ISCHAEMIC BRAIN DAMAGE: THE ROLE OF EXCITATORY ACTIVITY AND OF CALCIUM ENTRY

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The pattern of selective vulnerability occurring after cerebral ischaemia is similar to that after status epilepticus (Brierley, 1976; Corsellis and Meldrum 1976). In the hippocampus, the pyramidal neurones in the CA1 and CA3 zones and the hilar polymorphic neurones are particularly vulnerable and the dentate granule cells are relatively resistant. During status epilepticus induced experimentally in rats by the i.v. administration of bicuculline, allylglycine or kainic acid (Evans, Griffiths and Meldrum, 1983, 1984; Griffiths, Evans and Meldrum, 1983, 1984), selectively vulnerable neurones show condensation of perikaryal cytoplasm and swelling of mitochondria. The changes are evident after 30–90 min of continuous seizure activity. Severe condensation with a pyknotic nucleus and multiple, small, structureless vacuoles occur in a minority of vulnerable neurones after 90–150 min (Evans, Griffiths and Meldrum, 1984; Griffiths, Evans and Meldrum, 1984).

The oxalate–pyroantimonate method for the electron microscopic visualization of calcium reveals massive calcium deposits in swollen mitochondria, both in focal dendritic swellings and in the perikaryal region (Griffiths, Evans and Meldrum, 1984). We have compared the acute changes in status epilepticus with those seen during reperfusion after 30 min of forebrain ischaemia (Simon, Griffiths et al., 1984). The similarity in the changes suggests that enhanced activity, probably taking the form of burst firing in vulnerable neurones, contributes to the pathological outcome.

Adult Wistar rats, prepared under halothane anaesthesia and maintained on nitrous oxide–oxygen, were peripherally paralysed and snared placed round the common carotid arteries. After a period of stabilization, the arteries were occluded and the mean arterial pressure was reduced to 50 mm Hg. After 30 min, flow was restored and mean arterial pressure maintained at 100–120 mm Hg. The brain was perfusion-fixed with a modified Karnovsky’s solution containing 2% paraformaldehyde, 2.8% glutaraldehyde and potassium oxalate 90 mmol litre$^{-1}$. Vibratome slices of dorsal and ventral hippocampus were washed in 7.5% sucrose containing potassium oxalate 90 mmol litre$^{-1}$. The modified pyroantimonate technique for precipitating calcium as calcium pyroantimonate was performed (Borgers, Thone and Van Neuten, 1981), and slices were embedded in araldite, cut at 50–80 nm and stained with uranyl acetate and lead citrate.

After 30 min ischaemia and no reperfusion, hippocampal neurones showed normal ultrastructural morphology except for clumping of nuclear chromatin. This was observed equally in pyramidal neurones and dentate granule cells and probably reflects the intracellular acidosis associated with ischaemia. Occasional neurones showing swollen mitochondria with dense calcium pyroantimonate deposits were apparently indicative of partial ischaemia, that is, a focally maintained minimal flow permitted the accumulation of calcium.

After 30 min of reperfusion, cytopathology was severe and generalized, affecting pyramidal neurones and dentate granule cells equally. Nuclear outlines were irregular and the density of the cytoplasm was increased. In nearly all neurones there were swollen disrupted mitochondria containing dense calcium pyroantimonate deposits. Swollen astrocytic processes occurred throughout the pyramidal cell layer and hilar region.

After 2 h reperfusion, a pattern of selective pathology was evident. Dentate granule cells were uniformly normal in morphology. Pyramidal neurones showed marked variation, some appearing...
essentially normal, others showing "ischaemic cell change", with severe condensation obliterating fine structural detail except for multiple vacuoles containing calcium deposits (fig. 1). Ischaemic cell change was commonest in pyramidal cells in CA1 (adjacent to the subiculum) and in basket cells and polymorphic neurones in the hilar region. Focal dendritic swellings containing swollen mitochondria with dense calcium deposits were evident in the distal parts of the basal dendrites of CA1 and CA3. Such focal swellings nearly always showed one or more asymmetric (excitatory) synaptic terminals. These appearance were closely similar to those seen after 90 min of seizure activity (fig. 2).

Evidently, the movement of calcium from the extracellular space into neurones during the first minutes of ischaemia (Harris et al., 1981) does not lead to intracellular calcium deposits that are visualized by this method. However, during reperfusion, extracellular calcium is replenished and mitochondrial oxidative metabolism re-established so that, with only partial repolarization of the plasma membrane or burst firing, calcium enters neurones and is accumulated actively by mitochondria. This occurs non-selectively in the first 30 min of reperfusion. Subsequently, normal resting potentials are restored and outward transport of Ca^{2+} by membrane Ca–Mg ATPase brings cytosolic [Ca^{2+}] to normal values, allowing efflux of Ca^{2+} from mitochondria. However, in some selectively vulnerable neurones, burst firing in the subsequent 90 min leads to a continued accumulation of Ca^{2+}.

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**Fig. 1.** Electron micrograph of CA1 region of the hippocampus in a rat after 30 min ischaemia and 120 min reperfusion. N = normal neurone; v = nerve terminal packed with vesicles containing calcium pyroantimonate deposits (a normal appearance); I = neurone showing ischaemic cell change; multiple vacuoles contain calcium pyroantimonate deposits. Bar indicates 1 μm.

**Fig. 2.** Electron micrograph of stratum oriens in CA3 region of hippocampus in a rat after 90 min of seizure activity. Focal dendritic swellings contain grossly swollen mitochondria with dense calcium pyroantimonate deposits. Synaptic terminals making excitatory (asymmetric) contacts are identifiable in relation to the focal swellings (arrowheads). Similar changes are found after 30 min ischaemia and 2 h recovery (Simon et al., 1984).
Evidence for a role of excitatory transmitters acting on a particular sub-class of receptors has been provided in a further series of experiments with similar ischaemic and reperfusion periods. 2-Amino-7-phosphonoheptanoic acid is a specific antagonist of excitation at the “N-methyl-D-aspartate receptor” and is a potent anticonvulsant agent (Meldrum et al., 1983). A chronically implanted guide tube permitted the focal injection of 2-amino-7-phosphonoheptanoic acid (20 µg in 1 µl) into one dorsal hippocampus immediately before the ischaemic episode. The brain was perfusion fixed after 30 or 120 min of reperfusion, and hippocampal blocks embedded in paraffin and serially sectioned for light microscopy.

The pathological changes were similar to those observed in the study described above, except that the injection of buffer solution appeared to delay recovery in the dentate granule cells so that, at 120 min, some of these still showed dark staining. In the hemisphere receiving 2-amino-7-phosphonoheptanoic acid, the hippocampus showed little or no pathological abnormality at 120 min, in dramatic contrast to the buffer-injected side. The protection involved all cell types within a sphere of 1–2 mm surrounding the injection site and on occasion protection of pyramidal neurones extended more anteriorly and posteriorly within the hippocampus. This protective action of 2-amino-7-phosphonoheptanoic acid implies that excitation at the “N-methyl-D-aspartate preferring receptor” plays an important part in determining the appearance of ischaemic cell change. Agonists at the NMDA-preferring receptor activate a membrane calcium conductance (Dingledine, 1983) and induce paroxysmal burst firing (Herrling, Morris and Salt, 1983). Spread of burst firing from CA3 neurones to CA1 neurones depends on an excitatory amino acid transmitter released by the Schaffer collaterals. It appears that suppression of burst firing during the recovery period by blocking one class of excitatory receptor site prevents excessive calcium entry and thereby protects against the acute occurrence of ischaemic cell change. These observations provide a novel therapeutic approach to cerebral ischaemia.

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References


