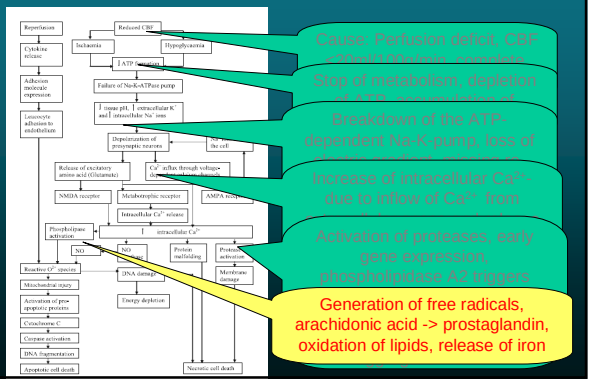

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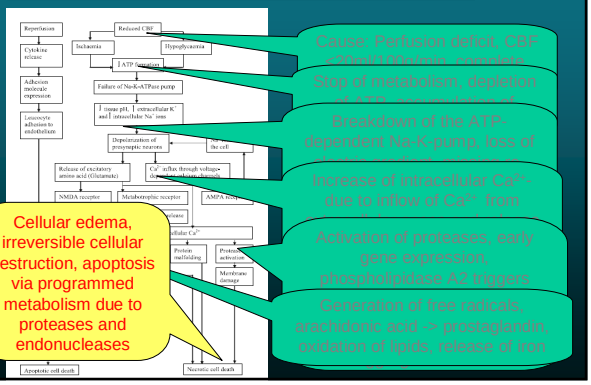

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The concept of so-called neuroprotection

- Neuroprotective treatment
  - Glutamat-Antagonist
    - NMDA-Receptor-Antagonist (e.g. Magnesium, Lubeluzole)
    - AMPA-Receptor-Antagonist (e.g. ZK200775)
    - Glycin-Antagonist (e.g. GV-150526A)
  - GABA-Agonist (e.g. Clomethiazol)
  - GABA-Analoga (e.g. Pirazetam)
  - Calcium antagonist (e.g. Nimodipin)
  - calcium channel blockers (e.g. BMS-204352)
  - Adenosin agonist (e.g. Acadesin, Propentofyllin, Pentoxifyllin)
  - 5-HT_{1A} -Agonist (e.g. Ipsapiron, BAY X 3702)
  - growth factors (e.g. Trafermin)
  - Membrane-stabilizer (e.g. Citicholin, Tirilazad)
- Anti-inflammatory treatment
  - Cytokin inhibitors (e.g. IL-1-Rezeptor-Antagonisten)
  - Immune modulation (e.g. Tacrolimus, Cyclosporin)
- free radical scavengers
- barbiturates

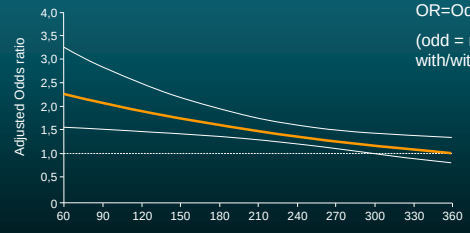
Hoyte, L., J. Kaur, and A.M. Buchan, Lost in translation: taking neuroprotection from animal models to clinical trials. Exp Neurol, 2004. 188(2): p. 200-4.

Treatment with thrombolysis and its therapeutic effect

OR/NNT combined endpoints (mRS1, NIHSS 1, BI≥95)

0–90 min: OR 2,8; NNT»4 91-180 min: OR 1,5; NNT»9

181-270 min: OR 1,4; NNT»21 271-360 min: OR 1,2; NNT»45



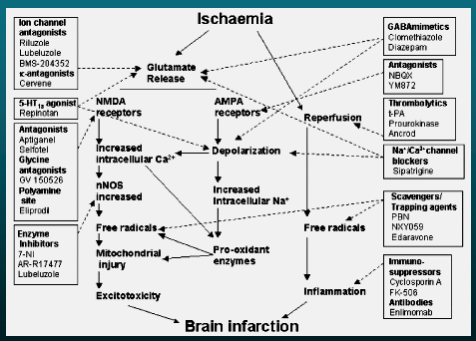
OR=Odds ratio

(odd = ratio of number with/without event)

From RCT:
NINDS
ECASS I + II
ATLANTIS

Brott et al., 2003

The concept of so-called neuroprotection



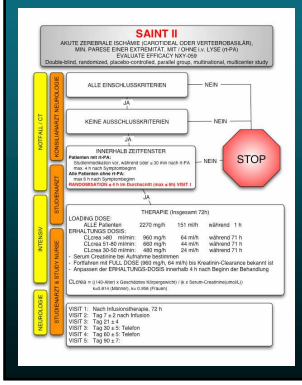
A. Richard Green, Tim Ashwood, Tomas Odergren, David M. Jackson; Pharmacology & Therapeutics 100 (2003) 195– 214

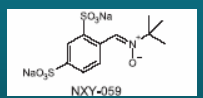
Lit.:Wahlgren NG; Cerebrovasc Dis 2004

Neuroprotective clininal trials in UHBS

N patients	Trial; years	Drug	Result
3	TESS; 94-95	Tirilazad	Negative - harmful
4	TEAST; 97-98	Trafermin (fibroblast growth factor)	harmful
20	POST 010; 00-01	BMS 204352	Negative safe

SAINT-2 new trial





Investigational compound

Nitron, traps free radicals

i.v. therapy starts within 6 hours of stroke onset

Infarct size reduction in rat MCAO 62-69 %

What is wrong with neuroprotection ?

„Is it not erroneous to lump together infarcts of all shapes, sizes, times, severities, and locations due to various occlusions (or no occlusions or site of occlusion unknown) and trust the statisticians to make sense of it all through randomization into underpowered trials?“

Furlan AJ, Stroke 2002;33:1450-501.

Reasons for neuroprotectants failure

- Pharmacologic reasons
  - pharmacologic target not relevant to humans
  - wrong dose
  - wrong treatment duration
  - unsuitable pahrmacokinetics
- Unsuitable study design
  - Use of wrong measurements (outcomes)
  - delayed treatment
- Statistical Power[1]
  - overestimation of therapeutic effect (>10%)
  - underestimation of clinical relevant effects
- Pathophysiological heterogeneity[2] of the disease
  - no biological substrate to salvage
  - no penumbra
  - lack of reperfusion

[1]Stroke 2001;32:669-74; [2]Stroke 2002;33:1545-50.

Future design for neuroprotective trials

More standardized stroke syndromes for inclusion in trials

Animal models should be standardized

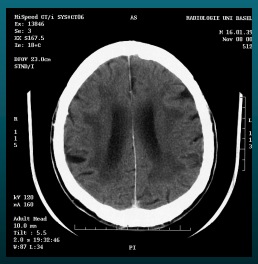
Short time window

Salvageable tissue in the DWI/PWI MRI

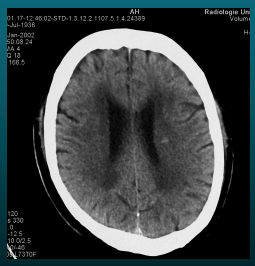
Molecule that passes BBB

Co-administration of thrombolytic therapy

Leucoaraiosis

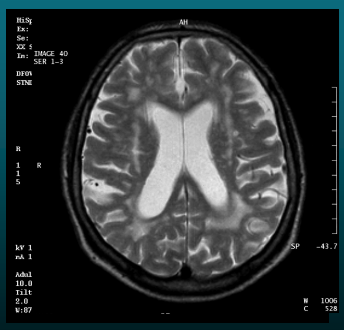


JJ160139, m 61 yrs
Hypertension



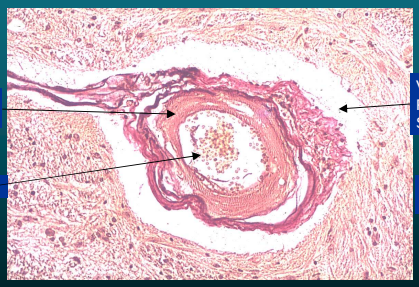
RE030736, m 66-years,
no hypertension

Subcortical hypertensive arteriosclerotic enzephalopathy



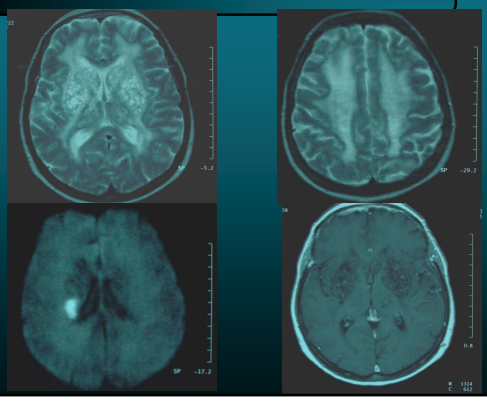
JJ160139, m 61 yrs, hypertension

Pathology – miccroscopic appearence of affected vessels (arterioles)



from A. Probst, Neuropathology, Basel

FM, 020136, f



Vascular Dementia - Epidemiology

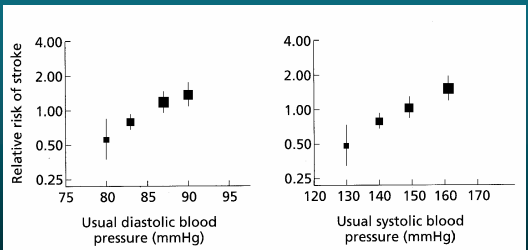
- Prevalence (%) in Europe/USA:

1989 Boston	AD	8.7	VaD	0.9
1990 London		3.1		0.1
1991 Stockholm		6.0		3.0
1995 Rotterdam		4.5		1.0
1997 Odense		4.7		1.3

- Ratio AD/VaD: <0.1-0.5 !

Review, Acta Psych Scand 2001;104:4-11

Blood pressure and risk of stroke



per 5-6mmHg diastolic or 10-12 systolic BP-elevation: 38% relative Risk increase

(Lit.: Journal of Neurology, Neurosurgery and Psychiatry. 1991; 54: 1044-1054)

Animal models: **Genetically predisposed rats**

Spontaneously Hypertensive rats (SHR)

Origine:

Okamoto at the Kyoto School of Medicine in 1963 from an outbred Wistar Kyoto male with marked elevation of blood pressure mated to female with slightly elevated blood pressure

Characteristics:

Hypertension, insulin resistance, hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia.

Spontaneously Hypertensive Stroke Prone rats (SHR-SP)

Characteristics:

82 % of males will develop cerebrovascular lesions (cerebral hemorrhage or infarction) over 100 days of age.
Hypertension,nephropahy, insulin resistance, hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia.

Animal models: **Genetically predisposed rats**

Anatomical abnormalities:

- Variable branching pattern of the distal MCA
- Morphological arrangement of vascular smooth muscle cells are disorganized in the basilar artery of the SHR-SP: influence collateral circulation, rheological changes of blood flow, or vulnerability of the arterial wall to high blood pressure,
- Abnormalities in the BBB: plasma components leakage through arteriols: induce fibrinoid necrosis of small arteries, severe brain edema and lacunar infarction.
- No atheromatous lesions.

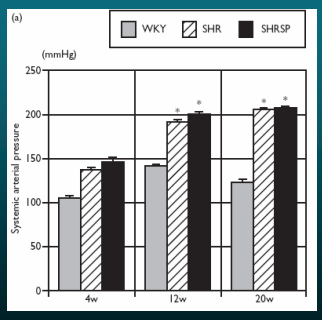
Vascular physiology:

- Response of the cerebral artery to substances causing endothel-dependent vasodilatation impaired in SHR-SP.

Genetic loci involved:

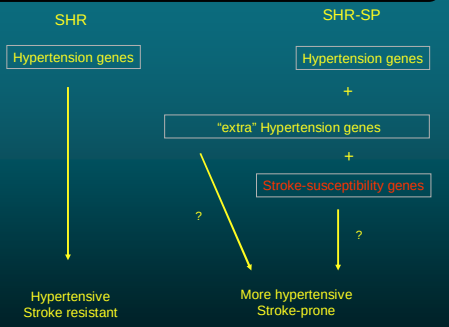
- Chromosomes 1 and 18: genes involved in blood pressure
- Chromosome 5: blood pressure independent, co-localized with genes encoding atrial and brain natriuretic factor

Blood pressure according to different strains



Lin J.-X. et al., Neuroreport 2001;12:1835-1839

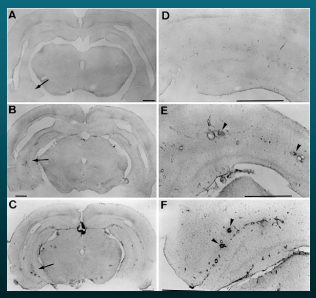
Hypothetical hypertension genes of SHR and SHR-SP



Nabika T. et al., Cellular and Molecular Neurobiology 2004;24:639-646

Vascular permeability in WKY, SHR and SHR-SP

Horse-radish peroxidase accumu-lation



Normotensive
Stroke-resistant WKY

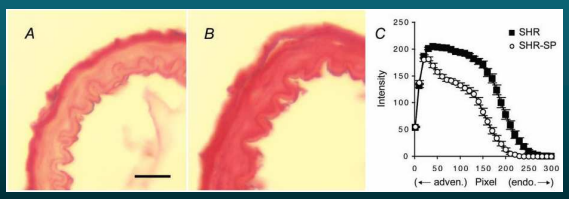
Hypertensive
Stroke-resistant SHR

Hypertensive
Stroke-prone SHR-SP

Ueno M. et al. Acta Neuropathol 2004;107:532-538

Differences between SHR and SHR-SP strains

Collagen staining reveals significant differences in the distribution of collagen in MCA from SHR-SP compared with SHR:

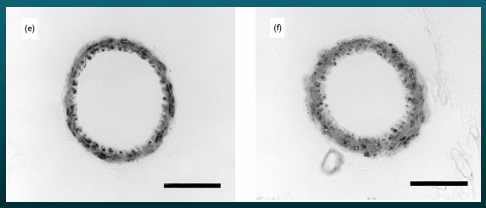


SHR-SP SHR

Izzard A.S. et al., Am J Physiol Heart Circl Physiol 2003;285 H1489-1494

Differences between Wistar and stroke prone SHR

Smooth muscle actin staining, increased in SHR-SP with hypertrophic arteries (Anterior cerebral artery)

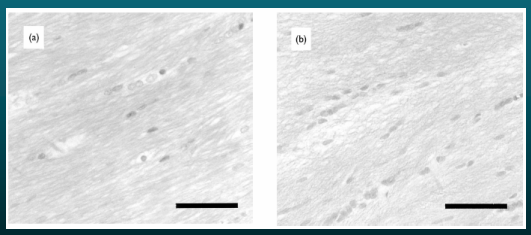


WKY SHR-SP

Lin J.-X. et al., Neuroreport 2001;12:1835-1839

Klüver-Barrera staining of white matter in corpus callosum

Disturbed structure of the white matter im CC from SHR-SP: disarrangement of nerve fiber, marked vacuoles und disappearence of myelinated fibers



WKY SHR-SP

Lin J.-X. et al., Neuroreport 2001;12:1835-1839

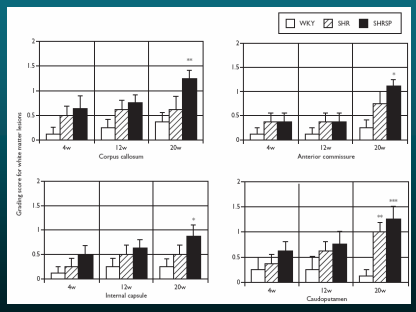
Appearance of white matter lesions in the brain of SHR, SHR-SP

Normal (grade 0)

disarrangement of the nerve fibers (grade 1)

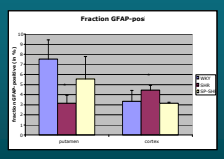
formation of marked vacuoles (grade 2)

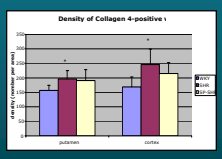
disappearance of myelinated fibers (grade 3)

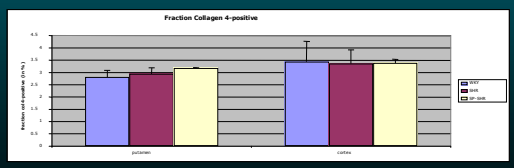


Lin J.-X. et al., Neuroreport 2001;12:1835-1839

Col4 and GFAP in normotensive and hypertensive rats







The End