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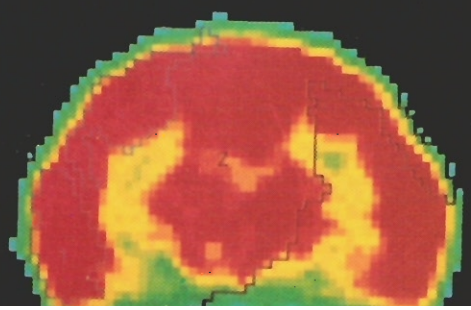

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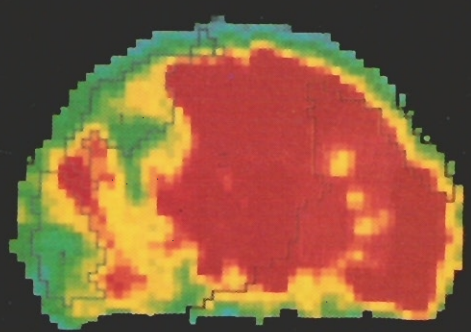

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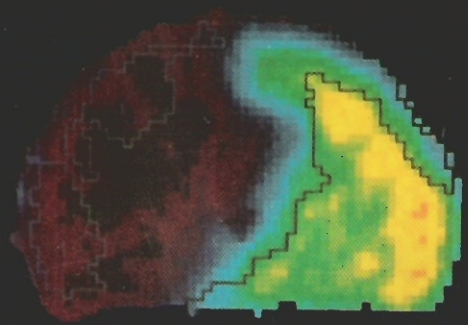

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Review Article

Emerging Insights into the Genesis of Cerebral Ischaemia and Stroke.

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Summary. *Stroke is a global problem with increasing significance because of the ageing population. Except for age, hypertension is by far the most important risk factor for stroke. Hypertension predisposes to a number of intracerebral and extracerebral vascular lesions which may cause cerebrovascular events by different mechanisms. The high metabolic need and low energy reserve make the brain very vulnerable to ischaemia. During the last decade a number of experimental studies - supported by PET studies in man - suggest the presence of a therapeutic window, i.e. the time during which the neurons can be saved. The penumbra is the zone surrounding the core of the infarct where the flow is decreased and the neurons are lethargic and may be electrically silent but still viable. The presumed role of calcium, excitatory amino acids, free radicals, platelet-activating factor, acidosis and brain temperature in the process of neuronal death is briefly reviewed.*

Keywords : Ischaemia, Stroke, Hypertension, Atherosclerosis, Positron Emission Tomography (PET), Middle Cerebral Artery Occlusion (MCA), Reperfusion, Free radicals, Nitric Oxide (NO).

Introduction

As a consequence of the high rate of oxygen metabolism and the lack of tissue oxygen stores, interruption of oxygen delivery to the brain causes immediate cell dysfunction and rapidly leads to cell death. Oxygen delivery to the brain is defined as the product of the oxygen content of arterial blood and the cerebral blood flow. Inadequate oxygen delivery (hypoxia) can result from inadequate cerebral blood flow (Ischaemic hypoxia), inadequate partial pressure of oxygen in arterial blood (hypoxic hypoxia), or inadequate oxygen-carrying capacity of arterial blood (anaemic hypoxia). The most common cause of brain hypoxia is ischaemia or inadequate cerebral blood flow. The level of cerebral blood flow at which the brain begins to exhibit energy failure is fairly well defined. Reduction of cerebral blood flow below 15ml/min/100g of tissue results in failure of electrical activity, and a reduction to less than 10ml/min/100g of tissue results in loss of the transmembrane ionic gradient. Cellular energy depletion appears to be a triggering event for many of the damaging biochemical processes occurring during ischaemia.

There are many causes of cerebral ischaemia in humans, including head trauma, stroke and cardiac arrest. Cerebral ischaemia may be further divided into focal and global categories. In global ischaemia, blood supply to the entire brain is interrupted, e.g., cardiac arrest. In focal ischaemia, blood supply to a particular region of the brain is interrupted, usually representing the area supplied by a particular vasculature. Cerebral ischaemia may also be described as complete or incomplete. Complete ischaemia is defined as total absence of blood flow to the entire brain or region of the brain. Incomplete ischaemia, on the other hand is defined as a severe reduction of cerebral blood flow in a focal or global pattern. Ischaemia may result in reversible cell injury or may be sufficient to cause tissue death (infarction) depending on the duration and severity of ischaemia. Not all regions of the brain are affected in the same manner during global ischaemia. The most sensitive, i.e., selectively vulnerable, neurons are located in the CA1, CA3 and CA4 regions of the hippocampus, portions of the caudate and cerebellum and layers 3, 5 and 6 of the neocortex. The mechanisms responsible for this selective vulnerability are not clear. In focal ischaemia, the anatomical location and extent of ischaemic damage depend on the distribution of the blood vessels whose flow is limited and on the presence of collateral circulation. Damage resulting from focal ischaemia commonly occurs in a graded fashion because collateral circulation partially perfuses the area surrounding the ischaemic core. This ischaemic 'penumbra' may receive blood flow that is inadequate to preserve normal cellular function but adequate enough to allow recovery. The concept of the ischaemic penumbra is important because the compromised status of these areas may be improved if effective early intervention is achieved. The purpose of our study which is being conducted at Max Planck Institute for Neurological Research, Cologne, in conjunction with the Department of Biomedical Science, University of Malta, in part, is to find early indicators for many of the damaging biochemical processes occurring during ischaemia.

Types and origins of stroke

Stroke or apoplexy is defined as an abrupt onset of neurological symptoms and signs indicating a disturbed cerebral circulation and lasting for at least 24 h or leading to death earlier. If lasting for less than 24 h, the episode is called a transient ischaemic attack (TIA) and is not included in the stroke definition. In Europe and North America about 80 % of strokes are caused by cerebral haemorrhages, the figures for haemorrhages being somewhat higher in East Asia.

Infarction can be caused by occlusion of large extracranial or small intracranial arteries. Occlusions can be caused by locally formed thrombi, by emboli coming from the heart or from the carotid and vertebral arteries. Arterial dissection, induced by trauma or occurring spontaneously, may be an underdiagnosed cause of brain infarction, particularly in young patients.

Arterial dissection is often associated with localized headache or facial pain at or before the onset of neurological signs. Cerebral infarcts can also occur in connection with hypotensive episodes.

Cardiac emboli have been reported to account for between 15 and 50 percent of cases; the wide range illustrates that the diagnosis often is uncertain. Atrial fibrillation is a well recognized risk factor for cerebral embolism and the most common causes are rheumatic and ischaemic heart disease. The frequency of stroke increases with the duration of fibrillation. Cardiac embolism as a cause of stroke increases with age and may constitute about half of the cases in patients > 75 years of age.

As an intrinsic intracranial source of strokes, lacunae reflect arterial disease of the small penetrating arteries supplying the internal capsule, basal ganglia, thalamus and paramedian regions of the brain stem. They are thought to account for about 15 to 20 percent of strokes. It is currently debated whether some lacunae are of embolic origin.

Most intracerebral haemorrhages occur in the supratentorial compartment, mostly involving the basal ganglia and the thalamus. The second most common location is the subcortical white matter of the cerebral lobes. Less than 15 percent of the haemorrhages are located in the cerebellum or pons. The symptoms will depend on the location and the size of the haematoma. Although the onset is usually abrupt, both the focal deficit and the level of consciousness usually undergo a gradual worsening due to further bleeding and /or secondary swelling. Haematomas of moderate or large size are accompanied by decreased levels of alertness.

Diagnosis

The neurological symptoms and signs will depend on the vascular territory involved. Most infarcts are supratentorial with the vascular territory of the middle cerebral artery being most often affected. The clinical features sometimes suffice to differentiate acute haemorrhage from infarction. Classification of the ischaemic stroke into subtypes can, to some extent, be performed on clinical grounds. However, it is not possible, clinically, to definitely separate haemorrhages from infarction, as revealed by brain computed tomography (CT), which is now routinely used in most stroke centres. Small haemorrhages can give comparatively minor symptoms or even transient symptoms. Whereas haemorrhages are seen on CT scan immediately after onset, brain infarcts may not be visible during the first days and, if small, may not be detected at all. A lumbar puncture, previously the investigation of choice to separate haemorrhage from brain infarction, is now less frequently performed in centres where CT is available, but might be of value in selected cases.

Ancillary Methods

1) Doppler techniques and ultrasound imaging techniques

The Doppler techniques depend upon the reflection of a beam of sound of very high frequency by moving red blood cells. Doppler techniques are widely used for the diagnosis of cerebrovascular occlusive disease. The most commonly used technique is that of plotting the Doppler frequency shift against time. When stenosis is present, blood flow velocity increases in the stenotic portion of the vessel and is detected by an increase in the frequency of the Doppler shift signal. Turbulence distal to the stenosis produces a characteristic visual pattern. To visualize the carotid arteries, a two-dimensional ultrasound imaging technique is used. Currently, the two techniques (Doppler flow and imaging) are combined in real time, defining structure and flow. This technique is called Echo-Doppler.

Transcranial Doppler ultrasonography is a non-invasive procedure for the assessment of intracranial cerebral circulation, allowing measurement of blood velocity in cerebral arteries at the base of the brain. However, since the diameter of the arterial lumen is unknown the blood flow cannot be determined. Despite this limitation, the method can be useful in answering specific questions such as detection of haemodynamically significant intracranial arterial stenosis. It is particularly useful in following changes in patients with subarachnoid haemorrhage who develop spasm, monitoring of brain-injured patients and intraoperative and postoperative monitoring of neurosurgical patients (Petty et al., 1990).

2) Magnetic Resonance Imaging (MRI)

The underlying principle of MRI is that many nuclei respond to the application of strong magnetic fields by absorbing and re-emitting radio waves, that can be detected and analysed and thus used to generate spectra indicating the concentration of various chemical species of these nuclei. Protons are among the most sensitive and abundant nuclei in biological tissues and have been widely used for MRI. Bone is not visualized and areas normally obscured by bone on CT scans are easily imaged. The resolution of grey and white matter is superior to that of CT scanning and cerebral infarction is evident much earlier, usually within 2 to 6 hours. However, CT is superior in early identification of brain haemorrhages and will probably continue to be the screening method at admittance of stroke patients. The recent development in MR angiography, MR diffusion weighted imaging, MR spectroscopy and functional MRI together with the higher time resolution and more widely accessible MRI than PET have made these techniques powerful tools in experimental and clinical stroke research (Baron, 1993; Neil, 1993).

3) Cerebral angiography

Cerebral angiography involves the selective introduction of a water soluble contrast medium into the carotid or vertebral arteries. It is used predominantly to detect and evaluate extracranial anomalies, particularly carotid stenosis, to diagnose arterial dissection and arterial and arteriovenous aneurysms.

4) Single photon emission computerized tomography (SPECT) and positron emission tomography (PET)

In acute stroke, the normal coupling between cerebral

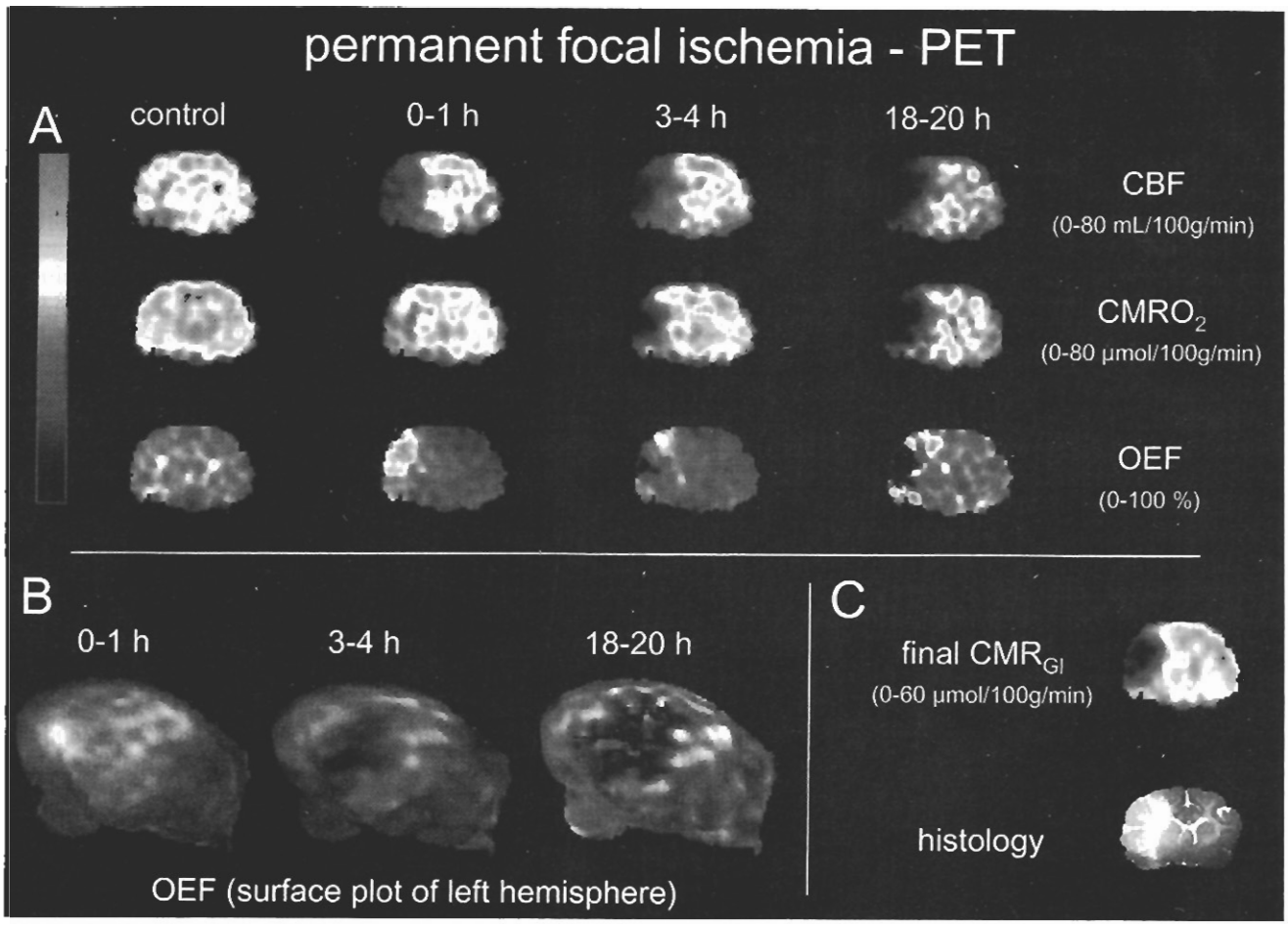

Fig 1.
A: Sequential quantitative PET images of an individual cat representing cerebral blood flow (CBF), cerebral oxygen consumption ($CMRO_2$) and oxygen extraction (OEF) before (control) and at 3 time points after permanent left middle cerebral artery (MCA) occlusion (0-1h, 3-4 h, 18-20 h). Progressive deterioration of oxygen consumption in the MCA territory corresponds with the spreading of the area with increased OEF and finally leads to hemodynamic and metabolic derangement.
B: Reconstructed OEF surface views of the left hemisphere (masked by control CBF) at the same 3 time points showing dynamic penumbra with progressive metabolic derangement.
C: PET image of glucose consumption (CMR_{Gl}) representing the area of final CMR_{Gl} suppression at 18-20 h after MCA occlusion and corresponding histological cross section showing the area of infarction at the same time point.
Adapted from Graf at al (1994) with permission.

blood flow and metabolism is not upheld. Without a concomitant determination of the oxygen extraction or cerebral metabolism, it is not possible to determine the state of the tissue by cerebral blood flow studies alone. This has been illustrated in studies using PET alone (Wise et al., 1983; Brooks, 1991; Heiss et al 1992). In the early stage, the blood flow and the metabolism might be low but oxygen extraction increased, suggesting that the tissue is still viable (Fig1). Later, particularly if the blood flow is restored, hyperaemia occurs while the oxygen extraction is low or nil indicating that the tissue is severely damaged. The rate of spontaneous reperfusion increases gradually with time and occurs within the first two weeks after stroke onset in 77 per cent of patients with cortical infarcts (Jorgensen et al., 1994).

Examples of remote metabolic depression after focal stroke have been observed with PET in man. Reduced metabolic activity can be seen in the cerebral hemisphere contralateral to cerebral infarcts, ipsilateral to thalamic and lenticulo-capsular lesions, the thalamus ipsilateral to a cortical infarct, visual cortex distal to lesions of the optic radiation and the cerebellar cortex contralateral to supratentorial infarcts (crossed cerebellar diaschisis). The exact mechanism underlying these metabolic changes that usaually are accompanied by changes in CT or MRI is not completely understood (Pappata et al., 1987; Ginsberg, 1990).

Risk Factors
The stroke incidence increases markedly with age. In a Swedish unselected population only 20 per cent of first-ever stroke patients were below the age of 65 and half of the patients were more than 75 years of age (Johansson et al., 1992). With the current ageing of the world population, stroke is likely to remain a major medical problem.

Hypertension
Hypertension is the most important risk factor for stroke. Hypertension predisposes to different types of intracerebral and extracerebral arterial lesions which may cause cerebrovascular events by different

mechanisms (Johansson, 1992). Hypertension leads to three main types of vascular changes: compensatory structural adaptation, degenerative vascular changes and, in the presence of other risk factors, to atherosclerosis.

Structural Adaptations to hypertension
When the blood pressure is increased abruptly to high levels in a previously normotensive individual, the autoregulatory capacity of the cerebral resistance vessels might be overcome and the blood flow increases. A stepwise increase in blood pressure will be tolerated. In chronic hypertension the vascular bed will adjust functionally and structurally to the increased load. The smooth muscle hypertrophy/hyperplasia will help to sustain wall tension and maintain adequate contractile function. In the cerebrovascular bed, an increased media thickness and lumen reduction has been observed over a large range of arterial sizes in vivo and in vitro, starting at rather large arteries. These changes will shift the autoregulatory curve to the right and protect the blood-brain barrier (Strandgaard,1978; Johansson, 1989). Although the structural adaptations basically are beneficial in protecting the vessels and preventing haemorrhage and permeability changes, they constitute a risk for ischaemia distal to any stenosis or occlusion or when the blood pressure is rapidly decreased because of the increased peripheral resistance and reduced collateral capacity. In connection with an abrupt decrease in blood pressure in hypertensive individuals, infarction may occur in the border zones between the territories of the main cerebral arteries. These 'water-shed' infarcts constitute approximately 10 percent of all human brain infarcts (Torvik, 1984).

Degenerative changes in hypertension
Degenerative changes in the small intracerebral blood vessels will occur when the compensatory mechanisms are not sufficient to protect the smaller intracerebral vessels. When the vessels yield to the high pressure, extravasation of plasma constituents seems to be the first step in hypertensive degenerative lesions in the vascular wall and in the development of brain lesions (Johansson,1992). Degenerative changes in the intracerebral arteries can lead to focal brain oedema, small lacunar infarctions or intracerebral haemorrhages due to intracerebral microaneurysms (Johansson,1992).

Hypertension, atherosclerosis and other risk factors
Hypertension is a risk factor for atherosclerosis. Vascular changes related to atherosclerosis or ageing are more pronounced in hypertensive individuals. The predominant sites, and usually earliest localization of atherosclerotic changes, are in the extracranial arteries. The second most common site is in the circle of Willis and with time, the changes may also occur in the smaller intracerebral arteries. This is reported to occur earlier in hypertensive than in normotensive individuals.

Other risk factors include diabetes, cigarette smoking, impaired cardiac function and high hematocrit and plasma fibrinogen levels. About 10 percent of strokes are preceded by transitory ischaemic attacks. There are substantial race differences with higher incidence in Japan and China, and in the US in the black population than in the white. There is an association between stroke and alcohol intake in men, which partly but not completely can be attributed to coexistent hypertension. Although epidemiological studies are lacking, drug abuse, particularly amphetamine, heroin and 'crack', a cocaine preparation that is inhaled, have repeatedly been reported to be associated with stroke in young individuals.

Several studies suggest that family occurrence of stroke is an independent risk factor. Homocysteinaemia, long known to be a risk factor in homozygotes, has in several recent studies been reported to be a risk factor in heterozygotes also.

Animal models of cerebral ischaemia
Animal models are required for detailed study of the pathophysiology of cerebral ischaemia. An 'ideal' animal model would have the following features:

- The animal model should closely mimic the development of the clinical insult in humans.
- The experimental insult should elicit similar responses in each individual animal tested; i.e., the insult should cause a reproducible injury.
- The model should be closely related physiologically; e.g., temperature, blood glucose, and blood pressure should be tightly controlled.
- The experimental animal pathology should be similar to that in humans.

Obviously, there are no 'ideal' animal models and it must be clearly understood that no animal model exactly reproduces cerebral ischaemia in man. Animals do not readily develop the cardiovascular disease that commonly underlies stroke, nor do they duplicate the age, nutritional status, or drug history of patients who suffer from stroke or cardiac arrest. Furthermore, animals are usually anaesthetized, are paralyzed, and have undergone surgery, particularly craniotomy, all of which may profoundly alter tissue response. Reproducible models are not easily achieved because of anatomical and physiological variability, both within and among species. In addition, lack of strict control of physiological variables, e.g., brain temperature, can lead to misleading results. In spite of these limitations, numerous animal models (rats, rabbits, gerbils, cats, dogs, goats, pigs and monkeys) have been developed and provide meaningful data that can be applied to the understanding of cerebral ischaemia in humans.
Experimental ischaemia is induced in animals by occluding vessels that perfuse the brain. Focal ischaemia is modeled by middle cerebral artery occlusion (MCA); in some models this is accompanied by occlusion of the ipsilateral common carotid artery. Models of ischaemia can be permanent (no reperfusion) or transient (allowing reperfusion). Models of global ischaemia are utilized to simulate ischaemic injury resulting from cardiac arrest. In large animals, global ischaemia has been modeled in numerous ways, including inducing cardiac arrest, using a neck tourniquet, cross-clamping the proximal aorta, and raising the intracranial pressure to levels greater than mean arterial blood pressure. Our experimental approach, for induction of focal cerebral ischaemia in cat

models involves occlusion of the proximal middle cerebral artery. Briefly, after enucleation, a small burr hole of 3-mm diameter is drilled transorbitally above the optic foramen in the posterior wall of the left orbit. The trunk of the left MCA is prepared just above the optic nerve by cutting dura and arachnoid membranes under microscopic control, and an occluding device (Graf et al., 1986) is implanted around the MCA trunk. This device consists of an outer cannula whose tip forms a hook to be put around the MCA, and an inner occluder, which can slide into the hook through the cannula. By pushing the inner occluder toward the silicon-coated wall of the hook, the MCA is compressed gently and firmly between the occluder and the hook wall, yielding total arterial occlusion, and by pulling back the occluder, the MCA occlusion is easily relieved.

Animal models are necessary to study cerebral ischaemia, and despite significant shortcomings, animal models have provided and will continue to provide insights into the pathophysiology of cerebral ischaemia which may lead to the development of effective pharmacological interventions.

Effects on brain tissue, mechanisms of neuronal death, post-ischaemic reperfusion

As already mentioned earlier, the high metabolic need and low energy reserve make the brain very vulnerable during ischaemia. When the perfusion pressure falls below critical levels, ischaemia develops and will progress to infarction if the flow is sufficiently reduced and the reduction persists long enough. In experimental studies on focal brain ischaemia, surprisingly extensive restoration of metabolism and function has been observed after ischaemic periods as long as 60 min or more. The length of time tolerated varies with factors such as the degree of reflow after ischaemia, and plasma concentrations of glucose, corticosteroids and catecholamines (see Figure 2).

Currently the penumbra is looked upon as a dynamic process with impaired and unstable perfusion and metabolism which is the target for possible therapeutic intervention (Heiss and Graf, 1994). There is some disagreement as to the time during which the tissue may be rescued but this is likely to be related to the degree of perfusion deficit and may be up to a couple of hours. However, with long recovery periods, permanent selective neuronal damage is seen already in ischaemia of 5-15min duration (Ito et al., 1975). This 'maturation' of neuronal damage following transient global ischaemia, which has been verified in a number of studies, indicates an on-going process in the post-ischaemic period.

Clot resolution or recanalization of an occluded artery may occur spontaneously but the frequency is unknown. Animal as well as clinical studies have demonstrated that this will often lead to postischaemic hyperaemia. Wheras hyperaemia has been reported to be essential for the functional recovery in global ischemia, hyperaemia has been associated with pronounced oedema and severe brain damage after focal ischaemia. Prevention of hyperaemia reduces the vasogenic oedema as well as the ischaemic brain damage in focal ischaemia in

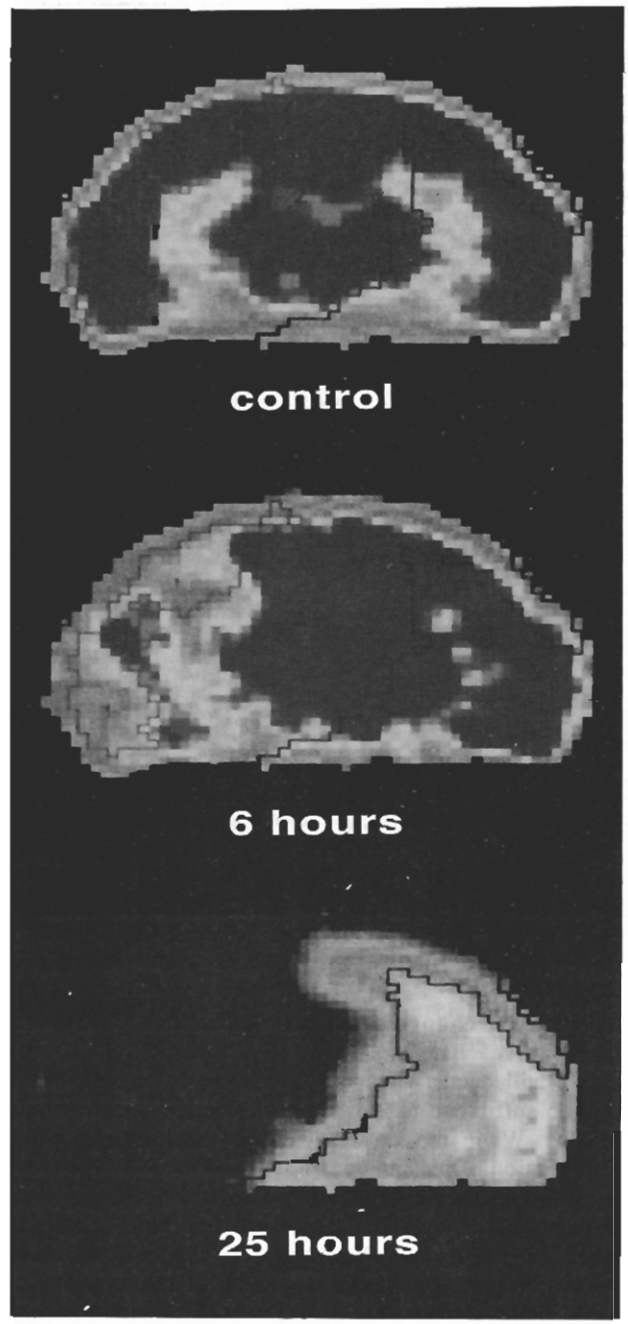

Fig 2. Sequential PET images of an individual cat representing CMR_{Gl} before (control) and at 2 time points after left middle cerebral artery occlusion (6 hours and 25 hours). Progressive deterioration includes in this case the hemisphere contralateral to the ischemic focus, probably due to malignant edema formation and rise of intracranial pressure.
[By courtesy of Dr. R. Graf. Max Plank Institute for Neurological Research, Cologne, Germany.]

experimental animals. If confirmed in man, these observations could be of importance in clinical trials with fibrinolytic agents and during operations on severely stenotic vessels where gradual rather than abrupt opening of an occluded vessel should be aimed at. However, in the clinical situation it would be a difficult task to decide if and when attempts to reduce the blood flow should be tried. An aggravating effect of postischaemic hyperemia could be related to any of the currently discussed mechanisms for nerve cell death following ischaemia such as free radicals, lactacidosis,

excitatory amino acids and possibly to some additional intrinsic or extrinsic factors. However, so far clinical studies have not confirmed that hyperaemia aggravates the lesions, rather hyperaemia has been proposed to be a prognostically good sign (Jorgensen et al., 1994; Marchal et al., 1993). Preliminary reports from studies on thrombolytic therapy in stroke indicate that it might be a smaller problem than expected. One possible explanation could be that the reflow does not occur so rapidly as under experimental conditions. Further studies are needed to clarify the possible adverse and beneficial effects of hyperaemia in stroke.

One other problem that must be anticipated in ischaemia is the underlying neuronal damage. Since energy is required to uphold the ion gradients across nerve and glial cell membranes, energy failure will lead to a shift with efflux of K^+ from cells and influx of Na^+, Cl^- and Ca^{2+} . This ion shift will lead to an accumulation of water within the cells, an intracellular oedema. Accumulation of metabolites within the cell will add to this oedema, which in the early stage is completely reversible. Ischaemia leads to a diffuse transmitter release since energy is needed to keep transmitters stored in their granules. The electrical activity of the neurons stops when the blood flow is decreased to about one-third of the normal values (under normal temperature and blood glucose levels) but some basic cell function is still present and the cells can regain their function if the blood supply is restored. There are various hypotheses as to the triggering mechanisms for neuronal death and some will be presented below. Current research indicates that these mechanisms combine in the process that finally kills neurons. The neuronal damage can be of two types: selective neuronal vulnerability affecting groups of neurons with a characteristic distribution within the brain, and infarction, affecting not only neurons but also blood vessels and glial cells.

The calcium hypothesis

That cell death may be a specific consequence of disturbance in intracellular Ca^{2+} homeostasis has long been discussed (Schanne et al., 1979). The concentration of calcium is 10,000 times higher in the extracellular fluid than in the cells and influx of calcium into the cells together with release of calcium from intracellular sources leads to uncontrolled activation of a number of calcium-dependent reactions (Siesjo, 1992; Morley et al., 1994). The combination of energy failure and calcium influx/release can lead to an extensive breakdown of phospholipids and proteins, to proteolytic degradation of cytoskeleton components and to free radical formation.

The excitotoxic hypothesis

Glutamate and some other excitatory amino acids have a transmitter function in the brain but can also be toxic to the neurons. First, exposure of neurons to glutamate may cause an acute neuronal swelling resulting from the depolarization-mediated influx of Na^+ Cl^-, and water. The degree to which this event contributes to neuronal injury is unclear, but it has been suggested that water entry causes osmotic lysis, which may disrupt neuronal function.

In order to prevent a high extracellular concentration of glutamate and other excitatory amino acids, these are taken up by efficient re-uptake mechanisms after release. The uptake mechanisms are energy dependent and during ischaemia the excitatory amino acids may accumulate in high concentrations. Current evidence indicates that ischaemic neuronal damage may be caused by enhanced release or diminshed uptake of glutamate or other excitatory amino acids enabling an enhanced calcium influx through channels gated by excitatory amino acid receptors (Rothman and Olney, 1986; Hossmann, 1994). The elevation of intracellular Ca^{2+} is known to activate lipases, phospholipases, proteases, and protein kinases, each of which, if not properly regulated, can easily be envisaged to produce considerable cellular damage. Antagonists which block N-methyl-d-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-isoxazoleproprionic acid (AMPA) glutamate receptors have been shown to ameliorate neuronal damage in experimental ischaemia (Choi, 1990; Graham et al., 1993).

Free radicals

The role of free radicals in the ischaemic nerve cell pathology has been debated for over a decade (Siesjo,1992; Chan, 1994). It has been suggested that free radicals are important particularly in the reperfusion period with good access to oxygen in an already damaged tissue. However, recent studies suggest a role also in permament ischaemia and free radical scavengers have been shown to reduce the infarct size in experimental brain infarction. Likewise, studies have shown that transgenic mice, overexpressing the enzyme Cu-Zn-superoxide dismutase, develop smaller infarcts than control animals (Chan et al., 1991). Calcium will enhance lipolysis and accumulation of arachidonic acid and interact with the free radical mechanisms in the degradation of lipids, proteins and DNA. Metals, including iron, increase the rate of lipid peroxidation and it has been suggested that free radical damage may also be related to alterations in iron-binding.

Another factor thought to be of importance for ischaemic injury is platelet-activating factor which may act at least in part by generating free radicals (Lindsberg et al., 1991). Since the metabolic needs of the brian increase with temperature, lowering of the brain temperature will lead to better survival of neurons during ischaemia. Free radicals also stimulate the release of glutamate in rat hippocampal slices and neuronal cultures. This suggests that free radical formation and glutamate release are mutually related and cooperate in a series of molecular events that link ischaemic injury to neuronal cell death.

The role of nitric oxide

Nitric oxide (NO) has been regarded as one of the mediators playing a key role in the pathophysiologic mechanism of focal cerebral ischaemia (Dalkara and Moskowitz, 1994; Dawson, 1994). As expected from the complex and diverse actions of NO, studies attempting to modify NO production in focal cerebral ischaemia report conflicting results (Dalkara and Moskowitz, 1994; Dawson, 1994). Nitric oxide acts as an intercellular messenger molecule in the brain; it simply diffuses out of a cell where it has been

synthesized into the neighbouring cells where its targets exist (Dawson and Snyder, 1994). Like authentic neurotransmitters, NO must diffuse across the extracellular space to exert its biological effects, and thus extracellular NO concentration probably is a good indicator of physiologic NO activity. With the invention of NO-sensitive electrodes, real time measurements of extracellular NO concentration has become feasible. The first attempts to apply them to focal cerebral ischaemia have reported an increase in tissue NO concentration (Malinski et al., 1993; Ohta et al., 1996) and NO is thus implicated as a mediator of tissue injury. Within 3 to 24 minutes after MCA occlusion, NO increases dramatically from approximately 10nM to 2.2 (M within cortex as detected by a porphyrinic microsensor (see Figure 3). Brain nitrite, cGMP levels and brain NOS activity increase as well (Malinski et al., 1993). Nitrite, NO and nitric oxide synthase (NOS) activity return to baseline levels within an hour. One speculation holds that NOS activity increases due to a rise in intracellular Ca^{2+}/calmodulin complex (Dawson and Snyder, 1994). Brain NO is synthesized from L-arginine and oxygen by NOS requiring NADPH, flavins and tetrahydrobiopterin. Among some isoforms, constitutive NOS is habitually present in neurons and endothelial cells in brain tissue, and is calcium/calmodulin dependent, ready to be activated by a small increase in cytosolic Ca^{2+} concentration (Dawson and Snyder, 1994). In ischaemic tissue, cytosolic Ca^{2+} can be raised by Ca^{2+} influx through NMDA receptor-mediated Ca^{2+} channels and voltage-sensitive Ca^{2+} channels (Siesjo, 1992). These Ca^{2+} channels require depolarization of the plasma membrane to let Ca^{2+} in, as in the case in ischaemic tissue. Intracellular Ca^{2+} stores, such as endoplasmic reticulum, must also be quoted as possible sources of a possible rise in cytosolic Ca^{2+} in the ischaemic tissue (Silver and Erecinska, 1990; Mitani et al, 1993).

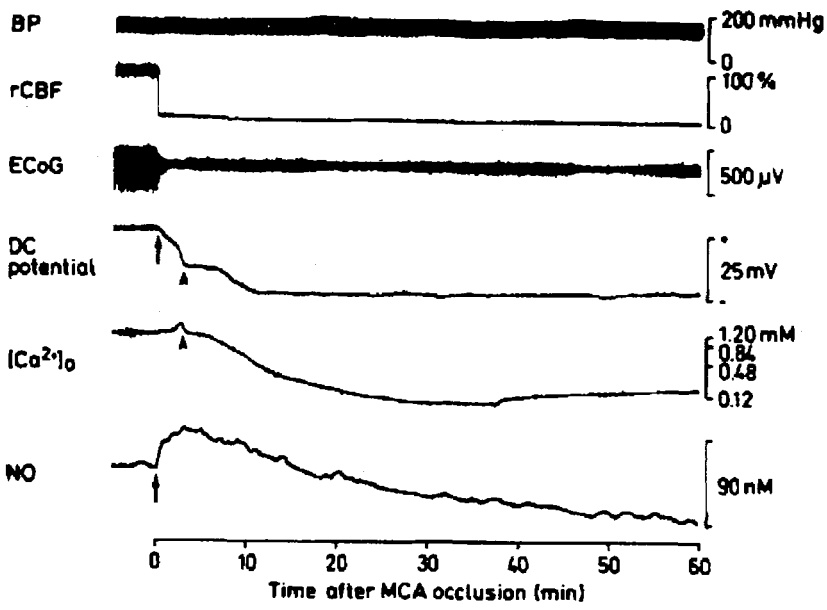

Fig 3. Actual recording of systemic arterial blood pressure (BP), regional cerebral blood flow (rCBF), electrocorticogram (EcoG), direct current (DC) potential, extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$), and nitric oxide concentration (NO) in the ectosylvian gyrus. Middle cerebral artery (MCA) occlusion caused an immediated drop in rCBF accompanied by a steep negative shift of the DC potential and a sudden increase in NO (see arrows). Note that$[Ca^{2+}]_o$ showed a small elevation in the initial phase when NO increased, and that the steep fall $[Ca^{2+}]_o$ occurred much later at 160s after MCA occlusion. *Adapted from Ohta et al (1997) with permission.*

Although in normal brain NO seems to be a nontoxic mediator of cerebral vasodilation, recent data suggest that NO may have neurotoxic effects if present in abnormally high concentrations (Dawson et al., 1991). In endothelium and brain, ionized calcium is the intracellular messenger initiating the reduced nicotinamide-adenine dinucleotide-dependent oxidation of arginine to produce NO. It is well known that cerebral ischaemia causes an increase in extracellular concentration of glutamate and aspartate, and these excitatory amino acids bind to and stimulate NMDA receptors in brain (Beneviste et al ., 1984). Excessive activation of the NMDA receptors allows influx of Ca^{2+} into neurons, which may stimulate production of superoxide anion via a prostaglandin pathway and NO via stimulation of NOS. Stimulation of non-NMDA glutamate receptors is also thought to generate NO (Southam et al, 1991). Moreover, inhibition of NOS in neuronal culture ameliorates glutamate toxicity (Dawson et al., 1991). One postulated mechanism of NO toxicity is that at high concentrations, superoxide anion and NO may react to form the peroxynitrite anion, which decomposes at acidic pH into strong oxidants. Thus excessive NO generation might act as an agent of cell death in stroke by reacting with superoxide.

In conclusion, the discovery of NO has provided a further opportunity to explore the pathophysiology and treatment of cerebral ischaemia. Controversy abounds, which may reflect the importance of NO to the diversity of factors (both known as well as unknown) which impact cerebral ischaemia. However, it would be of interest to determine whether drugs that alter NO synthesis and metabolism will be of use in the treatment of ischaemia. More to the point, one still needs to demonstrate whether NO is relevant to the pathophysiology of global ischaemia. Nevertheless, answers should be forthcoming from a number of developments, namley;

- selective inhibitors for the neuronal or vascular isoforms of NOS
- transgenic mice in which neuronal or endothelial NOS are selectively knocked-out
- discovery of new methods for both directly measuring NO and assessing NOS activity routinely.

Models of ischaemia and reperfusion

It is evident from evaluating different animal models of ischaemia and reperfusion that a number of factors are important in determining the extent of neurological damage that occurs as a direct result of ischaemia. These include but are not limited to the amount of blood flow reduction, the duration of time that flow is reduced, the regional location of the flow reduction, and the metabolic state of the brain before the ischaemic period. Multiple mechanisms may contribute to the injury, and the relative contribution of each mechanism depends on the specific situation. For example, calcium-induced damage from the release of excitatory neurotransmitters is generally most prominent with moderate reductions of blood flow in regions with high NMDA receptors (Monaghan et al., 1985) and high excitatory amino acid innervation. With severe or complete reduction of blood flow, calcium entry through non-NMDA receptor-operated channels and voltage-sensitive channels becomes prominent (Siesjo et al., 1985). With reduced blood flow over prolonged periods, continued anaerobic glycolysis enhances the contribution of acidosis to ischaemic injury (Rehncrona et al., 1981). Therefore, when different potential mechanisms of ischaemia and reperfusion injury in brain are evaluated, it is important to recognize that the mechanism of injury will depend on the degree, duration, and localization of reduced blood flow and other aspects of the experimental model. Furthermore, the issue of radical-mediated injury is complicated because the various potential mechanisms are interrelated with radical injury. Acidosis can potentiate lipid peroxidation, calcium accumulation stimulates phospholipase activity, which may generate radicals via arachidonic acid metabolism; and NMDA receptor activation generates NO which in turn is capable of reacting with superoxide to form the cytotoxic oxidants as exemplified before.

Thus it is an oversimplification to consider an array of parallel pathways that can be independently blocked for assessing the individual contribution of these injury mechanisms in the brain.

Pharmacological intervention in ischaemia

Despite major advances in deciphering the biochemical events involved in the pathophysiology of cerebral ischaemia, little has been done to arrest, prevent, or reverse ischaemic injury. It has been realized that the extent of irreversible damage is governed by both the duration of ischaemia and the severity of ischaemia (complete versus incomplete). A number of pharmacological agents have been utilized for cerebral ischaemia in both animal models and humans, but to date, no agent has been shown to be of unequivocal value; i.e., there is no effective treatment for cerebral ischaemia, presently. However, a brief outline of the pharmacological agents together with their proposed pharmacological strategies for the treatment of cerebral ischaemia are listed below.

1) Calcium channel blockers

These drugs block the entry of calcium ions into the ischaemic neurons. These drugs do not themselves antagonize the effects of calcium ions; instead they prevent this ion from gaining access to its intracellular site of action. By blocking the entry of calcium ions, they may inhibit the essential role of this cation in the activation of lipolytic enzymes, protein kinases, and phosphatases during ischaemia. Improved cerebral blood flow, improved or no change in neurological outcome, decreased brain lactic acidosis, and decreased infarct size all have been reported following calcium channel blocker treatment in various studies (Wong et al., 1990).

2) Vitamin E

α-Tocopherol, a well known antioxidant, has beneficial effects on brain oedema and ischaemia. It inhibits the activities of phospholipase A_2 and lipoxygenase and plays a fundamental role in the stabilization of polyunsaturated fatty acid chains in membrane phospholipids. Vitamin E interacts with cellular membranes and prevents lipid peroxide formation by acting as a hydrogen donor (Traystman et al., 1991).

3) CDP-amines

CDP-amines are key intermediates in the biosynthesis of phosphatidylcholine and phosphtidylethanolamine. The therapeutic actions of CDP-amines are thought to result from restorative effects on phospholipid synthesis in the ischeamic brain. CDP-amines attenuate the fatty acid increases and counteract the disruption of cerebral mitochondrial lipid metabolism induced by hypnoxia. They have been reported to inhibit the activities of phospholipases A1 and A2. CDP-amines have also been reported to increase oxygen consumption and glucose incorporation into amino acids and phospholipids followed by a decrease in lactate production (Murphy et al., 1990).

4) Glutamate antagonist MK-801

The use of MK-801 for the treatment of ischaemia is controversial. It has been used successfully for the treatment of cerebral ischaemia in experimental models. MK-801 may exert its antagonistic effects via a site related to the ion channel. The onset of NMDA receptor blockage with MK-801 is more rapid in the presence of glutamate. These two effects may be relevant to the efficacy of MK-801 in cerebral ischaemia, which provokes a marked elevation in extracellular concentrations of glutamate. In addition to direct receptor blockade, MK-801, may protect neurons with severe, but not complete, energy failure by preserving ionic gradients across the plasma membrane and enhancing amino acid uptake (Buchan, 1990).

5) Superoxide dismutase

SOD has been proposed as a therapeutic agent for reperfusion injury because of its ability to scavenge superoxide anion. However, Cu-ZnSOD, is a large water soluble molecule (32 kDa) and therefore cannot penetrate the blood-brain barrier in significant quantities. In addition, SOD has a circulatatory half-life of only 8 mins in rats. In an effort to overcome these problems, Traystman (1991) conjugated this enzyme and administered it intravenously in rats as liposome-entrapped SOD and polyethylene glycol-conjugated SOD.

SOD delivered in liposomes has been shown both to increase brain SOD activity and to reduce infarct volume in a rat model of focal cerebral ischaemia. In contrast, polyethylene glycol-conjugated SOD does not appear to increase brain SOD activity but has been shown to reduce infarct volume in animal models of focal cerebral ischaemia.

6) Platelet-Activating factor antagonist

Large amounts of platelet activating factor (PAF) are produced by brain tissue and endothelium cells in response to ischaemia and reperfusion. PAF is a powerful vasoconstrictor and has many cytotoxic properties. PAF antagonists that are present in an extract of Ginkgo biloba leaves (ginkgolide B) appear to reduce edoema and neuronal damage in several mammalian species (Braquet et al., 1989).

7) U74006F, 21-Aminosteroids (Lazaroids)

U74006F, a nonglucocorticoid 21-aminosteroid, is a potent inhibitor of lipid peroxidation. It has a beneficial effect in animal models of severe head injury, posttraumatic spinal cord ischaemia, and cerebral ischaemias. The mode of action of 21-aminosteroids is not known, but it may act by inhibiting iron-dependent lipid peroxidation. The 21-aminosteroids significantly reduce Na^{2+} accumulation, K^{+} loss, and water entry into ischaemic brain. The effect was found to be most consistent and prominent in tissues surrounding the infarct site (Hall et al., 1990).

8) Cholesterol-lowering agents and oestrogen

Two agents that are in routine clinical use - inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase or 'statins' (Cholesterol-lowering agents), and oestrogen have been shown to reduce brain vulnerability to focal ischaemic insults (Endres, 1998). It is thought that the former may work largely by upregulating the activity of endothelial NOS; the mechanism of oestrogen-induced neuroprotection is unknown, but it may involve antioxidant actions or amplification of trophic mechanisms.

Future therapeutic directions

Advances in methods of brain imaging, such as magnetic resonance imaging and positron emission tomography, should allow more accurate delineation of tissue regions at risk because of impairment of blood supply, but not irreversibly damaged, and so will enhance the targeting of countermeasures in time and space. One can anticipate continued refinement in thrombolytic strategies, aiming to limit side effects at locations remote from the offending thrombus and thereby reducing bleeding complications, as well as more powerful approaches aimed at reducing brain oedema, or favourably altering the distribution of blood flow in cerebral arteries.

With regard to neuroprotection, it is predicted that several paths of research will gain momentum, aimed at blocking excitotoxicity in ways superior to that achievable with unselective NMDA antagonists, or moving away from central preoccupation with excitotoxicity and neuronal Ca^{2+} overload to target other processes and other ionic imbalances.

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